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Colonization Capability of Fluorescent Pseudomonads in the Rhizosphere of Tomato

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ABSTRACT

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Strains of fluorescent pseudomonads isolated from tomato roots differed markedly in their rhizosphere colonization ability. The rhizosphere population of 64 strains tested on 21-day-old tomato plants following seed bacterization ranged from 0 (nondetectable) to 5.52 log cfu/ g wet root, with most strains in the range of 3-4 log cfu/g wet root. The rhizosphere population of strain FP10 (a better rhizosphere colonizer) declined with plant growth and colonized mostly sections of roots near the point of attachment with the stem under greenhouse and field conditions. Seed bacterization followed by root treatment before transplanting increased rhizosphere populations of strain FP10 and enhanced also the distribution of the strain along the root. With seed bacterization, an increase in inoculum levels resulted in a proportionate increase of strain FP10 colonizing the rhizosphere. Strain FP10 had a higher density of rhizosphere population and colonized more root segments in the autoclaved soil than in the nonautoclaved soil. The rhizosphere colonization of strain FP10 was greater at 20 C than at 28 C. Colonization of strain FP10 in the rhizosphere of tomato growing in four of five soils tested was similar, but in an acidic Tachi loam soil, the rhizosphere population decreased more rapidly than in the other four soils. Adjustment of pH in Tachi soil from the original 4.1 to a range of 5.3-7.1 increased significantly the rhizosphere population of strain FP10.

Key words: tomato, fluorescent pseudomonads, rhizosphere colonization

INTRODUCTION

Fluorescent pseudomonads are potential agents for controlling soil-borne plant diseases and for promoting plant growth (13,19,23). Colonization of the root system by fluorescent pseudomonads is considered to be an important factor in their ability to suppress diseases and to enhance plant growth (19,23). Therefore, population dynamics of fluorescent pseudomonads in the rhizosphere of plants have been studied by many investigators. Most studies revealed that specific strains of fluorescent pseudomonads readily colonized root systems after being introduced onto seeds and persisted in varying population sizes during plant growth, however, the population was greatest on portions of root near the inoculum source and decreased toward the root tip (1,4,16,20,21). The root colonization of fluorescent pseudomonads was affected by many factors such as soil type (8,12), soil temperature (16), soil matric potential (8,15), soil microflora (6,18), plant variety (7,22), percolating water (1,6,15,17) and inoculum dose (3,16,21). In an attempt to evaluate the potential use of fluorescent pseudomonads for control of bacterial wilt of tomato caused by *Pseudononas solanacearum* (Smith) Smith, a serious soilborne disease in Taiwan, studies were conducted to screen strains of fluorescent pseudomonads isolated from tomato root for the ability to colonize the rhizosphere of tomato and to determine some factors affecting their rhizosphere colonization. A preliminary report of this work has been published (5).

MATERIALS AND METHODS

Bacterial strains

Sixty-four strains of fluorescent pseudomonads were isolated using King's B medium (11) from roots of healthy tomato plants collected from various localities in Taiwan. They were purified by selecting single colonies that produced fluorescent pigments from repeated plating on King's B medium.

Selection of antibiotic resistant mutants

To monitor the bacterial population size on seeds and in rhizosphere, all strains were selected for resistance to rifampicin and nalidixic acid. These antibiotic resistant mutants were obtained by streaking cells of bacterial

cultures first on King's B medium containing 100μg/ml rifampicin. Resistant colonies were isolated and then streaked on King's B medium containing 100 μg/ml rifampicin and 250 μg/ml nalidixic acid. Colonies that appeared on the medium containing both antibiotics were restreaked several times on the same medium to verify their resistance to the two antibiotics. The antibiotic resistant mutant from each strain was used in all experiments. All wild type strains and antibiotic resistant mutants were stored in screw-capped test tubes containing sterile distilled water at room temperature.

Seed bacterization

Inocula of the antibiotic resistant fluorescent pseudomonads were prepared by washing the 24–28 hr cultures from King's B medium plates with 5 ml of 1% methyl cellulose per plate. Tomato (*Lycopersicon esculentum* M., cultivar Known You No. 301) seeds, previously surfaces disinfested with 100-fold dilution of chlorox for 25–30 min and then rinsed with sterile water for three times, were soaked in the bacterial inoculum (100 seeds/10 ml) for 1hr at room temperature. The soaked seeds were then transferred onto filter papers and air dried in a laminar flow. The treated seeds were used immediately and the number of bacterial cells coated on seeds was determined just before use. Ten seeds, with three replicates, from each treatment were placed in 100 ml of 0.1 M $MgSO_4.7H_2O$ and agitated for 20 min. The resulting suspension was plated on King's B medium containing 100 μg/ml rifampicin and 250 μg/ml nalidixic acid with a spiral plater (Model D, Spiral System, Inc., Bethesda, Maryland, U.S.A.). Colonies were counted after 48 hr incubation at 30 C. The mean number of colony-forming units (cfu) per seed for each treatment was calculated based on colony counts in the three replicated determinations.

