A method for assessing the root-colonizing capacity of bacteria on maize

F. M. SCHER,[1] J. S. ZIEGLE, AND J. W. KLOEPPER[1]

Advanced Genetic Sciences, P.O. Box 1373, Manhattan, KS, U.S.A. 66502

Accepted October 21, 1983

SCHER, F. M., J. S. ZIEGLE, and J. W. KLOEPPER. 1984. A method for assessing the root-colonizing capacity of bacteria on maize. Can. J. Microbiol. **30**: 151–157.

A closed test tube assay was developed for measuring the root colonization capacity of bacteria in raw soil–sand. Bacteria were coated onto seeds at inoculum levels of 10^3–10^9 colony-forming units (cfu) per seed, and root-colonizing bacterial strains successfully grew along the emerging radicle. Quantification of specific bacterial populations on roots was highly reproducible between experiments with the assay. Of 54 strains tested, 24 colonized roots at populations of 10^4 to 10^6 cfu/g root. All root-colonizing strains were Gram negative. Two fluorescent pseudomonad strains that failed to colonize maize roots in raw soil did colonize in autoclaved soil. Captan seed treatment and temperature of incubation (14 and 24°C) had no substantial effect on subsequent root colonization. Root population densities determined in the sand–soil assay were comparable with those determined with plants grown in soils under greenhouse conditions.

SCHER, F. M., J. S. ZIEGLE et J. W. KLOEPPER. 1984. A method for assessing the root-colonizing capacity of bacteria on maize. Can. J. Microbiol. **30**: 151–157.

Un test avec tube à essai fermé a été développé pour mesurer la capacité de colonisation des racines par des bactéries dans du sol ou du sable non-autoclavés. Des bactéries ont été enrobées sur des graines à des niveaux d'inoculum de 10^3–10^9 unités formant des colonies (ufc) par graine, et des souches bactériennes colonisatrices de racines se sont développées avec succès sur les radicules en émergence. La quantification des populations bactériennes spécifiques sur les racines s'est avérée hautement reproductible entre les diverses expériences de l'essai. Sur 54 souches testées, 24 ont colonisé les racines à des populations de 10^4 à 10^6 ufc/g de racine. Toutes ces souches colonisatrices de racine étaient Gram-négatives. Deux souches pseudomonad fluorescentes, qui n'avaient pas colonisé les racines de maïs dans du sol non-autoclavés, ont réussi la colonisation dans des sols autoclavés. Le traitement de graines au captan, de même qu'à des températures d'incubation de 14 et de 24°C n'ont eu aucun effet sur la colonisation subséquente des racines. Les densités de population sur les racines, déterminées dans des essais sol–sable, furent comparables à celles qui furent déterminées à partir de plantes croissant dans des sols sous conditions de serre.

[Traduit par le journal]

Soil microbiologists and plant pathologists have recently succeeded in altering the native microflora of plant roots to achieve either biological control of soil-borne diseases (Kloepper et al. 1980; Scher and Baker 1980, 1982; Sivasithamparam and Parker 1978; Smiley 1978, 1979; Weller and Cook 1983) or increased plant growth (Kloepper and Schroth 1978, 1981*b*; Suslow and Schroth 1982). When the proper bacterial strain is used, plant roots are extensively colonized by the introduced strain, which suggests a close bacteria–plant association that allows for beneficial plant growth or disease protection.

While root colonization alone is not predictive of a beneficial plant response, it is a logical starting point for assays designed to select either growth-promoting bacteria or biological control agents. However, the current techniques which are required to ascertain bacterial root colonization capacity are laborious and often produce highly variable results.

Bennett and Lynch (1981) developed a closed test tube assay for measuring root colonization capacity of bacteria under gnotobiotic conditions which proved useful for studying specific microbial interactions in the rhizosphere. However, in sterile soils, pseudomonad populations are enhanced (F. M. Scher, unpublished), but plant growth promotion may not be realized (Kloepper and Schroth 1981*a*). Therefore, it is not possible to extrapolate from results obtained in sterile environments to those expected under field conditions.

We report here the development of a raw soil assay for bacterial root colonization of maize and present data on the usefulness of the technique. An abstract of this work has been published (F. M. Scher, J. S. Ziegle, and J. W. Kloepper. 1983. Phytopathology, 73: 814 (Abstr.)).

