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Spatial-Temporal Colonization Patterns of a Rhizobacterium on Underground Organs of Potato

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ABSTRACT

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The spatial-temporal colonization patterns of *Pseudomonas fluorescens* strain A1-B were determined at all stages of plant development on belowground parts of field-grown potatoes in two soil types. The populations of A1-B persisted on inoculated seed pieces during the season but declined from an initial density of 10^8 cfu per seed piece to about 10^6 by harvest. Strain A1-B was also detected throughout the season in nonrhizosphere soil in relatively high numbers. Irrigation caused a gradient of declining bacterial density beneath the seed pieces, but the downward dispersal was localized (4–6 cm in silty clay-loam and 10–12 cm in sandy loam soils). Strain A1-B colonized roots, underground portions of shoots, stolons, and progeny tubers in a lognormal distribution pattern, with greatest populations on plant parts nearest inoculated seed pieces. Mean population densities on root segments before irrigation were about 1 log greater in sandy loam soil than in silty clay-loam. Spatial distribution patterns and population densities on roots were substantially altered after irrigation in contrast to those in nonrhizosphere soil. Mean population sizes of strain A1-B on sampled root segments were about 1 log greater after irrigation. The advancing root tips were sporadically colonized by strain A1-B with low population sizes, and this was affected by soil type. Colonization of root tips, underground portions of shoots, and stolons was greatest on those located nearest the source of original inoculum. Strain A1-B colonized developing progeny tubers; mean population sizes (log cfu per tuber) were about 35 times greater in the sandy loam (3.0) than in the silty clay-loam (1.5). Strain A1-B was not restricted to any particular root zone. Forty-one percent of A1-B cells were associated with rhizosphere soil, 54% were loosely adhered to the root surface, and 5% were tightly adhered or internal to the root surface. Samplings of root segments adjacent to seed pieces revealed that strain A1-B accounted for 0.16 and 12.58% of the total detectable aerobic and fluorescent pseudomonad bacterial component, respectively.

Root-colonizing bacteria may enhance plant health by inhibiting the pathogenic activities of both major (4,5,9,10, 13,23,28–31) and minor (8,16,25,32) soilborne pathogens. Suggested mechanisms of inhibition include production of antibiotics (9,10), iron-chelating compounds (4,5,14,31), and competitive preemption of nutritionally favorable sites (25). All of these mechanisms presumably depend on the establishment of the antagonist in relatively high numbers at the potential infection sites. Knowledge of the colonization capabilities of these antagonists and the stability of the populations at specific root sites is therefore essential to understanding microbial interactions and for targeting specific strains against specific pathogens. There have been no detailed, comprehensive studies of the population dynamics of root-colonizing bacteria in field conditions where

spatial-temporal patterns were monitored on individual roots throughout a season.

Most studies on root colonization with introduced bacteria have been done in greenhouse and laboratory settings under a variety of environmental conditions. Although the plants were grown under relatively artificial conditions, the experiments have provided important insights on the dynamics of root-bacterial interactions. For example, strains varied in rhizosphere competency when tested under gnotobiotic conditions (2,20). In studies using natural field soil, certain fluorescent pseudomonads colonized entire potato roots (up to 10 cm) in the absence of irrigation water and showed strain-specific patterns of colonization (18). These populations were quantitatively related to initial seed piece inoculum levels and were affected by soil temperature. Population densities on roots also were related to in vitro osmotolerance of the strains. The distributional analysis of the rhizosphere populations in the above study and in a combined greenhouse-field investigation revealed a lognormal distribution of seed-introduced bacteria among individual roots (18) and among individual plants (19). This work provided the basis for the development of appropriate root-sampling techniques and use of valid statistical analysis procedures.

The ability of specific bacteria to colonize roots and an assessment of the spatial-temporal patterns can best be evaluated in field studies where the full, natural component of soil microorganisms and fauna are present. It also is difficult in greenhouse and laboratory studies to simulate the temperature and moisture regimes that naturally occur in the field or to preserve a soil structure that plays an important role in affecting the movement of solutes and microorganisms. Except for a study with seminal roots of immature wheat plants (27), the few assessments of root colonization patterns of bacteria in the field have been made by bulk sampling of whole root systems or occasional sampling of specific root segments and were reported as average numbers of colony-forming units (cfu) per unit length or weight of root (7,11,13,17,28,30). These latter studies provided insights concerning population trends of root-colonizing bacteria but were not designed to yield critical information on the specific numbers of bacteria on individual roots and their spatial distribution.

The purpose of this study was to determine the population dynamics including the spatial-temporal patterns of a root-colonizing bacterium, *Pseudomonas fluorescens* strain A1-B, on underground parts of potato under field conditions. A preliminary report has been published (1).

MATERIALS AND METHODS

Bacterial strains and inoculum preparation. *P. fluorescens* strain A1-B was selected from several previously described antagonistic rhizobacterial strains (17,26) because of its superior ability to colonize and persist in the rhizosphere. Strain A1-B was isolated from the periderm of a developing progeny tuber in a commercial potato planting. A stable, rifampicin-resistant mutant of strain A1-B was used in all colonization experiments reported here. Dry seed piece inoculum was prepared with a final bacterial concentration of 5×10^8 cfu g^{-1} (15). Suberized, uniform-sized seed pieces (about 56 g) were dusted with inoculum immediately before planting, resulting in average populations of 10^8 cfu per seed piece.

Experimental design of colonization studies. Experiments were done on the University of California Agricultural Experiment Station at Tulelake and in a commercial potato field near Bakersfield, CA. Soil type at Tulelake was an Osborn silty clay-loam with 10% organic matter (peat) in the surface horizon. The Bakersfield soil was a Hesperia sandy loam with negligible organic content. The soil pH at both sites was about 7.0. Treated seed pieces were planted at a spacing of 23 cm on beds 91 cm apart and at a depth of 18 cm. The plot designs consisted of eight blocks 15 m × three rows at Tulelake and eight blocks 5 m × two rows at Bakersfield. Cultivar Russet Burbank was used at Tulelake and cultivar Russet Centennial was used at Bakersfield. Irrigation was by sprinkler. Soil temperature at Tulelake was monitored continuously in the zones of seed piece placement (18 cm) and primary root growth (36 cm) with a Bristol model 2T500-IB double-probe recorder (The Bristol Company, Waterbury, CT). Pairs of tensiometers were placed at corresponding depths in four locations within the plot, and soil moisture tensions were recorded daily. Soil temperature just below the seed pieces (22 cm) at Bakersfield was recorded at planting and at each sampling date.