Screening strains for rhizosphere colonization capacity

All strains were tested for the ability to colonize the rhizosphere of tomato by a laboratory plastic bag assay modified from the sand-soil test tube method of Scher *et al.* (18). Plastic bags (25 cm long and 7.5 cm wide) were each filled with 50 g washed, dry fine sand and added with 10 ml water. The sand was then overlaid with 150 g field soil (Tali loamy sand soil, pH 7.8, organic matter 0.25%) adjusted to 15% moisture content. One bacteria-coated tomato seed was sown per bag in soil about 1 cm below the surface. Bags were sealed with tapes, and upper potion of the bag was punctured with a fine needle to produce 5–6 small holes. Bags were incubated in a growth chamber at 25 C for 21 days before being cut open to remove plants. The whole root system from each plant was excised and loose soil was shaken away. After weighing, the root system was places in 10 ml of 0.1 M $MgSO_4.7H_2O$ and shaken for 20 min. The suspension was plated on King's B medium containing 100 μg/ml rifampicin, 250 μg/ml nalidixic acid and 50 μg/ml cycloheximide (KB-RNC) with the spiral plater. Mean cfu/g wet root was determined from five replicated plants for each treatment.

Greenhouse pot experiments

Fifteen strains varied in the rhizosphere population density as determined by the plastic bag assay were further tested in the greenhouse. A bacteria-treated tomato seed was sown in each pot (11 cm diameter, 9cm high) filled with the field soil. Pots were placed in the greenhouse (25.8–34.2 C) and watered every day. At 21 days after seeding, plants were carefully removed from pots. The rhizosphere population of the 15 strains in each plant was then determined with the same procedure as described above. Population dynamics of three selected strains, FP3, FP10 and FP34, in the rhizosphere were also determined by the same method.

Distribution of strains FP3, FP10 and FP34 on roots were determined for 14- and 28-day-old seedlings. The entire root system was removed from the pot and gently shaken to remove all but the tightly adhering soil. Only the tap root with side branches removed was assayed. The tap root was cut into 2-cm segments. The root segment closed to the stem was placed in test tube containing 6 ml of 0.1 M $MgSO_4.7H_2O$ and the other segments were placed separately in eppendorf tubes containing 1 ml of the same solution. After vortexing for 20 sec, the suspension was plated on KB-RNC with the spiral plater. Five replicates were used per treatment in the greenhouse pot tests.

Field experiments

The experiment was conducted during October 1990 to February 1991 in the experimental field of Taiwan Seed Improvement and Propagation Station. Shinshieh, Taichung. Tomato seeds treated separately with strains FP3, FP10 and FP34 were sown in seedling raising trays (60 x 40 cm in size divided into 240 wells per tray) filled with BVB substrate (Bas Van Büren, No. 4, Maasland, Netherlands), at thedensity of one seed per well. Thirty days old seedlings were used for transplanting. The day before transplanting, a group of seedlings raised from bacteria-treated seeds for each treatment were root-treated with the same bacterial strains by adding the cell suspension (10^8 cfu/ml) to BVB medium in which seedlings were growing at the rate of 10 ml per well. The other group was not root-treated. Seedlings were transplanted into field plots, consisting of two 20-plant rows per plot (7.6 x 1.5 m) and three plots per treatment. The plots were arranged in a randomized complete block design. At 10-day or other intervals, eight plants randomly selected from three plots per treatment were sampled. Plants were dug with a shovel from the soil area (approximately 10 cm in diameter and 15 cm in depth) surrounding the plant. Plants were shaken to remove loose soil from roots. The root system severed from each plant was weighed, dissected into small segments

and placed in appropriate amount of 0.1 M $MgSO_4.7H_2O$. The rhizosphere population of the fluorescent pseudomonads in the root system was then assayed as described above.

Strain FP10 was examined for its distribution on roots from plants grown for 90 days after transplanting. Eight plants randomly selected from three plots (2-3 plants per plot) were sampled. The entire root systems were carefully removed from soil and loose soil was shaken away. Five to seven larger roots (30-50 cm in length) were excised from each plant. Each root was cut into four equal segments. The rhizosphere population of strain FP10 on each segment was then determined as described.

Effect of seed inoculum level on the rhizosphere colonization

Cells of strain FP10 were washed from the 24-hr culture grown on King's B medium plate with 5 ml of 1% methylcellulose. The bacterial suspension was diluted 4, 16 and 64-fold with 1% methylcellulose. Dry and surface-disinfested tomato seeds were soaked in each of the diluted bacterial suspensions (100 seeds/10 ml), and mean cfu/seed for each treatment was then determined as previously described. The bacteria-treated seeds were planted in pots containing a field soil. The rhizosphere population of strain FP10 was determined 21 days after planting as described for the greenhouse pot experiment.