Materials and methods

Bacterial strain selection and characterization

Bacterial strains were isolated from maize, soybean, sorghum, and wheat rhizosphere soils and from fallow soil on Pseudomonas agar F (PAF), (Difco, Detroit, MI, U.S.A. 48232), and spontaneous rifampicin-resistant mutants were selected. Strains were characterized for Gram-stain reaction, motility, gelatin hydrolysis, and fluorescent pigment production (Gerhardt 1981). Gram-positive bacteria were further tested for endospore (Salle 1973) and catalase production. *Escherichia coli* (strain K12) was employed for comparative studies.

[1]Current address: Allelix Inc., 6850 Goreway Drive, Mississauga, Ont., Canada L4V 1P1.

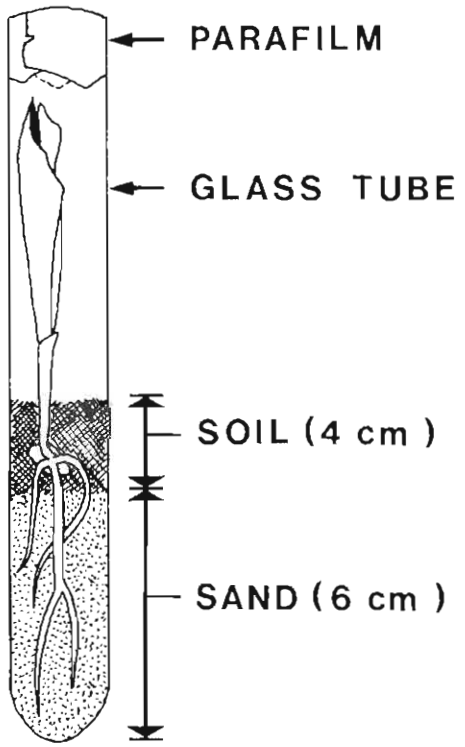

FIG. 1. Apparatus for assessing root colonization capacity of bacteria on maize. Bacterial-treated maize seeds were planted in the soil and plants were harvested after 3–4 weeks.

Bacterial seed treatment

Bacterial inoculum was prepared by washing a 24-h culture from a PAF plate with 10 mL of 0.1 *M* $MgSO_4$. Maize seeds (*Zea mays* L. cv. Pioneer 3183) were soaked in the bacterial suspension for 2–3 h at 25°C on a rotary shaker (80 rpm). Control seeds were soaked in 0.1 *M* $MgSO_4$ washed from a noninoculated PAF plate. Captan-treated seeds were used in all experiments unless otherwise stated.

Inoculum levels on seeds were determined by agitating three seeds from each treatment in 9 mL of 0.1 *M* $MgSO_4$, and plating the suspension onto PAF containing 100 μg rifampicin/mL (PAF-R) with a spiral plater (Spiral Systems, Inc. Bethesda, MD, U.S.A. 20014). Mean colony-forming units (cfu) per seed were determined by averaging the log values of populations in three replicates per treatment after 24–48 h incubation at 30°C.

Root colonization assay

Glass test tubes (25 × 200 mm) were filled with coarse sand to a depth of 6 cm (35 g). Distilled water (5 mL) was added to each tube and the sand was overlaid with 2 cm (6 g) field soil adjusted to 15% moisture. Soil used was a sandy loam collected from a field previously cropped to potato in Riley County, Kansas. Soil characteristics were as follows: pH, 7.7; N, 7 ppm; P, 24 ppm; K, 190 ppm; organic matter, 1.2%; diethylenetriamine pentaacetic acid (DTPA) extractable Fe, 8 ppm. One bacterial-treated seed was added per tube and covered with another 2 cm (6 g) field soil. Tubes were sealed with parafilm (Fig. 1) and incubated in a growth chamber (14 or 24°C, 4000 lx) for 3–4 weeks without added water. Plants were carefully removed from the tubes and all root segments in the sand (Fig. 2) were excised, weighed, and mixed with a Vortex blender for 20 s in 9 mL of 0.1 *M* $MgSO_4$. The suspension was spiral plated onto PAF-R containing 50 μg cyclohexamide/mL (to inhibit fungal growth).

