

Population Dynamics of Soil *Pseudomonads* in the Rhizosphere of Potato (*Solanum tuberosum* L.)

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Rhizosphere population dynamics of seven *Pseudomonas fluorescens* and *Pseudomonas putida* strains isolated from rhizospheres of various agricultural plants were studied on potato (*Solanum tuberosum* L.) in field soil under controlled environmental conditions. Rhizosphere populations of two strains (B10 and B4) were quantitatively related to initial seed piece inoculum levels when plants were grown at -0.3 bar matric potential. At a given inoculum level, rhizosphere populations of strain B4 were consistently greater than those of strain B10. In vivo growth curves on 4-cm root tip-proximal segments indicated that both strains grew at similar rates in the potato rhizosphere, but large populations of strain B10 were not maintained at 24°C after 7 h, whereas those of strain B4 were maintained for at least 40 h. Although both strains grew more rapidly in the rhizosphere at 24°C than at 12°C , their rhizosphere populations after seed piece inoculation were generally greater at 12 or 18°C , indicating that in vivo growth did not solely determine rhizosphere populations in these studies. In vitro osmotolerance of seven *Pseudomonas* strains (including strains B4 and B10) was correlated with their abilities to establish stable populations in the rhizosphere of potato. Stability of rhizosphere populations of the *Pseudomonas* strains studied here was maximized at low (i.e., 12°C) soil temperatures. These results indicate that *Pseudomonas* strains differ in their capacity to maintain stable rhizosphere populations in association with potato. This capacity, distinct from the ability to grow in the rhizosphere, may limit the establishment of rhizosphere populations under some environmental conditions.

Rhizosphere bacteria affect growth and development of higher plants by such mechanisms as nitrogen fixation, biological control of phytopathogens, and phytohormone elaboration. The ability of a rhizosphere bacterial strain to establish a significant population size along an elongating root system is a key determinant in its effect on plant growth (20). Although there is great interest in the manipulation of natural microbial communities and specific beneficial microorganisms in the rhizosphere, little is known about the dynamic processes determining the rhizosphere population size (hereafter referred to as populations) of these microbes. Quantitative studies of the dynamics of bacterial populations in the rhizosphere are few (1-3), and the need for more studies has been emphasized in recent reviews of the bacteria-root interaction (4, 9, 15, 17, 20).

It has been previously suggested that the number of organisms in the rhizosphere is dependent on the substrate supply from the roots. The efficient utilization of these substrates by a bacterial strain determines, in part, its population in the rhizosphere (3). The maximum population achieved per unit mass of root, defined as colonization potential by Bennett and Lynch (1), is characteristic of the bacterial strain and independent of original inoculum when determined from a described sterile sand system. The inoculum independence of populations determined in this and other systems (2, 3, 19) is consistent with the assumption that rhizosphere bacterial populations are largely defined by the replication of individual bacterial cells in association with the plant root. As such, the term rhizosphere colonization, determined experimentally by quantifying rhizosphere bacterial populations at one or more time points during the life of a plant, is often used as a term analogous to bacterial

growth in the rhizosphere. Although populations representing the colonization potential of individual bacterial strains are thought to be determined by their abilities to utilize the available carbon of root exudates (1, 2), the parameters determining rhizosphere bacterial populations in field soil have not been identified. The dynamics of these rhizosphere populations must be governed by many interacting processes influencing instantaneous population size. These processes may include bacterial growth, survival, death, emigration, and immigration as influenced by the chemical, biological, and physical environment of the rhizosphere.

In this study, we describe the population dynamics of two plant growth-promoting *Pseudomonas* strains in the rhizosphere of potato (*Solanum tuberosum* L. cv. White Rose). Several parameters, including soil moisture and temperature, initial inoculum, and phenotypic differences exhibited by these and other *Pseudomonas* strains in vitro, are considered in relation to their rhizosphere population sizes. Bacterial survival and in situ growth are considered as processes determining the population dynamics of these strains in the potato rhizosphere.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study and their influences on plant growth are described in Table 1. All strains are fluorescent pseudomonads, taxonomically classified in the species *Pseudomonas fluorescens* Migula or *Pseudomonas putida* (Trevison) Migula. None of the strains fit clearly into any one described biovar within these species, and several strains exhibit phenotypes intermediate to the two species descriptions (D. Hildebrand, personal communication).