Effect of soil type on the rhizosphere colonization

Five field soils were collected from Tanan, Taichung (loam, pH 5.1, organic matter 2.79%); Tali, Taichung (two soil samples, one was sandy loam, pH 5.6, organic matter 1.47%; another was loamy sand, pH 7.8, organic matter 0.25%); Tachi, Taoyuan (Loam, pH 4.1, organic matter 2.96%) and Taitung city (silt loam, pH 7.2, organic matter 2.05%). Tomato seeds treated with strain FP10 were sown in pots containing BVB substrate. Four days after germination, seedlings were transplanted in pots filled with different soils. Rhizosphere populations of strain FP10 were determined 18, 24, 36 and 56 days after transplanting.

Effect of soil pH on the rhizosphere colonization

The pH of the Tachi soil was adjusted with 1N NaOH from original 4.1 to 5.3, 5.9 and 7.1. Tomato seeds treated with strain FP10 were sown in BVB substrate, and 30 days old seedlings were then transplanted in pots containing the soil of different pH. The rhizosphere population of strain FP10 was determined 14 days after transplanting.

Effect of soil microflora on the rhizosphere colonization

Tomato seeds coated with strain FP10 were planted in pots containing a field soil with or without autoclaving (121 C, 50 min.). Ten days after germination, the rhizosphere population of the whole root system and distribution on the tap root of strain FP10 were determined.

Effect of temperature on the rhizosphere colonization

About 30 days old seedlings raised from strain FP10-treated tomato seeds in BVB substrate were transplanted to pots filled with a field soil. Pots were incubated in the growth chamber at 20 and 28 C. The rhizosphere population of strain FP10 for the whole root system was assayed 10, 20, 30, 45 and 55 days after transplanting. The distribution of strain FP10 on the tap root was determined only at 10 days after germination.

RESULTS

Rhizosphere colonization capacity among strains of fluorescent pseudomonads

The 64 strains varied greatly in their ability to colonize the rhizosphere of tomato when determined by the laboratory plastic bag assay. Rhizosphere populations on 21-day-old seedlings following seed bacterization ranged from 3.06 to 4.83 log cfu/g wet root for 36 strains and from 5.18 to 5.52 log cfu/g wet root for four strains. The other 24 strains were not detectable on roots (Table 1). The number of bacterial cells coated on seeds ranged from 5.37 to 8.58 log cfu/seed. Rhizosphere populations of strains resulting from seeds coated with higher cell number were not necessarily greater than those from seeds coated with lower cell number.

When 15 strains varying in the colonization capacity were selected and compared by the greenhouse pot and laboratory plastic bag assays, rhizosphere populations of a given strain determined by the two tests were not different significantly (Table 2). Strains that colonized well in the rhizosphere in the bag assay were also good colonizers in the pot test.

Population dynamics in the rhizosphere

In the greenhouse pot experiment, population of three strains tested in the rhizosphere declined gradually with plant growth (Fig. 1). Strains FP10 and FP3 were better colonizers because both strains decreased more slowly than FP34. After 60 days, FP10 and FP3 in the rhizosphere were detected at 4.26 and 2.89 log cfu/g wet root, respectively, whereas FP34 was not detected. Under field conditions, rhizosphere populations of FP10 and FP3 decreased until 53 days after transplanting and remained nearly stable thereafter, whereas the population of FP34 declined very rapidly reaching the non-detectable level within 30 days after transplanting (Fig. 2). In each strain, the rhizosphere population was consistently higher at each sampling time when introduced by seed bacterization followed by root treatment than by seed bacterization alone.

Colonization on root segments

In the greenhouse pot experiment, rhizosphere population of the three strains tested were detected only

TABLE 1. Colonization capacity[1] of 64 strains of fluorescent pseudomonads in the rhizosphere of tomato following seed bacterization