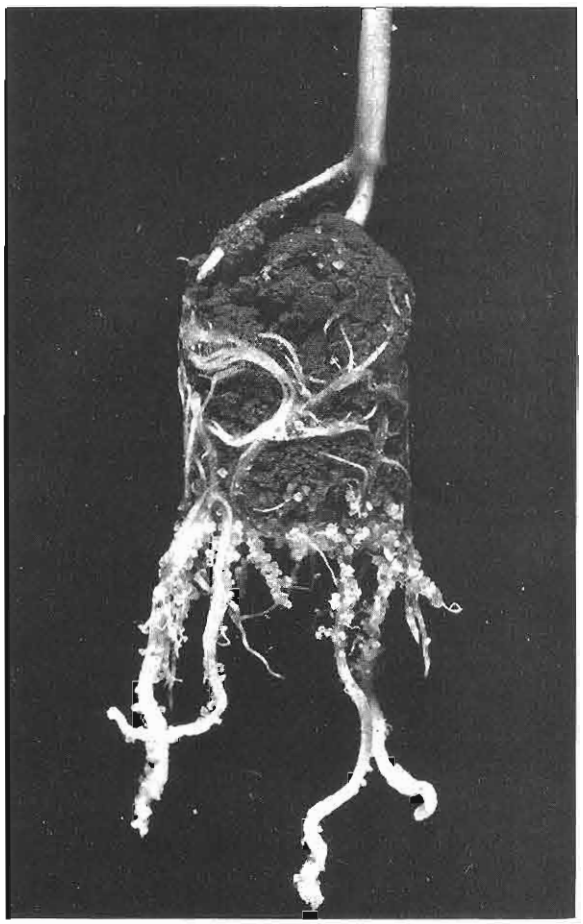

FIG. 2. Maize roots after 3 weeks in sand–soil assay. Bacteria present on root segments in sand were enumerated by the dilution plate method.

Fifty-four bacterial strains (28 Gram negative and 26 Gram positive) were tested for root colonization capacity on maize. Log determinations of mean cfu per gram of root were made as previously described for seeds, except with five replications per treatment. All experiments were performed at least twice. Data were subjected to one-way analyses of variance, and treatment means were separated by least significant differences ($P = 0.05$).

Effect of captan on bacterial populations

The fungicide captan, present on seeds at purchase, was removed by rinsing seeds for 5 min in 80% ethanol and then for 2 min in H_2O. Bacterial suspensions of six selected strains were used to treat captan-coated or captan-free seeds. Seed and root population densities were assessed as described above; tubes were incubated 3 weeks at 14°C.

TABLE 1. Characteristics of bacterial strains tested in the sand–soil assay

Strain	Gram-stain reaction	Gelatin hydrolysis	Motility	Fluorescent pigment production	Catalase production	Endospore production
3	+	+	+	−	−	−
29A	+	+	−	−	+	+
29B	−	−	−	−	N	N
42B	−	−	+	−	N	N
45B	−	−	+	+	N	N
51	+	−	−	−	+	+
57	+	+	+	−	+	+
97	+	+	+	−	+	+
112	−	−	+	+	N	N
120	+	−	−	−	+	−
122	+	−	+	−	+	+
123A	+	+	+	−	+	+
123B	+	−	−	−	+	N
154B	−	−	+	−	N	N
160	+	+	+	−	+	+
280	−	−	+	+	N	N
296	−	−	+	+	N	N
300C	+	−	+	−	+	−
325	−	+	−	−	N	N
339	−	−	+	−	N	N
355	−	−	+	+	N	N
643	+	−	+	−	−	−
A2	−	+	+	−	N	N
A3	−	−	+	−	N	N
A7	−	−	+	−	N	N
A8	−	−	+	−	N	N
A19	−	+	+	+	N	N
A24	−	−	+	−	N	N
SS3	−	−	+	+	N	N
SS4	−	+	−	−	N	N
SS8	−	+	+	−	N	N
SS10	−	+	+	+	N	N
SS3	−	+	−	−	N	N
NR1	+	+	+	−	+	+
NR2	+	+	+	−	−	+
NR3	+	+	+	−	+	+
NR4	+	+	+	−	+	+
NR5	+	+	−	−	+	+
NR6	+	+	+	−	+	+
NR7	+	+	+	−	+	+
NR8	+	−	−	−	+	−
NR9	+	−	−	−	−	+
NR10	+	+	−	−	+	−
NB5	+	−	−	−	−	+
NB17	+	−	−	−	−	+
5KC1	−	+	+	+	N	N
5KC5	−	−	+	+	N	N
5KC17	−	−	−	−	N	N
5KC24	+	−	−	−	+	+
5KC29	−	−	+	−	N	N
2K	+	+	+	−	+	+
3K	−	−	+	+	N	N
10K	−	−	+	+	N	N
E. coli K12	−	N	+	−	N	N