Strains used as inoculum in rhizosphere population studies were spontaneous mutants of strains described in Table 1, selected for resistance to the antibiotic rifampicin (100

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TABLE 1. Rhizobacterial strain description

Strain	Source	Crop Plants (s)	Effect of seed inoculation on plant ^a
B4	Sugar beet rhizosphere, Shafter, CA	Sugar beet	Yield increases (22)
B10	Potato periderm, Tulelake, CA	Potato	Yield increases (14)
A1	Potato periderm	Potato, sugar beet	Yield increases (14, 22)
E6	Celery rhizosphere, Moss Landing, CA	Potato, sugar beet	Yield increases (14, 22)
BK1	Potato periderm, Shafter, CA	Potato	Yield increases (5, 14)
Blythe 1	Lettuce rhizosphere, Blythe, CA	Lettuce	Seedling growth stimulation (J. Loper, unpublished data)
51653	Lettuce rhizosphere, Salinas, CA	Lettuce	Seedling growth stimulation (J. Loper, unpublished data)
7SR1	Sugar beet rhizosphere	Sugar beet	Seedling growth inhibition (21)

^a Seedling growth stimulation after seed inoculation with these strains was observed only under greenhouse, not under field conditions.

µg/ml) (Sigma Chemical Co., St. Louis, Mo.). These mutant strains grew at the same rate as parental strains in minimal medium and exhibited stable rifampicin resistance over at least 50 generations without selection.

Culture conditions. Bacterial cultures were routinely grown on King medium B (KBM) agar plates (13). Half-strength KBM broth with 0.4% agar was used as a semisolid medium to evaluate bacterial motility during incubation at 21°C. Rate of lateral spread on this motility medium was quantified by measuring changes in colony radius as a function of time. Minimal medium was composed of (per liter): 1.5 g of K₂HPO₄, 0.2 g of MgSO₄ · 7H₂O, 5 g of glutamine, and 15 ml of glycerol. Osmotolerance of bacterial strains was evaluated by observation of growth in minimal medium supplemented with NaCl, KCl, Na₂SO₄, or sucrose at levels corresponding to specific osmotic potentials, as determined from tabular values (12, 18). Stock cultures were stored at 4°C on nutrient agar slants (Difco Laboratories, Detroit, Mich.) supplemented with 2.5% glycerol.

Growth in KBM broth, in supplemented or in unsupplemented minimal medium broth, was monitored by changes in optical density (λ = 640 nm). A₆₄₀ increased linearly with CFU per milliliter to an optical density of 0.5.

Rhizosphere population studies. (i) **Soil characteristics.** All experiments were conducted in Hesperia fine sandy loam soil collected from the Cotton Research Station near Shafter, Calif. The water-holding capacity of this soil is described by a moisture-release curve (Fig. 1) generated with a pressure plate extraction system (Soil Moisture Equipment Co., Santa Barbara, Calif.).

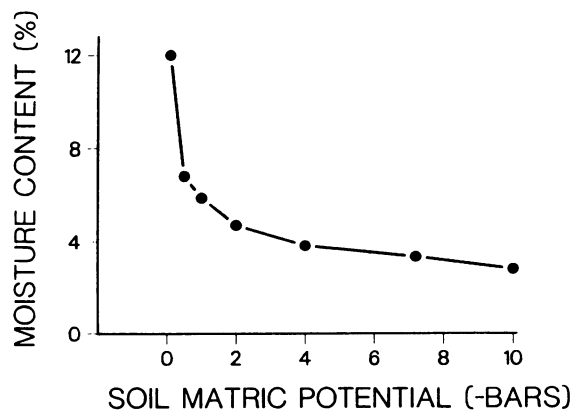


FIG. 1. Moisture-release curve of Hesperia fine sandy loam soil collected from the Cotton Research Station near Shafter, California.

Water potential of soil used in rhizosphere population studies was adjusted by adding distilled water to air dry field soil to bring the final moisture content (on a per gram basis) to a matric potential extrapolated from the moisture-release curve. Soil moisture was adjusted to an initial moisture content of 9% (corresponding to -0.3 bar) in all experiments except when specified below. Moisture content varied 0.2% among replications. Soil matric potential was determined from each replication at the end of each experiment by determining percent moisture and extrapolating back to the standard soil moisture-release curve.

(ii) **Seed piece inoculation.** Bacterial inoculum was prepared as follows. Cultures (48 h) grown on KBM agar at 27°C were suspended in sterile water and adjusted to an A₆₄₀ = 0.1 optical density. Single-eye potato pieces were cut to a standard size (fresh weight, 8.7 ± 1.2 g), allowed to suberize for 2 days, and then dipped for 5 to 15 min in the bacterial inoculum. Potato seed pieces were planted to a depth of 2 cm in 400-ml plastic beakers containing a 250-ml volume of soil which had been previously adjusted to a specified soil moisture. The beakers were then covered with plastic wrap and placed in constant-temperature water baths or growth chambers. Evaporation from covered beakers was negligible over the time course of these experiments.