Strain	No. bacterial cells coated on seed (log cfu/seed)[2]	Rhizosphere population (log cfu/g wet root)[3]	Strain	No. bacterial cells coated on seed (log cfu/seed)	Rhizosphere population (log cfu/g wet root)
FP1	7.74	3.09	FP43	7.40	ND
FP2	7.41	4.11	FP45	7.97	3.40
FP3	8.03	5.52	FP47	7.06	ND
FP5	7.71	3.16	FP48	6.44	3.76
FP6	8.06	3.86	FP49	7.97	4.82
FP7	7.91	3.62	FP52	8.16	3.14
FP10	8.19	5.36	FP53	7.30	3.45
FP12	7.25	3.46	FP54	7.87	4.18
FP14	7.63	3.25	FP55	7.08	ND
FP15	5.37	ND[4]	FP56	6.92	ND
FP16	8.26	4.17	FP58	7.55	4.58
FP18	7.42	4.53	FP59	8.22	ND
FP19	8.30	4.83	FP60	8.17	ND
FP20	7.71	4.67	FP61	7.43	3.05
FP21	7.72	5.24	FP62	6.99	ND
FP22	7.32	4.37	FP63	7.91	3.86
FP23	7.94	4.79	FP64	6.47	ND
FP25	7.56	3.52	FP65	7.45	3.47
FP26	7.53	3.23	FP67	7.32	3.72
FP29	6.13	ND	FP68	6.87	3.56
FP30	6.09	4.33	FP70	8.38	ND
FP31	7.40	ND	FP71	7.76	ND
FP32	7.59	3.43	FP72	6.20	ND
FP33	7.29	ND	FP73	7.07	3.06
FP34	7.34	3.11	FP74	7.59	4.14
FP35	8.58	ND	FP77	7.72	3.49
FP36	7.94	ND	FP78	7.37	ND
FP38	7.01	3.79	FP80	8.09	ND
FP39	6.27	ND	FP81	8.38	ND
FP40	6.82	ND	FP84	7.15	4.39
FP41	7.94	ND	FP86	6.95	ND
FP42	7.94	5.18	FP87	7.31	3.59

1. Colonization capacity was determined by a laboratory pastic bag assay at 25 C.
2. Values are the mean of three replications with 10 seeds per replication.
3. Populations were determined on 21-day-old seedlings. Values are the mean of five replications with one plant per replication.
4. ND, not detectable.

on the first 2-cm segment of root adjacent to the inoculum source at both 14 and 28 days after planting, and higher rhizosphere population densities on this segment were achieved by strains FP10 and FP3 than by FP34 (Table 3). In the field experiment, only strain FP10 was examined for its distribution on root segments. When roots were sampled at 90 days after planting and each was divided into four equal segments with segment I being closest to the point of root attachment with the stem, rhizosphere population of strain FP10 were detected on segments I, II, and III if the strain was introduced by seed bacterization, but were detected on all segments if introduced by seed plus root treatments (Table 4). The rhizosphere population was highest at the base of the root and decreased toward the tip. Populations of strain FP10 on the same segments were greater when introduced by seed plus root treatments than by seed treatment only.

TABLE 2. Comparison of the laboratory plastic bag method with the greenhouse pot method for assaying the colonization ability of strains of fluorescent pseudomonads in the rhizosphere of tomato

Strain	No. bacterial cells coated on seed (log cfu/seed)[1]	Rhizosphere population (log cfu/g wet root)[2]	
		Bag	Pot
FP3	8.11	5.25 a	5.07 a
FP10	7.97	5.29 a	5.95 a
FP15	7.69	ND[3] a	ND a
FP20	8.19	4.29 a	5.24 a
FP22	7.58	4.50 a	5.06 a
FP23	8.27	4.74 a	4.74 a
FP25	7.51	3.40 a	3.39 a
FP34	7.79	3.89 a	4.03 a
FP35	8.13	ND a	ND a
FP36	7.46	ND a	ND a
FP41	7.94	ND a	ND a
FP42	7.91	4.84 a	5.26 a
FP48	6.96	3.41 a	2.87 a
FP65	8.22	3.54 a	3.73 a
FP72	6.88	ND a	ND a

1. Values are the mean of three replications with 10 seeds per replication.
2. Populations were determined on 21-day-old seedlings. Values are the mean of five replications with one plant per replication. Means between the two assays for a test strain followed by the same letter are not significantly different according to the t test ($P=0.05$).
3. ND, not detectable.

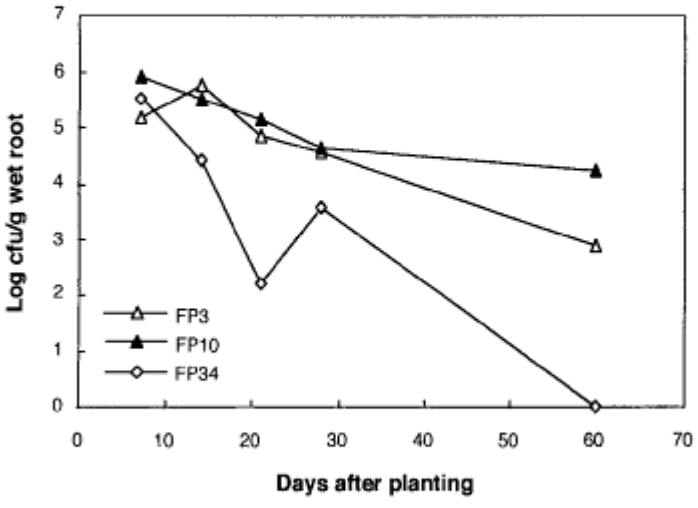

Fig. 1. Population dynamics of three strains of fluorescent pseudomonads in the rhizosphere of tomato following seed bacterization in the greenhouse pot test. Each value represents the mean of five replications.