Seed piece inoculum level. Populations of strain A1-B on planted, treated seed pieces were monitored by assaying six to eight seed pieces (each from a separate sampling block) at 10- to 30-day intervals from planting until about 80 days and again at harvest (about 130 days). Seed pieces were either processed immediately or placed in plastic bags, transported to the laboratory in an iced chest, and processed within 24 hr of sampling. Entire seed pieces were shaken in 100 ml of sterile washing buffer (0.1 M phospate buffer, pH 7.0, supplemented with 0.1% [w/v] peptone [Difco]) until washed clean of all white inoculum powder. Serial 10-fold dilutions of the washings were made to 10^{-3}, and a 0.1-ml aliquot of each was plated onto King's medium B (KB) agar (12) amended with 100 μg ml^{-1} of rifampicin (Sigma), 100 μg ml^{-1} of cycloheximide (Sigma), and 50 μg ml^{-1} a.i. of benomyl (Benlate). Colony counts were made after 48 hr of incubation at 25 C, and populations of A1-B per seed piece were determined.

Spatial distribution and survival of strain A1-B in nonrhizosphere soil. Nonrhizosphere soil at incremental depths beneath the seed pieces was assayed for the rifampicin-resistant bacteria to determine the extent of their downward distribution from the inoculum source. Samplings were made before and after sprinkler irrigations to measure the role of irrigation water in dispersal. Soil samples (1.5 g) were collected from the soil profile at points 12, 10, 8, 6, 4, 2, and 1 cm below the seed pieces. These samples were suspended in 10 ml of sterile washing buffer and run through three cycles of 2 min of agitation on a vortex mixer followed by 10 min of nonagitation, then serial 10-fold dilutions were made to 10^{-2}. One-tenth-milliliter aliquots of each dilution were plated on KB amended with the antibiotics (KB-rif) and incubated as described. Extreme care was used to collect samples free of rhizosphere soil. Four sets of samples, each from beneath a different seed piece in a separate sampling block, were taken at each sampling date. Colony counts were made, and numbers of strain A1-B per gram of soil at each depth were determined. The level of detection of this assay was estimated in the laboratory by adding suspensions of known densities of strain A1-B to six 1.5-g samples of Tulelake and Bakersfield soils each, allowing the samples to equilibrate for 4 hr, and assaying for bacterial populations as described previously.

Colonization of roots, underground portions of shoots, stolons, and progeny tubers. The sequential, spatial-temporal colonization patterns of strain A1-B on potato were determined by monitoring populations of strain A1-B on developing roots, underground portions of shoots, stolons, and progeny tubers. Beginning at seed piece germination (11–21 days after planting), individual emerging roots (arising adjacent to seed pieces) and shoots were excised, shaken to remove excess soil, and divided into sequential segments 4 cm long. The only exceptions to this were the initial two root-sampling dates at Tulelake when roots were segmented into 2-cm lengths. Single segments were placed into disposable 1.5-ml centrifuge tubes containing 0.25 ml of washing buffer (roots) or test tubes containing 1–2 ml of washing buffer (shoots). Root samples were agitated for 10 min with an Eppendorf mixer No. 5432 (Brinkmann Instruments, Inc., Westbury, NY), and shoots were agitated as described for soil samples described earlier. A single dilution was made by transferring 0.1 ml of washing mix to 1 ml of washing buffer, after which 0.1 ml of washing mix and diluted suspension were plated onto KB-rif. At each sampling date, those roots and shoots showing the most advanced stages of growth and of approximately equal sizes and lengths were selected for assay. Eight roots and six to eight shoots, each from a different plant from a separate sampling block, were collected at each date. At Tulelake, root samples were collected at about 3-day intervals for the first 25 days after seed piece germination, at 65 days postgermination, and at harvest. Shoots were assayed at 20, 32, and 76 days after planting. At Bakersfield, root and shoot sampling

was done at 2- to 3-wk intervals from germination to onset of general root senescence (90 days postplant) and at harvest (roots only). Processing of roots and shoots was done immediately after collection. At first, intact entire roots were lifted from the soil with a tile spade. However, once roots had grown longer than about 16 cm, it was necessary to dig a trench alongside the plants and gently excavate individual roots from the root tip upward to obtain whole roots. Roots subsequently arising from shoots at points >6 cm above the treated seed pieces were similarly assayed for populations of strain A1-B at 20, 30, and 77 days after planting at Tulelake and at 35, 49, 67, and 90 days after planting at Bakersfield.

The advancing tips of roots arising near the seed pieces were monitored for populations of strain A1-B at 15 and 19 days after planting at Tulelake and at 21 and 35 days after planting at Bakersfield. The apical 0.5-cm tips were excised from roots measuring 8 and 16 cm in total length at the first and second sampling dates, respectively, at each site. Eight tips per sampling date, each from a different plant growing in separate sampling block, were individually washed in 0.25 ml of washing buffer and plated as described previously. These assessments were made before the first postgermination irrigations at both field sites.

Stolons with points of origin on stems adjacent to and >6 cm above seed pieces were assessed separately for colonization by strain A1-B stolon initiation until maturity. Six entire, individual stolons from each position were excised from different plants in separate sampling blocks. Lengths of sampled stolons ranged from 1–4 cm at early sampling dates to 6–12 cm late in the season. Each stolon was measured, washed in 1–2 ml of washing buffer, and plated on KB-rif as described earlier for soil samples. Colony counts were made and numbers of colonizing strain A1-B per centimeter stolon determined.

Populations of strain A1-B on developing and mature progeny tubers were monitored by peeling the individual tubers and washing the peels in 50–100 ml of washing buffer, making two 10-fold serial dilutions, and plating each on KB-rif. Tubers arising from stolons with points of attachment to stems close to and those attached >6 cm above the seed pieces were assayed separately. Six tubers of about equal size, each from a different plant in a separate block, were assayed at each sampling date for each position. Progeny tubers were sampled 76 and 126 days after planting at Tulelake and 67, 90, and 135 days after planting at Bakersfield.