In one experiment, designed to follow populations of bacteria on root tip-proximal 4-cm root segments (see Fig. 5), inoculation was accomplished by dipping intact root systems into a dilute bacterial suspension. Seed pieces were planted in sterile sand and maintained under standard greenhouse conditions until 3 days after emergence. The pots were then immersed in water to facilitate removal of plants from sand with minimal injury. Potato seed pieces were excised from growing plants. Root systems of these plants were then blotted dry, dipped in a dilute bacterial suspension, planted directly into beakers containing soil of an adjusted matric potential, and placed in constant-temperature water baths or growth chambers.

(iii) **Environmental conditions of plant growth.** Growth chambers were maintained at 18°C under a cycle of 16 h of light and 8 h of dark. Constant-temperature water baths were placed in a greenhouse under fluorescent lights with the same light period. Three separate water baths, used simultaneously in these studies, were maintained at 12, 18, and 24°C. All water bath temperatures were regulated to ±1.0°C. Beakers containing soil and a single potato seed piece were suspended in each water bath such that the water level of the bath was above the soil level in the beaker. Soil temperature was consistent (±0.1°C) among the beakers in a given bath. A soil temperature gradient of less than 0.5°C developed from the bottom to the top of pots, when the air temperature

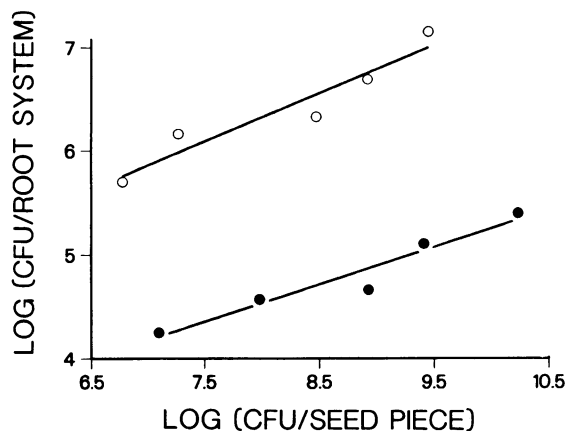


FIG. 2. Significant linear relationship between the logarithm of initial inoculum (\log_{10} [CFU per seed piece]), and that of subsequent rhizosphere populations (\log_{10} [CFU per root system]) for strain B4 (○) ($F = 5.5$; $P > F = 0.005$) and strain B10 (●) ($F = 42.8$; $P > F = 0.007$). The slopes of these linear functions are similar for strains B10 ($m = 0.35$) and B4 ($m = 0.36$). Populations at each point represent mean values of 10 replications.

was more than 15°C above the bath temperature. All experiments were done at soil temperatures of 18°C except when specified below.

(iv) **Harvesting procedures.** Two types of experiments were conducted, quantification of bacterial populations of entire root systems and quantification of those of isolated root segments. Ten replications, each consisting of a single potato plant in an individual beaker, were included in studies of intact root systems. Four or eight replications, each consisting of four segments (the seed piece-proximal and root tip-proximal segments from two individual roots) from a single potato plant in an individual beaker, were included in root segment experiments. Four replications, each consisting of a single potato seed piece, were used to quantify inoculum levels in all experiments. All experiments were harvested within 1 week after the emergence of potato plants, reducing the effects imposed as soil moisture was depleted by the growing plant.

Entire root systems were retrieved from the soil with sterile forceps. A diligent attempt was made to retrieve all roots from each sample. Roots were shaken gently to remove all but the most closely adhering soil and placed into 25 ml of washing buffer (0.1 M phosphate buffer [pH 7.0] supplemented with 0.1% [wt/vol] peptone [Difco]) in a 125-ml Erlenmeyer flask. Flasks were placed on a reciprocal shaker (200 rpm) for 30 to 60 min. No significant changes in numbers of bacteria in root washings were observed in up to 140 min of shaking. Placement of flasks in an ultrasonic bath did not increase the numbers of bacteria in root washings as has been reported in other systems (D. Haefele and R. Webb, *Phytopathology* 72:947, 1982).

Root segments were dissected from root systems retrieved from the soil as described above. Two intact primary roots, arising from the seed piece (not from the stem above the seed piece), were selected from each plant. Root length was recorded, and intact roots were dissected into 4-cm sections with sterilized forceps and razor blades, taking care not to cross-contaminate various sections. Any side branches were removed before dissection. Root sections were placed in 0.25 ml of sterile washing buffer in 1.5-ml Eppendorf microfuge tubes. Tubes were placed on a vortex shaker for 5 to 10 min.