NOTE: +, Positive reaction; −, negative reaction under test conditions; *N*, not determined. Tests listed here were conducted according to Gerhardt (1981) and Salle (1973) (endospore production).

TABLE 2. Bacterial seed and root populations on maize in sand–soil assay at 14°C

Gram-negative strains			Gram-positive strains		
Strain	cfu/seed	cfu/g root	Strain	cfu/seed	cfu/g root
29B	8.1×10^{6}	1.2×10^{4}	3	8.2×10^{5}	ND
42B	3.6×10^{6}	5.6×10^{5}	29A	4.9×10^{4}	ND
45B	9.1×10^{6}	1.5×10^{5}	51	5.7×10^{5}	ND
112	8.3×10^{6}	1.1×10^{5}	57	8.3×10^{5}	ND
154B	2.7×10^{6}	1.4×10^{5}	97	5.9×10^{5}	ND
280	9.1×10^{7}	4.2×10^{4}	120	5.8×10^{4}	ND
296	5.8×10^{7}	4.2×10^{4}	122	4.9×10^{5}	ND
325	7.6×10^{7}	1.1×10^{6}	123A	3.1×10^{5}	ND
339	5.8×10^{7}	2.2×10^{5}	123B	5.4×10^{6}	ND
355	1.0×10^{8}	2.2×10^{5}	160	1.4×10^{6}	ND
A2	3.0×10^{7}	2.8×10^{5}	300C	7.7×10^{4}	ND
A3	3.6×10^{6}	2.6×10^{4}	643	3.8×10^{6}	ND
A7	1.0×10^{7}	1.1×10^{6}	NR1	5.1×10^{6}	ND
A8	2.6×10^{8}	1.2×10^{5}	NR2	5.0×10^{5}	ND
A19	7.1×10^{4}	3.9×10^{4}	NR3	4.3×10^{4}	ND
A24	3.6×10^{6}	2.8×10^{4}	NR4	3.7×10^{5}	ND
SS3	6.5×10^{6}	ND	NR5	1.5×10^{8}	ND
SS4	1.7×10^{8}	1.4×10^{4}	NR6	8.2×10^{7}	ND
SS8	3.2×10^{7}	5.1×10^{4}	NR7	7.6×10^{6}	ND
SS10	5.9×10^{6}	1.6×10^{6}	NR8	4.5×10^{4}	ND
SS13	8.4×10^{6}	1.6×10^{5}	NR9	3.2×10^{4}	ND
5KC1	3.6×10^{5}	2.8×10^{5}	NR10	2.6×10^{6}	ND
5KC5	5.8×10^{6}	2.4×10^{5}	NB5	2.0×10^{3}	ND
5KC17	5.1×10^{7}	ND	NB17	3.8×10^{4}	ND
5KC29	1.0×10^{7}	2.5×10^{5}	5KC24	3.0×10^{3}	ND
3K	5.7×10^{6}	ND	2K	3.0×10^{4}	ND
10K	7.6×10^{5}	5.0×10^{5}			
E. coli K12	3.4×10^{5}	ND			

NOTE: ND, not detectable ($< 2 \times 10^{2}$ cfu/g root).

Root colonization at two temperatures

Maize seeds were treated with seven bacterial strains and planted in root colonization sand–soil assay tubes. Plants were harvested after incubation at 24°C for 1 week or at 14°C for 3 weeks. Root population densities were determined at these times since plant development was approximately equal between temperature treatments.