Spatial distribution of strain A1-B on and about roots. The spatial distribution of strain A1-B in the rhizosphere/rhizoplane of colonized roots at Tulelake was determined by separate assay of the tightly clinging rhizosphere soil and the root proper. The top 8 cm of six individual roots (each from a different plant in a separate block) with point of attachment adjacent to the seed pieces were sampled and divided into two equal 4-cm segments, then each segment was assayed separately. Root segments were shaken to remove loosely adhering soil, which was discarded. The tightly clinging soil (called rhizosphere soil herein) was then gently removed from the root with a fine, sterilized brush and suspended in 0.25 ml of washing buffer. Next, the soilfree root segment was placed in 0.25 ml of washing buffer and agitated for 10 min. The root segment was then removed from the vial and the washing suspension was saved. The once-washed segment was next washed twice in 1.0 ml of washing buffer to remove any residual, loosely adhering cells of strain A1-B and then triturated in 0.25 ml of washing buffer with a mortar and pestle. Serial dilutions to 10^2 were made from the rhizosphere soil suspension, first root washing, and the root triturate, and plated on KB-rif as described before. After incubation, colonies were counted to determine the relative numbers of strain A1-B inhabiting the rhizosphere soil, those loosely adhered to the root surface, and those tightly adhered or internal to the root surface.

Strain A1-B relative to population sizes of total aerobic and native fluorescent pseudomonad bacteria. Six roots arising adjacent to the seed pieces at Tulelake were excised at their point of attachment, and the initial segments of 1–4 and 4–8 cm were washed separately in 0.25 ml of washing buffer. Serial dilutions to 10^{-5} were made, and 0.1-ml aliquots of each were plated on KB-rif, KB alone, and a selective medium for isolation of fluorescent pseudomonads (22) to estimate populations of strain A1-B, aerobic bacteria, and fluorescent pseudomonads, respectively. Colonies of fluorescent bacteria were counted under ultraviolet irradiation (360 nm). Preliminary studies indicated that recovery rates of aerobic bacteria in the Tulelake soil were greater with KB than with general media such as 20% tryptic soy agar.

Data analysis. Rhizosphere populations of strain A1-B, total aerobic bacteria, and fluorescent pseudomonads were estimated from dilution plates, and counts ranged from one to 300 colonies per plate. The contribution of each plate count to the calculated mean was proportional to its dilution from the original washing mix. Bacterial populations were expressed as colony-forming units per centimeter of root or shoot, per individual stolon or tuber, per gram of soil, and as the logarithms (base 10) of these values. All data sets (e.g., populations on all root segments of equal position on a given sampling date) were analyzed for normality with the univariate procedure (Statistical Analysis Systems, release 79.6, SAS Institute, Inc., Cary, NC). Because populations of strain A1-B on roots, stolons, progeny tubers, and underground portions of shoots conformed more closely to a lognormal rather than a normal probability distribution, the log transformation (base 10) was applied to these data values before statistical analysis. Failure to transform data from lognormally distributed populations can lead to invalid use of analysis of variance procedures and to substantial overestimation of means (19). Populations of strain A1-B on seed pieces and in the nonrhizosphere soil were analyzed without transformation because of their more normal distributions.

RESULTS

Soil temperature and moisture. Soil temperatures near seed pieces at Tulelake fluctuated 1–3 C daily, with a mean daily temperature of 17.5 C and season lows and highs of 14 and 21 C, respectively. Soil water tension about the seed pieces cycled from low values of 0.06 bars shortly after irrigations to 0.22 bars just before irrigation. The average soil water tension at seed piece depth was 0.12 bars during the experiment. Soil temperature in the zone of primary root growth (36 cm below the surface) at Tulelake varied only 1–2 C during any 24-hr period, with a mean daily temperature of 16.5 C and a seasonal low and high of 15 and 19 C, respectively. Soil water tension in this zone ranged from 0.04 bars after irrigation to 0.13 bars before irrigation, with an average value of 0.07 bars. Irrigations at Tulelake were applied at intervals ranging from 16 days at the beginning of the season to 4 days during peak plant growth. Additional periodic applications of water were necessary to prevent frost injury. Soil temperatures just below the seed pieces (22 cm) at Bakersfield increased from 15.5 C at planting (20 February) to 23.8 C at harvest (15 June), after vine destruction.

Survival of strain A1-B on seed pieces. Strain A1-B persisted at relatively high population sizes on seed pieces at Tulelake and Bakersfield during all stages of plant development. Seed piece populations declined from average initial levels of 1×10^8 cfu at planting (both sites) to 4.4×10^5 cfu at Tulelake and 1.4×10^6 cfu at Bakersfield by harvest (Fig. 1). Most seed pieces (90%) at Bakersfield remained sound throughout the season, whereas most at Tulelake gradually decayed, leaving only partiallly intact periderms to sample by harvest.

Spatial distribution of strain A1-B in nonrhizosphere soil. Strain A1-B persisted in relatively high numbers in soil throughout the season (Table 1). Downward distribution of strain A1-B in nonrhizosphere soil beneath the inoculated seed pieces extended to a detectable depth of 4–6 cm in the silty clay-loam soil at Tulelake and 10–12 cm in the sandy loam at Bakersfield. Gradients of declining bacterial densities distal to seed pieces were established with the initial irrigations at both sites. Thereafter, the spatial distribution at Tulelake did not change with repeated irrigations, although the density of strain A1-B increased within the range. In contrast, both the density and physical distribution range of strain A1-B in nonrhizosphere soil increased during the season at

Bakersfield. Laboratory tests indicated that the efficiencies of recovery of strain A1-B in Tulelake and Bakersfield nonrhizosphere soils were about 70 and 95%, respectively.