Rhizosphere bacterial populations were quantified by dilution plating the root washings on KBM medium supplemented with rifampicin (100 $\mu\text{g/ml}$). Rifampicin-resistant colonies were enumerated after 2 days of incubation at 27°C. No naturally occurring rifampicin-resistant bacteria were seen when root washings of uninoculated controls were plated. The theoretical detection limits of these procedures were 125 CFU per root system or 2 CFU per root segment (0.5 CFU/cm).

(v) **Data analysis.** Rhizosphere bacterial populations were estimated from dilution plates, with densities ranging from 1 to 300 colonies per plate. The contribution of each individual plate count to the calculated mean (CFU per root system or segment) was proportional to its dilution from the original root washings. Bacterial populations were then expressed as CFU per root system and per root segment and as the logarithms (base 10) of these values.

Statistical analysis was accomplished by the univariate procedure and general linear models procedure provided by

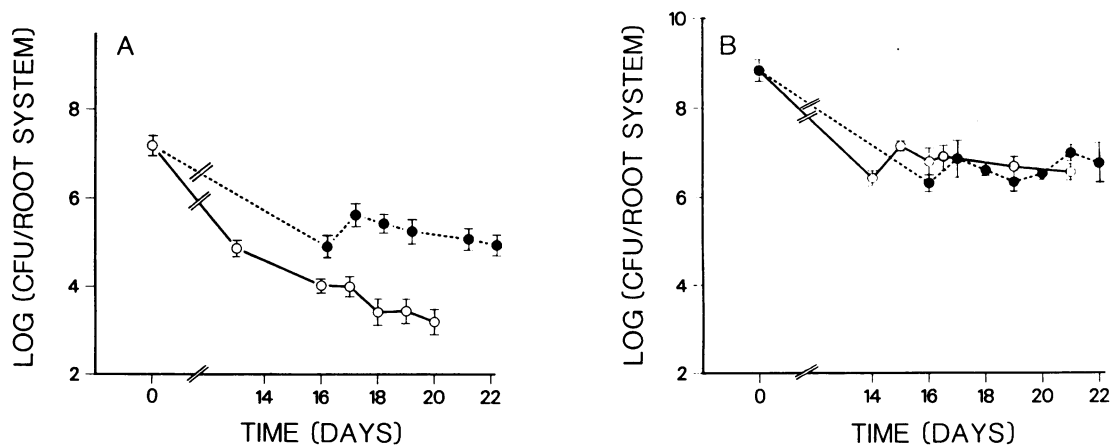


FIG. 3. Rhizosphere populations (\log_{10} [CFU per root system]) determined from entire potato root systems followed over time. Original seed piece inoculum levels are indicated here as populations at day zero. (A) Populations of strain B10 are consistently higher at 18°C (●) than at 24°C (○), although populations generally decrease with time at both temperatures. (B) Populations of strain B4 were not significantly different between 18°C (●) and 24°C (○) and were stable over the course of this experiment. Populations at each time point represent mean values of five replications. The vertical bars represent the standard error of mean populations.

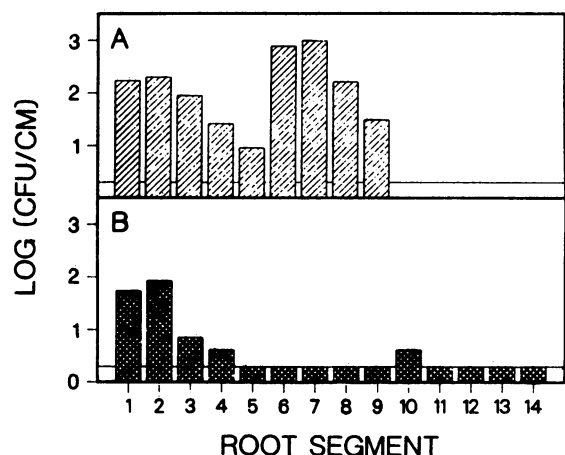


FIG. 4. Characteristic spatial distributions of rhizosphere populations of strains B4 (A) and B10 (B) on 1-cm longitudinal segments of a potato root. Root segment 1 is that closest to the seed piece. (A) Rhizosphere populations of strain B4 on a 10-cm root were found on all root segments, including the root tip-proximal segment (at the detection limit of \log_{10} [CFU/cm] = 0.3, designated by the horizontal line). (B) Rhizosphere populations of strain B10 on a 14-cm root were undetectable (designated at the detection limit) on most segments located more than a few centimeters from the seed piece.