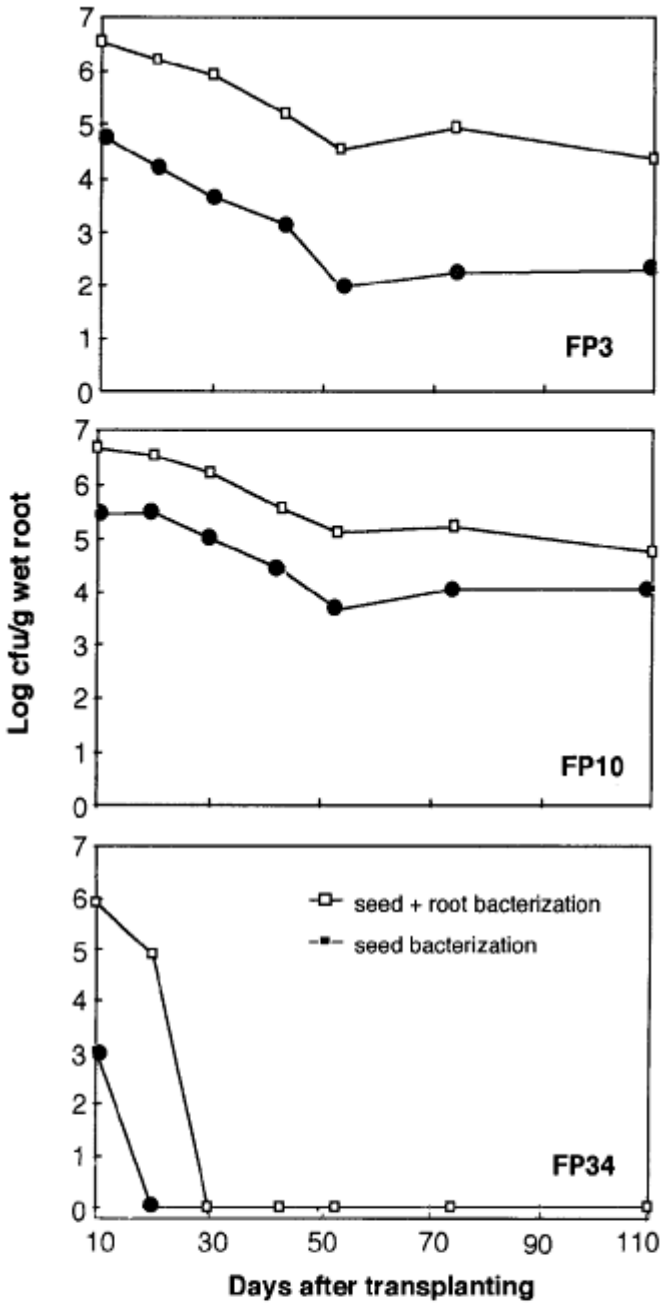

Fig. 2. Population dynamics of three strains of fluorescent pseudomonads in the rhizosphere of tomato in the field experiment. Tomato seeds or seeds plus roots were treated with each tested strain before seeding or transplanting. Each value represents the mean of eight replications.

Effect of seed inoculum level on rhizosphere colonization

The number of cells of strain FP10 coated on seeds influenced its population size in the rhizosphere. A direct relationship was observed between initial inoculum levels on seeds and subsequent populations detected in the rhizosphere 21 days after planting (Fig. 3).

Effect of soil type on rhizosphere colonization

Populations of strain FP10 in the rhizosphere of tomato grown in Tanan loam, Taitung silt loam, Tali (1)

TABLE 3. Rhizosphere populations of three strains of fluorescent pseudomonads on segments of tomato tap roots 14 and 28 days after seeding

Root segment (cm)[1]	Log cfu/cm root[2]					
	FP3		FP10		FP34	
	14	28	14	28	14	28
0–2	3.03	3.10	3.31	3.60	2.90	2.48
2–4	ND[3]	ND	ND	ND	ND	ND
4–6	ND	ND	ND	ND	ND	ND
6–8	ND	ND	ND	ND	ND	ND
8–10		ND		ND		ND
>10		ND		ND		ND

1. Commenced from the point of root attachment with stem (0 cm).
2. Values are the mean of five root segments.
3. ND, not detectable.