Colonization of roots by strain A1-B. The spatial distribution of strain A1-B on roots emerging from stems at loci 0–6 cm above seed pieces formed a gradient with greatest populations on the first segments (0–4 cm) of roots (about 103 cfu cm^{-1}) adjacent to the treated seed pieces (Table 2). Substantially smaller numbers of bacteria were detected on the more distal root segments, with none to few colony-forming units found on root segments 32–40 cm from the seed pieces. At Tulelake, population sizes on the distal (4–8, 8–12, and 12–16 cm) root segments were significantly greater ($P = 0.05$) just after the first postgermination irrigation (25 days postplant), indicating that downward movement of irrigation water enhanced distribution of strain A1-B along roots. In contrast, at Bakersfield, high mean populations of strain A1-B were established on all root segments up to 20 cm distal to their point of attachment (21 and 35 days postplant) prior to any irrigation (or rainfall). Preirrigation root populations (17–22 days postplant) were significantly greater ($P = 0.05$) on all segments at Bakersfield than at Tulelake. Maximum root populations occurred at about 30 days postplant at Tulelake and at 20–35 days postplant at Bakersfield, then the numbers of bacteria declined to fairly stable levels that persisted until root senescence. Small populations of strain A1-B were recovered from intact roots arising near seed pieces at harvest at both sites. Roots with points of origin on the stem 6–12 cm above the seed pieces were also colonized by strain A1-B (Table 3). Population sizes on these root segments were relatively low and similar in magnitude at the two sites. Native bacteria resistant to rifampicin were occasionally isolated from roots and soil. However, they were easily distinguished from strain A1-B by their much slower growth rate on KB-rif, usually appearing >48 hr after colonies of strain A1-B were formed, and by distinctive colony appearance.

Strain A1-B colonized the advancing tips (apical 0.5 cm) of roots measuring 8 and 16 cm in overall length before application of irrigation water. Mean root tip population sizes on roots 8 cm in overall length were significantly greater ($P = 0.05$) at Bakersfield (38.9 cfu tip^{-1}) than at Tulelake (7.1 cfu tip^{-1}). Populations on roots 16 cm long were markedly smaller at both sites, with mean sizes of 6.2 and 2.5 cfu tip^{-1} at Bakersfield and Tulelake, respectively. Strain A1-B was recovered from the tips of more 8-cm roots

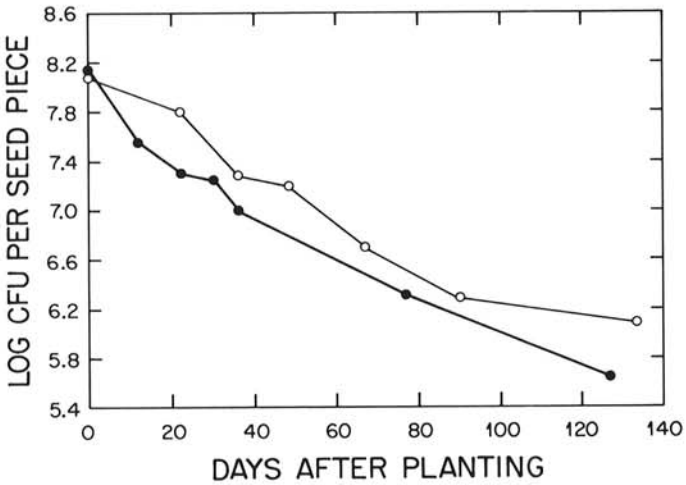

Fig. 1. Mean population densities of *Pseudomonas fluorescens* strain A1-B on inoculated potato seed pieces at Tulelake (o) and Bakersfield (•), CA.

TABLE 1. Distribution of *Pseudomonas fluorescens* strain A1-B in nonrhizosphere soil beneath inoculated potato seed pieces in Tulelake and Bakersfield, CA

Sample depth (cm)[a]	Log cfu/g soil[b] (days after planting)								
	Tulelake (silty clay-loam)					Bakersfield (sandy loam)			
	13	20	27	34	77	35	49	67	90
0–1	5.5	4.0	5.1	4.3	4.9	5.6	4.3	4.9	4.2
1–2	3.4	3.6	4.4	3.3	4.2	0.0	3.9	3.2	3.8
2–4	2.7	2.4	2.9	2.6	3.8	0.0	2.5	2.1	3.4
4–6	1.5	1.5	1.8	1.5	3.3	0.0	1.3	2.5	2.4
6–8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.9
8–10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5
10–12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7

[a] Incremental distances below inoculated seed pieces at which soil samples were taken.
[b] Average of four samples per depth increment at each date. Each sample was taken from beneath a different seed piece in a separate sampling block. Means calculated from arithmetic population size values. First sprinkler irrigations at 5 and 39 days postplant at Tulelake and Bakersfield, respectively.

TABLE 2. Rhizosphere population densities of *Pseudomonas fluorescens* strain A1-B on segments of roots arising from stems at loci 0–6 cm above the inoculated potato seed pieces at Tulelake and Bakersfield, CA

Root segment (cm)[a]	Log cfu/cm[b] (days after planting)															
	Tulelake (silty clay-loam)										Bakersfield (sandy loam)					
	11	14	17	20	22	29	32	35	76	126	21	35	49	67	90	135
0–2	2.1	3.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2–4	–	1.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–
0–4	–	–	2.7	2.4	2.2	2.8	2.8	1.6	2.0	2.1	3.6	3.4	2.7	2.4	3.6	2.5
4–8	–	–	1.0	0.8	0.6	1.5	1.5	0.8	0.8	0.1	1.9	1.2	0.9	0.3	0.8	1.2
8–12	–	–	0.5	0.2	0.1	1.2	1.1	−0.01	0.8	–	2.0	0.5	0.2	0.1	0.4	–
12–16	–	–	–	0.1	0.04	1.1	0.7	0.03	0.1	–	–	0.9	0.2	−0.03	0.2	–
16–20	–	–	–	–	–	0.9	0.9	0.0	0.3	–	–	1.1	−0.03	−0.03	0.5	–
20–24	–	–	–	–	–	–	0.9	0.1	0.2	–	–	0.6	0.0	0.0	0.1	–
24–28	–	–	–	–	–	–	–	−0.03	0.3	–	–	0.6	−0.1	−0.02	0.3	–
28–32	–	–	–	–	–	–	–	0.1	0.04	–	–	–	0.03	0.4	0.4	–
32–36	–	–	–	–	–	–	–	0.0	0.0	–	–	–	0.0	0.2	0.3	–
36–40	–	–	–	–	–	–	–	–	0.0	–	–	–	–	0.5	0.0	–

[a] Sequential, incremental lengths of segments into which individual roots were divided and separately assayed, commencing with point of root attachment of stem (0 cm).
[b] Mean of eight root segments for each increment of length (column 1) at each sampling date. Each segment from a different root on a different plant growing in a separate sampling block. Means calculated from log-transformed population size values. First sprinkler irrigation after germination of seed pieces at 25 and 39 days postplant at Tulelake and Bakersfield, respectively.

sampled at Bakersfield (six of eight) than at Tulelake (four of eight) but with equal frequency (four of eight) on 16-cm roots at the two field sites.