Statistical Analysis Systems (SAS release 79.6, SAS Institute, Inc., Cary, N.C.). Due to the lognormality of these and other data sets describing rhizosphere bacterial populations (14a), the logarithmic transformation was applied to all values before analysis by the general linear models procedure.

RESULTS

Rhizosphere populations of strains B4 and B10 on intact root systems. Rhizosphere populations of strains B4 and B10 on potato plants were quantitatively related to initial seed piece inoculum (Fig. 2). The relationship was nonlinear when plotted on arithmetic scale but linear when plotted on a logarithmic scale. The slopes of these linear functions were similar between the two strains, although rhizosphere pop-

ulations of strain B4 were consistently greater than those of strain B10 at each inoculum concentration.

The population dynamics of strains B4 and B10 on entire potato root systems differed after seed piece inoculation (Fig. 3). Populations of B10 decreased with time at both 18 and 24°C but were consistently higher at 18 than at 24°C (Fig. 3A). Rhizosphere populations of strain B4 were stable over the duration of these experiments and did not vary significantly with temperature (Fig. 3B).

Although matric potential was initially adjusted to -0.3 bar in these experiments, soil moisture was depleted to as low as -10 bar as the potato plant grew. The final soil moisture was determined from every replication of these time-course experiments and related to estimated rhizosphere populations. Rhizosphere populations of these strains were independent of final matric potential between -0.3 to -10.0 bar ($F = 2.22$; $P > F = 0.15$).

Rhizosphere populations of strains B4 and B10 on root segments. Rhizosphere populations of strains B10 and B4 on 1-cm-long segments of individual potato roots exhibited characteristic spatial distributions (Fig. 4). Populations of strain B4 were typically detected on all root segments (Fig. 4A). These populations fluctuated around a mean level over the entire length of the root but decreased to a level just above the detection limit on the 1-cm segment closest to the root tip. In contrast, populations of strain B10 typically decreased with distance from the inoculum source (the seed piece) and were usually undetectable on segments located more than a few centimeters from the seed piece (Fig. 4B).

The characteristic population distributions exhibited by strains B10 and B4 (Fig. 4) were confirmed in subsequent root segment experiments (Table 2). Rhizosphere populations of strains B10 and B4 were determined on 4-cm root segments selected from the seed piece-proximal and root tip-proximal portions of individual potato roots. Although root length varied from 12 to 29 cm in length, no significant quantitative relationship was observed relating root length to populations of B4 estimated from root tip-proximal segments.

Rhizosphere populations of strains B10 and B4 were determined from 4-cm root segments of seed piece-inoculated potato plants grown under three moisture (-0.1, -0.3, and -1.0 bar) and three temperature (12, 18, and 24°C)

TABLE 2. Rhizosphere populations of strains B4 and B10 on root segments of seed piece-inoculated potatoes

Matric potential (bars)	Rhizosphere populations ^a (\log_{10} [CFU/cm]) of the following strain:							
	B4				B10			
	12°C	18°C	24°C	Mean	12°C	18°C	24°C	Mean
Seed piece-proximal segment^b:								
-0.1	2.52	3.54	1.52	2.53	1.99	0.20	0.18	0.79
-0.3	3.50	3.88	3.57	3.65	1.97	0.28	0.04	0.76
-1.0	3.58	3.20	3.28	3.35	1.99	1.05	0.16	1.07
Mean	3.20ab	3.53a	2.79b		1.98a	0.51b	0.13c	
Root tip-proximal segment^c:								
-0.1	1.39	0.55	0.40	0.78	1.88	<D ^d	<D	ND ^e
-0.3	1.44	2.21	0.70	1.45	1.90	<D	<D	ND
-1.0	1.30	1.87	0.79	1.32	1.96	<D	<D	ND
Mean	1.38a	1.54a	0.63b		1.91a	<Db	<Db	

^a Mean population values of a given strain followed by the same letter do not differ significantly from each other as determined by Fisher's protected least-significant difference.

^b Determined from 4-cm root segments contiguous to the seed piece.

^c Determined from apical 4-cm root segments.

^d <D designates treatments in which populations were below the detection limit of 0.5 CFU/cm.

^e ND, Not determined.