Root colonization in autoclaved versus raw soil

Seed treated with thirteen bacterial strains were planted in raw or autoclaved (1 h at 121°C, 2 consecutive days) field soil in sand–soil assay tubes. The sand, seed, and test tubes were not autoclaved. Tubes were incubated at 24°C for 1 week and root population densities were determined.

Greenhouse pot assay

Seeds treated with four bacterial strains were planted in field soil at a 2-cm depth in 10-cm diameter plastic pots in the greenhouse or in test tubes (the standard sand–soil assay) in a growth chamber held at 14°C. Twenty plants (four replicates of five) from the greenhouse and five plants from test tubes were harvested after 4 weeks. One gram of root from greenhouse plants and all roots in sand from tubes were excised and assessed for bacterial population densities (cfu per gram of root) as above.

Results

Fifty-four bacterial strains exhibiting various characteristics (Table 1) were assessed for root colonizing capacity on maize by the sand–soil tube method described here. Initial bacterial population densities on seeds ranged from 10^{3} to 10^{9} cfu/seed and were strain dependent (Table 2). Of strains tested, 24 were root colonizers, herein defined as those strains with cfu $> 5 \times 10^{3}$/g root. Root population densities of colonizers ranged from 1.2×10^{4} to 1.6×10^{6}; 30 strains were not detected on roots ($< 2 \times 10^{2}$ cfu/g root).

Bacterial Gram-stain reaction correlated well with colonization capacity, i.e., all Gram-positive bacteria were noncolonizers, whereas all but four Gram-negative strains colonized maize roots. Ten of the 12 strains that produced fluorescent pigment (fluorescent pseudomonads) were colonizers. Motility and gelatin hydrolysis characterisitics of the 54 strains had no apparent correlation with their root colonizing capacity in this investigation.

Captan had no significant effect on bacterial seed and root population densities (Table 3), although it did re-

TABLE 3. Effect of captan on initial seed populations of bacteria and subsequent root colonization (in cfu per seed)

Strain	Initial seed populations		Subsequent root colonization	
	Captan	Captan removed	Captan	Captan removed
Control	ND	ND	ND	ND
355	1.4×10^{8}	5.0×10^{7}	2.3×10^{4}	2.0×10^{4}
280	2.0×10^{8}	1.5×10^{8}	8.9×10^{4}	2.4×10^{4}
45B	2.0×10^{8}	1.7×10^{8}	3.3×10^{5}	1.2×10^{5}
5KC29	2.2×10^{8}	1.4×10^{8}	3.5×10^{5}	4.1×10^{4}
NR7	1.4×10^{6}	5.4×10^{6}	ND	ND
NR10	1.2×10^{6}	6.0×10^{6}	ND	ND

NOTE: No significant ($P = 0.05$) effect of captan on seed or root populations was observed. ND, not detectable ($< 2 \times 10^{2}$ cfu). Captan was removed by rinsing seed for 5 min in 80% ethanol and then for 2 min in H_2O.

TABLE 4. Effect of two temperatures on root colonization (in cfu per gram of root)

Strain	Gram-stain reaction	cfu/seed	Root colonization at:	
			14°C	24°C
Control		ND	ND	ND
355	−	1.5×10^{9}	2.8×10^{4}	2.4×10^{5}
280	−	4.0×10^{7}	1.1×10^{5}	1.1×10^{4}
45B	−	7.8×10^{8}	7.2×10^{4}	3.9×10^{4}
5KC29	−	1.0×10^{9}	5.5×10^{5}	3.6×10^{5}
97	+	1.8×10^{6}	ND	ND
160	+	1.4×10^{6}	ND	ND
NR10	+	3.4×10^{6}	ND	ND

NOTE: Root populations of each strain were not significantly different at 14 and 24°C ($P = 0.05$). ND, not detectable ($< 2 \times 10^{3}$ cfu/g root).

sult in slightly lower seed population densities in some trials. Thus, captan-treated seeds were used to promote emergence in the raw soil employed in this assay.

When sand−soil assay tubes were incubated at 14 or 24°C, no significant difference in root population densities of seven bacterial strains was observed (Table 4). Four Gram-negative strains colonized roots at both temperatures, whereas none of three Gram-positive strains colonized roots.