There was great variability in population sizes of strain A1-B among root segments of a given sample set at all dates. Sample sets typically consisted of a few heavily colonized segments, with the remaining segments colonized lightly or not at all. Means calculated from arithmetic values for comparative purposes were two to 19 times higher than those computed from log-transformed values (Tables 2 and 3 and root tips).

Colonization of underground portions of shoots and stolons. The populations of strain A1-B on shoots declined in relation to the distance of the segments from the inoculated seed pieces (Table 4). Overall population sizes on shoots at Tulelake were greatest 32 days after planting. Populations remained relatively high on the segments 0–4 cm from seed pieces throughout the season in contrast to markedly reduced populations on segments 4–8 and 8–12 cm distal to the seed pieces. Conversely, underground shoot colonization at Bakersfield was relatively lower early in the season (35 and 40 days postplant), with highest populations occurring on all segments during the period of tuber initiation and development (67–90 days).

Strain A1-B colonized stolons arising from underground portions of shoots at points near and >6 cm above the seed pieces. Stolons attached to shoots at loci 0–6 cm above the seed pieces (near) were more heavily colonized than stolons with points of attachment from 6 cm above seed pieces to just beneath the soil surface (distal). Mean population sizes on near and distal stolons during the growing season at Tulelake were 15.8 and 3.2 cfu cm^{-1}, respectively, whereas average stolon populations of strain A1-B at Bakersfield were 126 and 5 cfu cm^{-1} for the same two respective positions.

Colonization of progeny tubers. Strain A1-B colonized developing progeny tubers and persisted throughout the season (Table 5). Tubers arising from stolons attached to shoots <6 cm above the seed pieces (near) were more heavily colonized than tubers arising from stolons with points of attachment to shoots from 6 cm above the seed pieces to just below the soil surface (distal). This was especially apparent at Tulelake, where mean population sizes of strain A1-B on near tubers (1.1×10^3 cfu) were more than 100 times greater than those on distal tubers (7.9 cfu) at 76 days postplant and at harvest when average population sizes on near tubers was 95 cfu while strain A1-B was not detected on distal tubers. Tuber population sizes (near and distal) were significantly greater ($P = 0.05$) at Bakersfield than at Tulelake at all sampling dates except for populations on near tubers sampled at harvesttime. Detectable populations of strain A1-B on tubers declined markedly during the last 45–50 days before harvest at both field sites.

Rhizosphere/rhizoplane populations of strain A1-B. The determination of the general location of strain A1-B about roots indicated that the bacterium was not restricted to any particular zone. A sampling of the segments of roots at 0–4 cm emerging near inoculated seed pieces showed that 41% of A1-B cells (200 cfu cm^{-1}) were associated with the rhizosphere soil, 54% (251 cfu cm^{-1}) were loosely adhered to the root surface, and 5% (25.2 cfu cm^{-1}) were tightly adhered or internal to the root surface as revealed in the triturated samples. The proportions and population sizes (cfu

TABLE 3. Rhizosphere population densities of *Pseudomonas fluorescens* strain A1-B on segments of root arising from stems at loci 6–12 cm above the inoculated potato seed pieces at Tulelake and Bakersfield, CA

Root segment (cm)[a]	Log cfu/cm[b] (days after planting)						
	Tulelake (silty clay-loam)			Bakersfield (sandy loam)			
	20	30	77	35	49	67	90
0–4	0.1	0.3	0.6	0.3	0.3	0.3	0.1
4–8	–	0.2	0.1	–	0.5	0.4	0.1
8–12	–	0.1	0.3	–	0.3	0.1	0.3
12–16	–	0.1	0.3	–	0.5	0.1	0.8
16–20	–	–	−0.03	–	0.5	0.2	0.6
20–24	–	–	–	–	0.1	0.0	0.5

[a] Sequential, incremental lengths of segments into which individual roots were divided and separately assayed, commencing with point of root attachment at stem (0 cm).
[b] Mean of eight root segments for each increment of length (column 1) at each sampling date. Each segment from a different root on a different plant growing in a separate sampling block. Means calculated from log-transformed population size values. First sprinkler irrigation after germination of seed pieces at 25 and 39 days postplant at Tulelake and Bakersfield, respectively.

TABLE 4. Population densities of *Pseudomonas fluorescens* strain A1-B on underground portions of shoots at Tulelake and Bakersfield, CA

Shoot segment (cm)[a]	Log cfu/cm[b] (days after planting)							
	Tulelake (silty clay-loam)			Bakersfield (sandy loam)				
	20	32	76	21	35	49	67	90
0–4	2.8	4.4	3.8	3.6	3.1	3.1	4.8	4.1
4–8	0.5	2.3	1.0	–	1.5	0.8	2.2	2.7
8–12	–	1.6	0.6	–	0.5	0.3	2.3	2.1

[a] Sequential, incremental lengths into which shoots were divided and separately assayed, beginning with point of shoot attachment at seed piece (0 cm).
[b] Mean of six to eight shoot segments per sampling date. Each segment from a different shoot from a different plant growing in a separate sampling block. Means calculated from log-transformed population size values.