TABLE 3. Analysis of variance of populations of strains B4 and B10 on root segments of seed piece-inoculated potatoes

Source of variation	Degrees of freedom	Sum of squares	Calculated <i>F</i> value ^a
Seed piece-proximal segments:			
Total	67	147.70	
Treatment	17	126.80	17.84*
Strain (S)	1	89.30	213.61*
Matric Potential (MP)	2	1.71	2.05
Temperature (T)	2	14.70	17.53*
MP × T	4	2.03	1.21
MP × S	2	5.27	6.30*
S × T	2	9.40	11.18*
S × MP × T	4	4.47	2.67*
Error	50	20.91	
Root tip-proximal segments:			
Total	67	57.97	
Treatment	17	46.66	12.14*
Strain (S)	1	5.83	25.78*
Matric Potential (MP)	2	0.43	0.95
Temperature (T)	2	21.49	47.52*
MP × T	4	1.52	1.68
Error	50	11.31	

^a Calculated *F* values significant at *P* = 0.05 are indicated by an asterisk.

conditions (Table 2). The mean population size of strain B4 was significantly greater than that of strain B10 on both seed piece- (\log_{10} CFU/cm = 3.18 versus 0.87) and root tip-proximal (1.18 versus 0.48) segments as determined by Fisher's protected least significant difference. Differences in temperature accounted for more of the variability of these experiments than differences in matric potential (Table 3), perhaps because temperature was more carefully controlled. Lower temperatures (12 and 18°C) generally favored populations of both B4 and B10. The population size of strain B10 was

generally greater at 12 than at 18°C, whereas that of strain B4 was not, indicating an interaction between strain and temperature (*S* × *T*; Table 3).

To determine whether the differences observed among populations of B4 and B10 on root segments were caused by their differential growth rates in the rhizosphere, the growth of these strains on root tip-proximal segments was monitored (Fig. 5). The optimal growth temperature in vitro was 30°C for strain B10 and 28°C for strain B4 (data not shown). Rhizosphere populations of both strains initially increased more rapidly at 24 than at 12°C. Populations of strain B4 continued to increase at 24°C and maintained high levels for the duration of the experiment (Fig. 5A). Populations of strain B10 decreased after 7 h at 24°C and continued to decrease for the duration of the experiment (Fig. 5B). Although the initial increase in population size of both strains was smaller at 12 than 24°C, these populations were maintained at 12°C by both strains. At 24°C, the initial increase in population size was similar for both strains, but populations of B10 subsequently decreased, whereas those of B4 were stable.

All of the experiments described above were repeated twice with similar results to those presented.

Relationship of rhizosphere bacterial populations to in vitro growth characteristics. The eight *Pseudomonas* strains described in Table 1 vary markedly in several characteristics, including their rates of lateral spread on motility medium, their resistance to osmotic stress, and their rhizosphere populations on potato. Their growth on minimal agar medium supplemented with NaCl, Na₂SO₄, or KCl to specified osmotic potentials is indicated in Table 4. The sensitivity of a given strain to the three osmoticants was similar, indicating that no specific toxicity accounted for the observed growth inhibition. Three strains, BK1, B10, and E6, were more sensitive to osmotic stress than the other five strains. This same relative sensitivity was noted when the eight strains were grown in shake culture, using KCl to induce osmotic stress. Their relative optical densities, measured 5 days after inoculation, were compared at -20 and -1 bar