Gram-positive strains NR1-10 failed to colonize roots ($< 2 \times 10^{2}$ cfu/g root) in raw and autoclaved soil, despite populations of over 1×10^{6} cfu/seed. Gram-negative strains SS3 and 3K, both fluorescent pseudomonads, failed to colonize maize roots in raw soil but reached population densities of $> 1 \times 10^{7}$ cfu/g root in autoclaved soil. Initial seed populations were approximately 2×10^{7} cfu/seed. Fluorescent pseudomonad strain 280, at 3×10^{7} cfu/seed, reached population densities of 9×10^{4} and 3×10^{7} cfu/g root in raw and autoclaved soil, respectively.

Bacterial root population densities in the sand−soil assay were not significantly different than those in pots of soil in the greenhouse (Table 5). Three Gram-negative strains colonized roots in both assays, whereas two Gram-positive strains did not colonize roots in either assay.

Discussion

The test tube assay for root colonization reported here is an efficient method for consistently determining bacterial population densities in the root zone. In this raw soil system, 24 of 54 strains tested were able to compete with native soil microorganisms and colonize maize roots. The advantage of this system over a sterile one is that the capacity of a bacterial strain to compete in soil, as well as its ability to grow on roots, is required for colonization.

This is well illustrated by the results in raw versus autoclaved soil. Two fluorescent pseudomonad strains (SS3 and 3K) failed to colonize roots in raw soil but successfully colonized in autoclaved soil, indicating that microbial competition had a negative effect on colonization by these strains in raw soil. In addition, strain 280 reached root population densities in autoclaved soil of over 2 log units greater than that in raw soil. Thus, use of autoclaved or sterile soil in colonization studies may lead to overestimates of the bacterial colonization which would occur under field (raw soil) conditions. We concur with Bowen (1980) that microbial root ecology can be fully understood only when raw soils are used.

Root population densities of colonizing strains varied in different raw soil types (F. M. Scher, unpublished) but were always distinguishable from those of non-colonizing strains. Results obtained in the sand−soil tube assay were comparable with those obtained in greenhouse pot tests with soils (Table 5). Thus, strains that colonized maize roots in the raw soil test tube assay were true root colonizers, as evidenced by

TABLE 5. Comparative root colonization in greenhouse pot and test tube assays

Strain	Gram-stain reaction	cfu/seed	Test tube, cfu/g root	Greenhouse, cfu/g root
Control		ND	ND	ND
355	−	1.3×10^{8}	7.9×10^{3}	3.9×10^{4}
280	−	1.0×10^{9}	4.2×10^{4}	1.2×10^{5}
5KC29	−	1.0×10^{9}	5.5×10^{5}	1.8×10^{5}
160	+	6.9×10^{3}	ND	ND
NR10	+	1.1×10^{5}	ND	ND

NOTE: Bacterial root populations for each strain were not significantly different between sand−soil and greenhouse assays ($P = 0.05$). ND, not detectable ($< 2 \times 10^{2}$ cfu).

their performance under simulated field (greenhouse) conditions.

Bacteria present on maize seed ($10^{3}-10^{9}$ cfu/seed) were either not detected on roots ($< 2 \times 10^{2}$ cfu/g root) or were present at population densities greater than 5×10^{3} cfu/g root regardless of presence or absence of captan (Table 3) or temperature of incubation (14 and 24°C, Table 4). These results were surprising, as we anticipated detection of "low-level" colonizers in the upper 10^{2} to 10^{3} cfu/g range, particularly with the Gram-positive strains at the warmer temperature. Gram-positive strains did not survive on seeds as well as Gram-negative strains; however, there was no obvious correlation between seed population densities (within $10^{4}-10^{9}$ cfu/seed range) and resulting root population densities in this investigation.

The lack of root colonization by Gram-positive strains, both in raw and autoclaved soil, was consistent with previous results in a gnotobiotic system (Rovira 1956). Rovira reported that 96 and 91% of colonizing bacterial stains on oats and tomato, respectively, were Gram negative. This profound difference in colonization capacity by Gram-positive and Gram-negative strains may be due to the generally longer generation time of Gram-positive than Gram-negative bacteria in soil (Bowen and Rovira 1976). Leben (1983) expressed the opinion that rapid multiplication of bacteria in the rhizosphere of cucumber was important for successful root colonization.