TABLE 5. Population densities of *Pseudomonas fluorescens* strain A1-B on progeny tubers at Tulelake and Bakersfield, CA

Tuber position[a]	Log cfu/tuber[b] (days after planting)				
	Tulelake (silty clay-loam)		Bakersfield (sandy loam)		
	76	126	67	90	135
<6 cm above seed pieces	3.1	2.0	4.3	4.8	2.3
>6 cm above seed pieces	0.9	0.0	2.5	2.4	1.5

[a] Position of loci on shoots from which stolons of sampled tubers arose.
[b] Average of six tubers per position per sampling date. Each tuber from a different plant from a separate sampling block. Means calculated from log-transformed population size values.

cm^{-1}) on the next 4–8 cm of root were 78% (15.8), 17% (3.2), and 5% (1.2) for the same three respective locations.

The sampling of roots arising adjacent to seed pieces revealed that population sizes (cfu cm^{-1}) of total detectable aerobic bacteria, fluorescent pseudomonads, and strain A1-B on the initial 0–4 cm were 5×10^5, 6.3×10^3, and 7.9×10^2, respectively, whereas populations of the same three respective bacterial groups on the next 4–8 cm were 4.8×10^5, 3.2×10^3, and 2×10^1. As such, strain A1-B accounted for 0.16 and 12.58% of the total aerobic and fluorescent pseudomonad bacterial component, respectively, on the segments at 0–4 cm and constituted 0.004 and 0.63% of the same respective bacterial groups on the segments at 4–8 cm.

DISCUSSION

The determination of the spatial-temporal colonization patterns of *P. fluorescens* strain A1-B on belowground parts of field-grown potato plants in two soil types showed that the bacterium colonized all plant parts and persisted in varying populations throughout the season. However, population sizes were greatest on plant parts nearest treated seed pieces and declined markedly with distance from the source of inoculum. This pattern applied to roots, shoots, stolons, and progeny tubers. Population sizes and spatial distributions within this general pattern were affected by soil type and water movement (Fig. 2).

There was significant variation in extent of colonization among plant parts at all sampling dates, with most samples colonized lightly or not at all and a few heavily colonized. Distributional analyses of raw colonization data confirmed that populations of strain A1-B were lognormally distributed among all plant samples, as has been previously shown for roots (18,19). The log transformation was thus applied to all population values before statistical analysis. Computation of root population means from arithmetic values in this study resulted in overestimations of population sizes from twofold to 19-fold. This explains, in part, the relatively low colonization figures compared with other studies, because previous root colonization studies other than the two cited above have not applied the log transformation to raw data.

Population sizes of strain A1-B on roots and root tips before irrigation and on progeny tubers and underground portions of shoots throughout the season were substantially greater at Bakersfield than at Tulelake. For example, average preirrigation (17–22 days postplant) population sizes on all root segments were significantly greater by a factor of 10 in the sandy loam soil (Bakersfield) than in the silty clay-loam (Tulelake). Similarly, season-long mean population sizes on progeny tubers near and distal from the seed pieces were 34 and 39 times greater, respectively, at Bakersfield than at Tulelake. Smaller soil pore sizes and increased adsorption to clay and organic colloids at Tulelake may have restricted the transport of strain A1-B on plant parts growing through the soil matrix and reduced passive distribution of the bacteria by water in the bulk soil (3,21). It is doubtful that site-related differences in colonization were due to the use of different potato cultivars, because in a previous study, cultivar type did not affect strain colonization (11).

Irrigation water redistributed strain A1-B along roots but, surprisingly, had a minimal effect in transporting bacteria in nonrhizosphere soil. The first irrigations at Tulelake and Bakersfield dispersed strain A1-B from the inoculum source and established bacterial gradients in the soil extending 6 cm beneath the seed pieces. Roots subsequently growing into this zone encountered mean populations of strain A1-B of about 2×10^3 cfu g^{-1} soil, which probably augmented primary colonization resulting from inoculation of emerging roots at the seed pieces. Average population sizes on the distal (4–8, 8–12, and 12–16 cm) segments of roots just after the first post-seed-piece germination irrigation at Tulelake were 10 times greater than before application of water. Periodic supplementation of root populations by water redistribution could serve to renew root tip populations of the antagonist, thereby enhancing subsequent colonization. An irrigation-associated increase in root population sizes was not detected at Bakersfield, probably because of the long interval between irrigation and sampling (10 days), during which elevated populations could have returned to preirrigation levels. The finding that strain A1-B persisted in the nonrhizosphere soils at both sites throughout the season has important implications for developing future biocontrol strategies; it indicates that antagonists can be introduced into soil and survive independently until contacted by roots or other plant parts.

Colonization of plant parts above the seed pieces appears to have been attained by transportation and progressive growth of strain A1-B on developing stems, roots, stolons, and progeny tubers. For example, before irrigation at Bakersfield, strain A1-B was recovered from all segments of underground portions of shoots. Key constraints preventing long-term maintenance of larger population sizes on all advancing plant parts (root, shoot, stolon, and tuber apices), and hence, better total colonization, probably include physical removal of bacteria as the growing part displaces and realigns soil particles in a tight sheath about itself (6), adsorption of bacteria to the soil solid phase (21), competition from native bacteria encountered in the soil, and bacterial generation times too slow to keep pace with plant growth. The possibility of bacterial dispersal by animals such as nematodes or arthropods cannot be discounted.

We make several assumptions and qualifications in critically evaluating the detection methodology used to quantitatively follow the population dynamics of strain A1-B. Presumably, all estimates of strain A1-B populations on plant parts are merely fractions of real population numbers because of the limitations inherent in root sampling and plating techniques. Many feeder or small roots bearing bacteria are lost during the sampling procedures, and considerable numbers of bacteria are lost when rhizosphere soil, which we found to contain many of the colonizing bacteria, falls off roots during lifting and processing. Also, variation in soil moisture affects the amount of soil clinging to roots, thus affecting the detection process. The inability to detect a bacterium on a plant part should not necessarily be construed as indicating that it is not present, because the populations may be below the detection limits of the techniques. Workers who have studied bacterial populations in soil generally agree that it is difficult to detect populations of a specific bacterium below 10^3 cells per gram of soil even when using selective media (24). Additionally, it has been suggested that members of antibiotic resistant bacterial populations, like those of strain A1-B, may spontaneously revert to antibiotic sensitivity when confronted with the selective pressures of the rhizosphere (7). They thus escape detection but still actively compete with resistant cells. Data from attempts to define detection limits by such methods as introducing a cultured bacterium to soil soon after followed by plating are of questionable value. For example, a bacterium that has been multiplying in the rhizosphere and producing extracellular polysaccharides may be more difficult to isolate because of adsorption to colloids.