TABLE 4. Rhizosphere populations of fluorescent pseudomonad strain FP10 on segments of tomato roots, when applied as seed bacterization or as seed bacterization plus root treatment at planting

Root segment[1]	Rhizosphere population (log cfu/cm)[2]	
	Seed bacterization	Seed bacterization + root treatment
I	1.64	3.29
II	0.26	1.57
III	0.14	0.99
IV	ND[3]	0.82

1. Each root was divided into four equal segments with segment I being the one closest to the point of root attachment with the stem.
2. Populations were determined 90 days after transplanting into the field. Values are the mean of eight replications, and each replication consisted of 5–7 larger roots (30–50 cm in length) excised from one plant.
3. ND, not detectable.

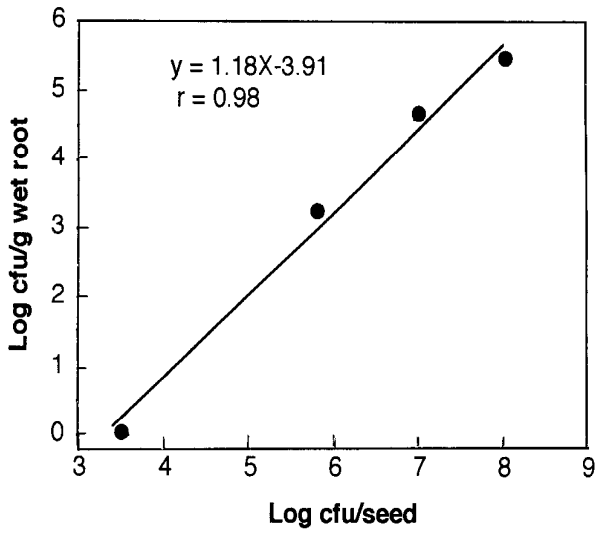

Fig. 3. Relation between concentration of fluorescent pseudomonad strain FP10 coated on seeds and colonization of the rhizosphere of tomato 21 days after planting. Each value represents the mean of five replications.

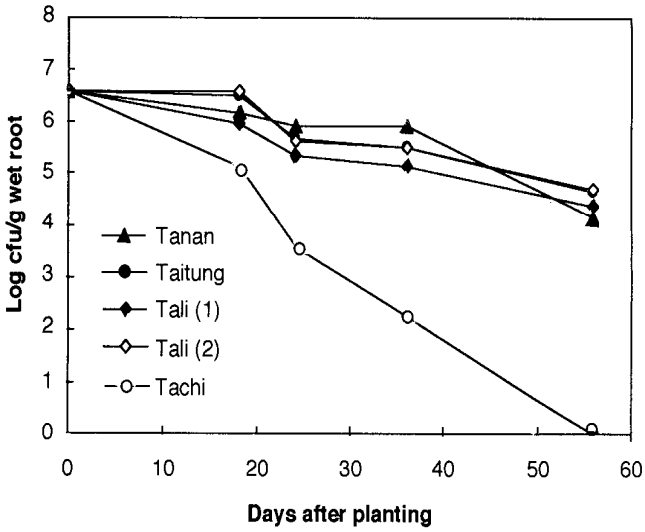

Fig. 4. Rhizosphere populations of fluorescent pseudomonad strain FP10 in the rhizosphere of tomato planted in different soils. Seeds were treated with FP10 and germinated for 4 days before transplanting. Each value represents the mean of five replications.

sandy loam and Tali (2) loamy sand soils were similar, but in Tachi loam soil, the rhizosphere population was much lower and decreased more rapidly than in the above four soils (Fig. 4) The rhizosphere population of strain FP10 was not detected 56 days after planting in Tachi soil, whereas about 4 log cfu/g wet root were detected in the other four soils.

Effect of soil pH on rhizosphere colonization

When 30-day-old seedlings raised from strain FP10-treated seeds were transplanted into Tachi loam soil adjusted to different pH, rhizosphere populations of the strain at 14 days after transplanting were not different in the soil adjusted to pH 5.3, 5.9 and 7.1, but were significantly higher than in soil with the original pH of 4.1 (Table 5).

Effect of soil microflora on rhizosphere colonization

Populations of strain FP10 at 5.75 and 6.88 log cfu/g wet root were detected from the rhizospheres of nonautoclaved and autoclaved soils, respectively, 10 days after germination, thus, colonization of the rhizosphere by strain FP10 was significantly greater in autoclaved soil than in nonautoclaved soil. Moreover, when the distribution of strain FP10 on the tap root was examined, only the first

upper 2-cm segment of root was colonized by the strain in the nonautoclaved soil, whereas all segments of root were colonized by the strain in the autoclaved soil (Table 6).

Effect of temperature on rhizosphere colonization

Population densities of strain FP10 in the rhizosphere declined with plant growth at both 20 and 28 C, but were consistently greater at each sampling time at 20 C than at 28 C (Fig. 5). On 10-day-old seedlings, strain FP10 colonized only the first segment of root near the inoculum source at 28 C, but colonized all root segments at 20 C, though the populations were low on all segments except the first upper segment (Table 7).