The colonization measured in our assay is primarily radicle root colonization of maize, which may not accurately reflect subsequent colonization of nodal roots. Nevertheless, to successfully compete later in the season, a bacterial strain must colonize the radicle immediately or soon after germination. Past results with radish (J. W. Kloepper, unpublished), sugar beet (Suslow and Schroth 1982), potato (Kloepper et al. 1980), and wheat (Weller and Cook 1983) demonstrated that most bacterial strains which colonized roots immediately after plant emergence continued to colonize the developing root system throughout the season. Therefore, we anticipate that many of the strains which are root colonizers in our test will be vigorous plant-root colonizers under field conditions.

Acknowledgements

We thank Dr. J. Uyemoto and Dr. M. Stayton for their critical review of the manuscript, and J. Raupp, K. Ingersoll, and T. Gosch for excellent illustrative, photographic, and technical assistance, respectively.

BENNETT, R. A., and J. M. LYNCH. 1981. Bacterial growth and development in the rhizosphere of gnotobiotic cereal plants. J. Gen. Microbiol. **125**: 95−102.

BOWEN, G. D. 1980. Misconceptions, concepts and approaches in rhizosphere biology. *In* Contemporary microbial ecology. *Edited by* D. C. Ellwood, J. N. Hedger, M. J. Latham, J. M. Lynch, and J. H. Slater. Academic Press, London.

BOWEN, G. D., and A. D. ROVIRA. 1976. Microbiol colonization of plant roots. Annu. Rev. Phytopathol. **14**: 121−144.

GERHARDT, P. (*Editor-in-chief*). 1981. Manual of methods for general bacteriology. American Society of Microbiology, Washington, DC.

KLOEPPER, J. W., J. LEONG, M. TEINTZE, and M. N. SCHROTH. 1980. *Pseudomonas* siderophores: a mechanism explaining disease-suppressive soils. Curr. Microbiol. **4**: 317−320.

KLOEPPER, J. W., and M. N. SCHROTH. 1978. Plant growth - promoting rhizobacteria on radishes. Proc. Int. Conf. Plant Pathol. Bact. 4th, 1978. pp. 879−882.

——— 1981*a*. Plant growth - promoting rhizobacteria and plant growth under gnotobiotic conditions. Phytopathology, **71**: 642−644.

——— 1981*b*. Relationship of in vitro antibiosis of plant growth - promoting rhizobacteria to plant growth and the displacement of root microflora. Phytopathology, **71**: 1020−1024.

KLOEPPER, J. W., M. N. SCHROTH, and T. D. MILLER. 1980. Effects of rhizosphere colonization by plant growth - promoting rhizobacteria on potato plant development and yield. Phytopathology, **70**: 1078−1082.

LEBEN, C. 1983. Association of *Pseudomonas syringae* pv.

lachrymans and other bacterial pathogens with roots. Phytopathology, **73**: 577–581.

ROVIRA, A. D. 1956. A study of the development of the root surface microflora during the intial stages of plant growth. J. Appl. Bacteriol. **19**: 72–79.

SALLE, A. J. 1973. Fundamental principles of bacteriology. 7th ed. McGraw-Hill. New York.

SCHER, F. M., and R. BAKER. 1980. Mechanism of biological control in a *Fusarium*-suppressive soil. Phytopathology. **70**: 412–417.

——— 1982. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to Fusarium wilt pathogens. Phytopathology, **72**: 1567–1573.

SIVASITHAMPARAM, K., and C. A. PARKER. 1978. Effect of certain isolates of bacteria and actinomycetes on *Gaeumannomyces graminis* var. *tritici* and take-all of wheat. Aust. J. Bot. **26**: 773–782.

SMILEY, R. W. 1978. Colonization of wheat roots by *Gaeumannomyces graminis* inhibited by specific soil microorganisms and ammonium-nitrogen. Soil Biol. Biochem. **10**: 175–179.

——— 1979. Wheat rhizosphere pseudomonads as antagonists of *Gaeumannomyces graminis*. Soil Biol. Biochem. **11**: 371–376.

SUSLOW, T. V., and M. N. SCHROTH. 1982. Rhizobacteria of sugar beets: effects of seed application and root colonization on yield. Phytopathology, **72**: 199–206.

WELLER, D. M., and R. J. COOK. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology, **73**: 463–469.