The results of this study now enable the development of a clearer perspective in defining the potential scope of influence and use of rhizobacteria to inhibit pathogens in the rhizosphere. With strains such as A1-B, it appears that colonization with concomitant development of populations sufficient to affect soilborne pathogens occurs principally on plant parts from about 16 cm beneath to 12 cm above the treated seed pieces, depending on the soil type. The large rhizosphere presence of strain A1-B relative to native bacteria on the initial root segments (0–8 cm), the persistent, high populations of strain A1-B on seed pieces, and the spatial distribution of bacteria in the soil below suggests that bacteria similar to strain A1-B could effectively exert a relatively permanent influence in the crown area of plants. Because juvenile potato tissues are most susceptible to attack by pathogenic organisms, greatest protection by crown area colonizing bacteria would most likely occur during the germination and seedling stages of development. This could entail control of seed-decaying bacteria (29), fungi attacking young roots and shoots, and the ill-defined "subclinical" pathogens. Plant parts developing subsequently in this zone, such as secondary roots, stolons, and progeny tubers, would also be subject to some degree of protection; however,

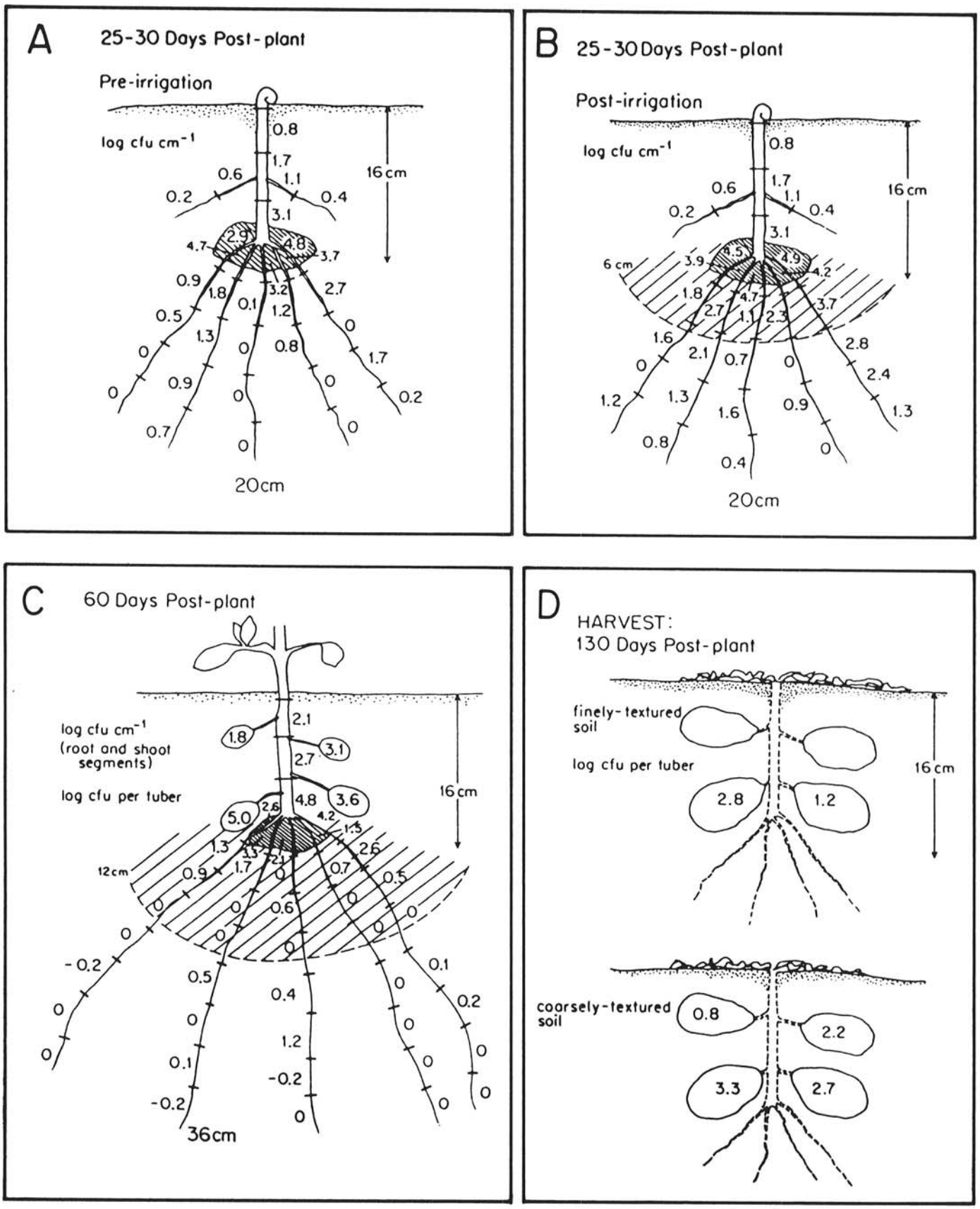

Fig. 2. Stylized depiction of spatial-temporal colonization patterns of *Pseudomonas fluorescens* strain A1-B on underground parts of potato at various stages of plant development: **A,** preirrigation populations of strain A1-B on 4-cm-long segments of roots and underground portions of shoots (log cfu cm^{-1}); **B,** same as (A), except after the first irrigation (note increased population sizes and distribution of strain A1-B along roots arising adjacent to seed pieces and dispersal of strain A1-B in nonrhizosphere soil beneath inoculated seed pieces); **C,** midseason populations on roots, shoots, and progeny tubers (log cfu per tuber) (note increased range of distribution of strain A1-B in soil beneath seed pieces); and **D,** populations of strain A1-B on progeny tubers at harvest in fine-and coarse-textured soils (note absence of detectable populations on tubers distal to seed pieces in the fine-textured soil).

colonization within this area was highly variable. Antagonism toward pathogens would be diminished outside this zone. For this reason, rhizobacteria like strain A1-B, when introduced on seed, would not be expected to give long-term control of pathogens such as *Verticillium* or *Fusarium* spp. that invade root tips of growing plants throughout the season.