TABLE 5. Effect of soil pH on rhizosphere populations of fluorescent pseudomonad strain FP10 following seed bacterization on tomato planted in the Tachi loam soil

pH level[1]	Rhizosphere population[2] (log cfu/g wet root)
4.1	4.69 b[3]
5.3	5.05 a
5.9	5.17 a
7.1	5.30 a

1. Original soil pH was 4.1 and was adjusted to other pH with 1 N NaOH.
2. Values are the mean of two experiments. Each experiment had five replications with one plant per replication. Populations were determined 14 days after transplanting of 30-day-old seedlings into the soil.
3. Means followed by the same letter are not significantly different (P=0.05) according to Ducan's multiple range test.

TABLE 6. Colonization of fluorescent pseudomonad strain FP10 on tap root segments of tomato after seeding of the bacteria-coated seeds in autoclaved and nonautoclaved soils

Root segment (cm)[1]	Rhizosphere population (log cfu/cm)[2]	
	Nonautoclaved soil	autoclaved soil
0–2	3.70	5.08
2–4	ND[3]	4.01
4–6	ND	3.44
>6	ND	2.77

1. Commenced from the point of root attachment with stem (0 cm).
2. Values are the mean of five replications with one plant per replication. Populations were determined 10 days after germination.
3. ND, not detectable.

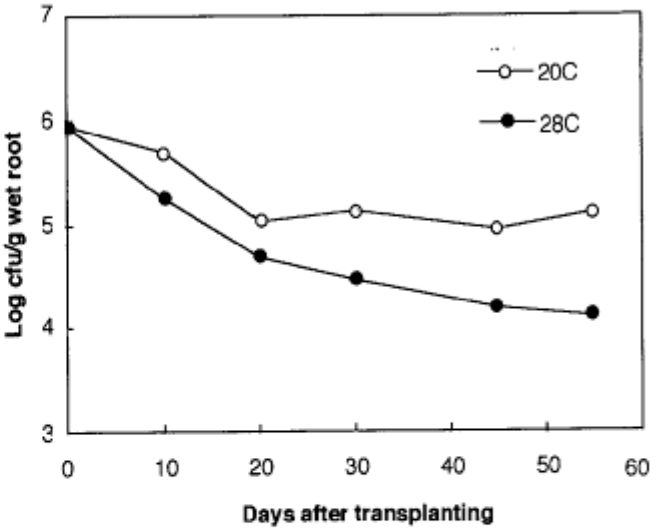

Fig. 5. Effect of temperature on population of fluorescent pseudomonad strain FP10 in the rhizosphere of tomato. About 30-day-old seedlings raised from seeds treated with FP10 were transplanted. Each value represents the mean of five replications.

TABLE 7. Effect of temperature on colonization of fluorescent pseudomonad strain FP10 on segments of tap roots of tomato after being introduced onto seeds

Root segment[1] (cm)	Rhizosphere population (log cfu/cm)[2]	
	20 C	28 C
0–2	4.2	4.7
2–4	0.7	ND[3]
4–6	0.9	ND
6–8	0.9	ND

1. Commenced from the point of root attachment with stem (0 cm).
2. Values are the mean of five replications with one plant per replication. Populations were determined 10 days after germination.
3. ND, not detectable.

DISCUSSION

The results of this study revealed that strains of fluorescent pseudomonads differed considerably in their population densities in the rhizosphere of tomato after being introduced as seed treatments, even though they were all isolated from tomato roots. The rhizosphere population densities of fluorescent pseudomonads determined by the laboratory plastic bag assay modified from the sand-soil test tube method of Scher *et al.* (18) were comparable to those determined by the greenhouse pot assay. Moreover, strains that were better rhizosphere colonizers as assessed by the bag assay also performed

better in their rhizosphere colonization under field conditions. Thus, the plastic bag assay may be used as a convenient method for screening strains for the ability to colonize roots. Scher *et al.* (18) reported that the test tube assay was an efficient method to assess the capacity of bacteria to colonize maize roots. We confirmed and extended the usefulness of a similar method for determining the colonization ability of fluorescent pseudomonads in the tomato rhizosphere.