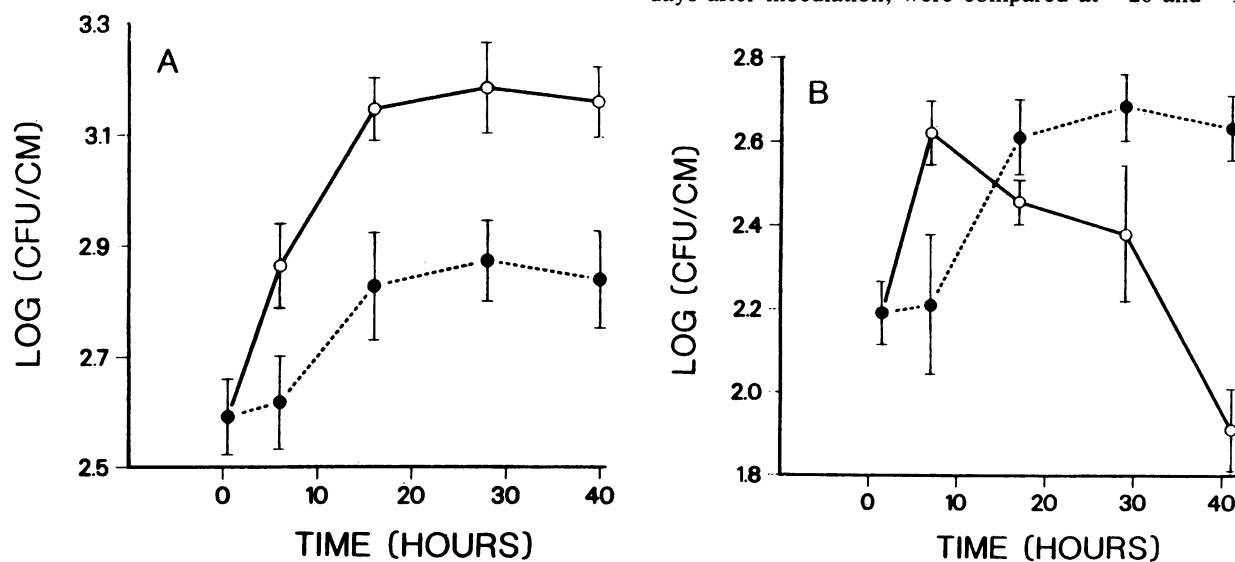


FIG. 5. Population dynamics of strains B4 (A) and B10 (B) on root tip-proximal segments of potato. (A) Strain B4 populations increased more rapidly and remained at higher levels at 24°C (○) than at 12°C (●). (B) Strain B10 populations increased more rapidly at 24°C (○) than at 12°C (●), but higher populations were maintained at the lower temperature. Analysis of variance of population values from this experiment indicates a significant strain effect ($F = 68.15$; $P > F = 0.0001$), no significant temperature effect ($F = 0.24$; $P > F = 0.6244$), and a significant interactive effect between strain and temperature ($F = 14.68$; $P > F = 0.0002$). The vertical bars represent the standard error of mean populations.

TABLE 4. Osmotolerance^a of bacterial strains on agar medium

Strain	Growth at specified osmotic potential ^b								
	-20 bar			-25 bar			-30 bar		
	NaCl	Na ₂ SO ₄	KCl	NaCl	Na ₂ SO ₄	KCl	NaCl	Na ₂ SO ₄	KCl
7SR1	+	+	+	+-	+-	+	-	-	-
51653	+	+	+	+-	+	+-	-	-	-
B4	+	+	+	+	+	+	+-	-	+-
A1	+	+	+	+	+	+	+-	-	+-
Blythe 1	+	+	+	+	+	+	+-	-	+-
BK1	-	+	+-	-	-	-	-	-	-
B10	-	+-	-	-	-	-	-	-	-
E6	+-	+	+-	-	+-	-	-	-	-

^a Osmotolerance was observed as relative growth under specified osmotic potentials. Symbols: +, growth; -, no growth; and +-, poor growth. All observations were consistent in two replications of two separate experiments.

^b Specified osmotic potentials were obtained by incorporation of one of three osmoticants (NaCl, Na₂SO₄, or KCl) into the growth medium at the appropriate concentration as determined from tabular values (12, 18).

and quantified as a fraction indicating osmotolerance (Fig. 6).

Mean rhizosphere populations of eight *Pseudomonas* strains, determined from 10 replications in each of two separate experiments, were unrelated to initial mean inoculum ($F = 0.02$; $P > F = 0.89$) and reflect strain differences. The mean rhizosphere populations of these strains did not correlate with their relative rates of spread on motility medium ($F = 0.01$; $P > F = 0.92$) but correlated with their relative abilities to grow under osmotic stress (Fig. 6). The ability of a bacterial strain to withstand osmotic stress was correlated with its capability to establish a large population size in the rhizosphere of potato.

DISCUSSION

Pseudomonas strains B4 and B10 differed markedly in their population sizes in the potato rhizosphere after seed piece inoculation. Rhizosphere populations of both strains were related to initial inoculum. However, at a given inoculum level, rhizosphere populations of strain B4 were consistently greater than those of strain B10. The rhizosphere populations of these strains could not be explained by their *in vivo* growth rates exclusively. *In vivo* growth curves on root tip-proximal segments indicated that both strains B4 and B10 grew at similar rates in the potato rhizosphere. However, high populations of strain B10 were not maintained at 24°C after 7 h, whereas those of strain B4 were maintained for at least 40 h. Rhizosphere populations of strain B4 were generally more stable than those of B10, perhaps reflecting the greater survival capacity of strain B4 at the soil-root interface in this system. Strain B4 was originally isolated from the soil type used in this study, whereas strain B10 was not. The effect of soil type on the relative growth and survival of these strains was not determined here.