LITERATURE CITED

1. Bahme, J. B., and Schroth, M. N. 1984. Colonization dynamics of a rhizobacterium on potato. (Abstr.) Phytopathology 74:806.
2. Bennet, R. A., and Lynch, J. M. 1981. Bacterial growth and development in the rhizosphere of gnotobiotic cereal plants. J. Gen. Microbiol. 125:95-102.
3. Bitton, G., Lahav, N., and Henis, Y. 1974. Movement and retention of *Klebsiella aerogenes* in soil columns. Plant Soil 40:373-380.
4. Colyer, P. D., and Mount, M. S. 1984. Bacterization of potatoes with *Pseudomonas putida* and its influence on postharvest soft rot diseases. Plant Dis. 68:703-706.
5. Elad, Y., and Baker, R. 1985. The role of competition for iron and carbon in suppression of chlamydospore germination of *Fusarium* spp. by *Pseudomonas* spp. Phytopathology 75:1053-1059.
6. Foster, R. C., Rovira, A. D., and Cock, T. W. 1983. Ultrastructure of the Root-Soil Interface. The American Phytopathological Society, St. Paul, MN. 157 pp.
7. Geels F. P., and Schippers, B. 1983. Selection of antagonistic fluorescent *Pseudomonas* spp. and their root colonization and persistence following treatment of seed potatoes. Phytopathol. Z. 108:193-206.
8. Geels F. P., and Schippers, B. 1983. Reduction of yield depressions in high frequency potato cropping soil after seed tuber treatments with antagonistic fluorescent *Pseudomonas* spp. Phytopathol. Z. 108:207-214.
9. Howell, C. R., and Stipanovic, R. D. 1979. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. Phytopathology 69:480-482.
10. Howell, C. R., and Stipanovic, R. D. 1980. Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. Phytopathology 70:712-715.
11. Howie, W. J., and Echandi, E. 1983. Rhizobacteria: Influence of cultivar and soil type on plant growth and yield of potato. Soil Biol. Biochem. 15:127-132.
12. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301-307.
13. Kloepper, J. W. 1983. Effect of seed piece inoculation with plant growth-promoting rhizobacteria on populations of *Erwinia carotovora* on potato roots and in daughter tubers. Phytopathology 73:217-219.
14. Kloepper, J. W., Leong, J., Teintze, M., and Schroth, M. N. 1980. Pseudomonas siderophores: a mechanism explaining disease-suppressive soils. Curr. Microbiol. 4:317-320.
15. Kloepper, J. W., and Schroth, M. N. 1981. Development of a powder formulation of rhizobacteria for inoculation of potato seed pieces. Phytopathology 71:590-592.
16. Kloepper, J. W., and Schroth, M. N. 1981. Relationship of in vitro antibiosis of plant growth-promoting rhizobacteria to plant growth and displacement of root microflora. Phytopathology 71:1020-1024.
17. Kloepper, J. W., Schroth, M. N., and Miller, T. D. 1980. Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology 70:1078-1082.
18. Loper, J. E., Haack, C., and Schroth, M. N. 1984. Population dynamics of soil pseudomonads in the rhizosphere of potato (*Solanum tuberosum* L.). Appl. Environ. Microbiol. 49:416-422.
19. Loper, J. E., Suslow, T. V., and Schroth, M. N. 1984. Lognormal distribution of bacterial populations in the rhizosphere. Phytopathology 74:1454-1460.
20. Lynch, J. M., and Clark, S. J. 1984. Effects of microbial colonization of barley (*Hordeum vulgare* L.) roots on seedling growth. J. Appl. Bacteriol. 56:47-52.
21. Marshall, K. C. 1971. Sorptive interactions between soil particles and microorganisms. Pages 409-445 in: Soil Biochemistry. Vol. 2. A. D. McLaren and J. J. Skujins, eds. Marcel Dekker, New York.
22. Sands, D. C., and Rovira, A. D. 1970. Isolation of fluorescent pseudomonads with a selective medium. Appl. Microbiol. 20:513-514.
23. Scher, F. M. 1986. Biological control of Fusarium wilts by *Pseudomonas putida* and its enhancement with EDDHA. Pages 109-117 in: Iron, Siderophores, and Plant Diseases. T. R. Swinburne, ed. Plenum Press, New York. 351 pp.
24. Schroth, M. N., Thomson, S. V., and Weinhold, A. R. 1979. Behavior of plant pathogenic bacteria in rhizosphere and non-rhizosphere soils. Pages 105-156 in: Ecology of Root Pathogens. S. V. Krupa and Y. R. Dommergues, eds. Elsevier North Holland, New York. 281 pp.
25. Suslow, T. V., and Schroth, M. N. 1982. Role of deleterious rhizobacteria as minor pathogens in reducing crop growth. Phytopathology 72:111-115.
26. Suslow, T. V., and Schroth, M. N. 1982. Rhizobacteria of sugar beets: Effects of seed application and root colonization on yield. Phytopathology 72:199-206.
27. Weller, D. M. 1984. Distribution of a take-all suppressive strain of *Pseudomonas fluorescens* on seminal roots of winter wheat. Appl. Environ. Microbiol. 48:897-899.
28. Weller, D. M., and Cook, R. J. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology 73:463-469.
29. Xu, G. W., and Gross, D. C. 1986. Selection of fluorescent pseudomonads antagonistic to *Erwinia carotovora* and suppressive to seed piece decay. Phytopathology 76:414-422.
30. Xu, G. W., and Gross, D. C. 1986. Field evaluation of interactions among fluorescent pseudomonads, *Erwinia carotovora*, and potato yields. Phytopathology 76:423-430.
31. Yuen, G. Y., and Schroth, M. N. 1986. Inhibition of *Fusarium oxysporum* f. sp. *dianthi* by iron competition with an *Alcaligenes* sp. Phytopathology 76:171-176.
32. Yuen, G. Y., and Schroth, M. N. 1986. Interactions of *Pseudomonas fluorescens* strain E6 with ornamental plants and its effect on the composition of root-colonizing microflora. Phytopathology 76:176-180.