Population densities of strains of fluorescent pseudomonads tested in rhizosphere of tomato declined with plant growth after seed bacterization. This observation was similar to those reported for specific strains of fluorescent pseudomonads in the rhizosphere of other plant species (8, 12, 14, 16, 20). The rate of the population decline, however, varied with strains and was related to their rhizosphere- colonizing capacity as assessed by the plastic bag assay. The rhizosphere populations of strains that were better rhizosphere colonizers declined more slowly than those of strains that were poor colonizers. Although the rhizosphere population of fluorescent pseudomonads introduced as seed treatments could be detected at different stages of tomato growth, they were distributed only on the segment of roots closest to the inoculum source. Microbial competition has been reported to have a marked effect on bacterial colonization on roots (4, 6, 18). A reason for the failure of strain FP10 to colonize along the entire length of tomato root was probably due to its inability to compete with native soil microorganisms. The possibility was supported by the finding that strain FP10 colonized all segments of root in autoclaved soil, but colonized only the first segment of root near the inoculum source in nonautoclaved soil. When bacteria-treated seeds were planted in raw soil and incubated at different temperatures, rhizosphere populations of strain FP10 were greater at 20 C than at 28 C. Moreover, strain FP10 was detected on all segments of roots at 20 C, but was detected only on the first segment from the base of root at 28 C. The effects of temperature on bacterial root colonization were complex (6). The better rhizosphere colonization at lower temperature observed in this study may have resulted in part from less competition from indigenous soil microorganisms, since microorganisms in the soil was less active at lower soil temperature (23).

Rhizosphere colonization by fluorescent pseudomonads may vary in different soil types (8, 12). In the present study, colonization of strain FP10 in the tomato rhizosphere was similar in four of five field soils tested. However, in the Tachi loam soil which had a low soil pH of 4.1, strain FP10 colonized markedly less compared to the other four soils with soil pH ranging from 5.1 to 7.8. The rapid reduction in the rhizosphere population of strain FP10 in the Tachi soil was probably due to the low soil pH, since a significant increase in rhizosphere population was observed when the soil pH was raised from the original 4.1 to a range of 5.3 to 7.1.

The reports on the relationship between the initial seed bacterial inoculum density and the resulting rhizosphere population density have been variable. Loper *et al.* (16) noted that rhizosphere populations of two strains of fluorescent pseudomonads were directly proportional to initial inoculum densities on potato seed pieces. Similar results were observed for specific strains of fluorescent pseudomonads on wheat, maize and barley (3, 9, 21). However, bacterial colonization of rhizosphere independent of initial inoculum levels on seeds has also been reported (2, 10, 18). In the present study, we confirmed the result of Scher *et al.* (18) in that rhizosphere population densities of strains of fluorescent pseudomonads resulting from seeds coated with higher inoculum levels wrere not necessarily greater than those of other strains resulting from seeds coated with lower inoculum levels. However, when a given strain, for example, strain FP10 was tested, the rhizosphere populations were positively correlated with the initial seed inoculum densities. Rhizosphere population densities of strain FP10 at various stages of tomato growth also were consistently higher by seed bacterization followed by root treatment at planting than by seed bacterization alone. Thus, the inoculum dose may influence the population size in the rhizosphere.

The intent of this study was to select superior rhizosphere-colonizing strains of fluorescent pseudomonads from tomato roots for used in evaluating their ability to control bacterial wilt of tomato. However, the strains tested were not able to colonize the entire root system and high population densities were not achieved as plant growth. Such strains probably would be inadequate to protect roots from infection by soil-borne *P. solanacearum* during the whole growing season. Therefore, more strains should be collected and screened in hopes of obtaining effective rhizosphere colonizers. Development of methods for enhancing the rhizosphere colonization may be another alternative. Our current research is directed to formulation of a soil amendment for enhancement of the rhizosphere colonization by fluorescent pseudomonads and for reduction of the pathogen density in the soil.

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摘　　要

陳金枝、徐世典、曾國欽．1992．螢光假單胞菌在番茄根圈之群集能力．植病會刊 1:130–139．
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由番茄根部分離的螢光假單胞菌菌株在番茄根圈之群集能力差異甚大，供試 64 個菌株被覆於番茄種子後，其在播種 21 天後的幼苗根圈之群集量從 0(偵測不到)至 5.52 log cfu/g wet root，而大多數菌株約在 3–4 log cfu/g wet root 左右。一個群集能力較佳的菌株 FP10 被覆於番茄種子後，於溫室及田間情況下其在根圈之族群密度均隨番茄生長期的增長而遞減，且在根部之分布大多集中於連接莖處附近；種子被覆再加上幼苗移植前根部浸漬 FP10 菌株可提高該菌

在根圈之群集量及延伸其在根部的分布。以種子被覆細菌法處理，FP10菌株在番茄根圈之菌量均隨著處理濃度的增加而提高。於低溫(20 C)或經高溫高壓滅菌之土壤環境中，FP10菌株在根圈之族群密度及在根上之分布較高溫(28 C)或未經滅菌之自然土中爲高及廣。FP10菌株在種植於台東坋質壤土、大南壤土、大里壤砂土及大里砂質壤土中的番茄根圈之群集能力差異不大，惟在較酸性(pH 4.1)的大溪壤土中，其根圈之族群密度較低且降低亦較快。大溪壤土的pH值由4.1提高至5.3–7.1可顯著增加菌株在根圈之族群密度。

關鍵字：番茄、螢光假單胞菌、根圈群集。