The findings of this study differ from those in which rhizosphere populations of specific strains were investigated in a sand system either gnotobiotically (1, 2) or in association with field soil (19), or in which total bacterial populations were investigated in field soil (3). In those studies, rhizosphere population size was independent of inoculum and was presumably determined primarily by the capacity of a bacterial strain to grow on substrates exuded by plant roots. Growth of a bacterial strain in those systems has been described as an intensity factor, describing the richness of substrate available for the initial rapid bacterial growth

phase, and a capacity factor, in which substrate supply balances the maintenance requirements of the bacteria, resulting in a steady-state bacterial population (3). The initial 1-day growth phase observed here was shorter in duration than the 2- to 5-day growth phase described with different strains in a gnotobiotic system (1, 2). In addition, the maximum populations attained by bacterial strains in this system were lower by several orders of magnitude than those attained by other strains in the gnotobiotic system described previously (1, 2). A steady-state population was attained by strain B4 but was attained by strain B10 only under certain environmental conditions. Apparently, both the intensity and capacity factors determining rhizosphere bacterial populations are influenced by the presence of competing rhizosphere microflora or other factors differentiating a sand and field soil system. The dynamics of rhizosphere bacterial populations in the system described here was more complex than those of other strains in previously described systems (1-3) and could not be explained on the basis of bacterial growth alone.

Soil temperature influenced rhizosphere populations of both strains B4 and B10. Although both strains grew more rapidly in the potato rhizosphere at 24 than at 12°C, their populations after seed piece inoculation were generally higher at 12 or 18°C. The beneficial influence of low temperature was apparently not on bacterial growth but on one or more processes limiting the establishment or maintenance of viable bacterial cells in this system. These limiting processes may include bacterial survival or transport along an elongating root system. Transport along a root system may have limited bacterial populations in these experiments, since there was no addition of surface water to the system nor were soils saturated. Percolation of surface water through the soil and saturated soil conditions are known to facilitate the movement of bacteria through the soil (10, 11, 16, 23). However, growth of potato roots is not significantly less in 12°C soils than in 24°C soils (8), making direct influences of temperature on bacterial transport along an elongating root system unlikely. Populations estimated from the rhizosphere at 12°C were generally more stable than those at 18 or 24°C, suggesting that the survival of bacterial cells was maximized

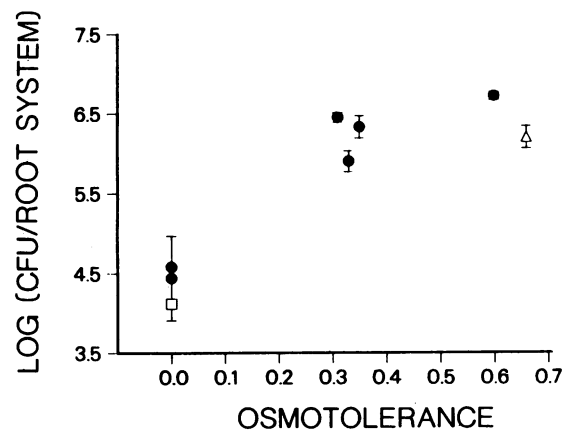


FIG. 6. Rhizosphere populations (\log_{10} [CFU per root system]) of eight bacterial strains were correlated with their osmotolerance, as quantified in shake culture ($F = 41.28$; $P > F = 0.003$). Osmotolerance values of strains B10 (\square) and B4 (\triangle) were 0.00 and 0.66, respectively. \bullet , Osmotolerance values of six additional strains described in Table 1. The vertical bars represent the standard error of mean populations.

at low temperatures. The greater rhizosphere populations observed in 12°C soils were analogous to soil populations of a *Rhizobium* sp. which were also maximized at low temperatures (7).

In vitro osmotolerance of eight *Pseudomonas* strains was correlated with rhizosphere population size. The variation in in vitro osmotolerance exhibited by these *Pseudomonas* strains may have been due to their various rates of growth under or rates of conditioning to osmotic stress. Osmotolerance of other bacterial strains was correlated previously with their survival in desiccated soils (6). Since the matric potential at the root surface of an actively transpiring plant may reach extremely low levels (-20 bar), far below that of the soil at large (24), the drought resistance of a rhizosphere bacterium may be critical to its survival. However, since bacteria are generally more sensitive to low matric potential than to low osmotic potential (12), no clear relationship can be drawn between the in vivo and in vitro characteristics exhibited here. It is possible that both characteristics, osmotolerance and the ability to establish stable rhizosphere populations, are manifestations of one or more phenotypic properties determining the capacity of a bacterial strain for growth and survival in the potato rhizosphere.

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