Effect of Seed Piece Inoculation with Plant Growth-Promoting Rhizobacteria on Populations of *Erwinia carotovora* on Potato Roots and in Daughter Tubers

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ABSTRACT

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The application of plant growth-promoting rhizobacteria (PGPR) to potato seed pieces prior to planting resulted in significantly reduced root zone populations of *Erwinia carotovora* (*Ec*) in field trials. The percentage of daughter tubers infested naturally with *Ec* from plants treated with PGPR also was significantly reduced. Reductions resulted from inoculations with PGPR strains singly or in mixtures of two or more strains and were observed in three distinct geographic areas. The results suggest that PGPR may be useful in management of potato blackleg and soft rot diseases. A modified medium developed for *Erwinia* spp. was more sensitive than McConkey-pectate medium. This medium eliminated pectolytic pseudomonads that can interfere with development of colonies of pectolytic *Erwinia* spp.

One of the mechanisms by which plant growth-promoting rhizobacteria (PGPR) (4,14,26) increase plant growth appears related to the ability to cause shifts in populations of various microorganisms that colonize root systems. Populations of PGPR up to 9×10^5 colony-forming units per centimeter (cfu/cm) of root are commonly detected on roots of potato and sugar beet (15,27) and are associated with marked changes in populations of other microorganisms found on roots (28). With potato, root colonization by PGPR is associated with reductions up to 93% in root zone populations of Gram-positive bacteria and 64% of fungi (18). These microorganisms are not "classical" plant pathogens, but normal root zone microflora, some of which can exert a negative effect upon plant growth (28).

The impact of PGPR upon native root zone microflora prompted an investigation to determine if PGPR could also reduce the root zone population of a major plant pathogen. *Erwinia carotovora* was selected since it is a common root zone inhabitant of potato (2,6,23–25) and weeds (7,11,18), and a reduction of its rhizosphere population might lead to a reduction in tuber infestation. Also, two strains of fluorescent *Pseudomonas* spp. were previously found to inhibit soft rot development in laboratory tests (3). No differentiation was made in this study between *Erwinia carotovora* subsp. *carotovora* (*Ecc*) and *E. carotovora* subsp. *atroseptica* (*Eca*), since Molina and Harrison (22) demonstrated that both cause typical potato blackleg and both are involved in seed piece decay and soft rot. Therefore, the designation *E. carotovora* (*Ec*) refers here to *Ecc* and/or *Eca*. A modification of Miller-Schroth's medium (21) was employed in these studies, and its effectiveness in detecting *Ec* was compared to McConkey-pectate medium. Preliminary results have been published (19).

MATERIALS AND METHODS

Effect of PGPR on root zone populations of *Erwinia carotovora*. Fluorescent pseudomonad PGPR strains, which were previously demonstrated to significantly increase potato yield in field tests (15), were used in three field plots in 1979. Dried inocula were prepared containing 10^8 cfu/g as previously described (16) and were dusted onto potato seed pieces immediately prior to planting at a rate of 0.5 kg per 46 kg of seed pieces. Control seed pieces were dusted with powders prepared the same way except that water was substituted for the bacterial suspensions. Field 1 was on sandy loam, pH 7.2, near Shafter, CA, and fields 2 and 3 were on peat soils, pH 7.0, in Tulelake, CA. The PGPR strains tested were A1, B10, TL3B2, and BK1 in Field 1; B10 and E6 in field 2; and A1 and B10 in field 3.

Field plots were arranged in randomized blocks with four-row plots, each 7.6 m (25 ft) long. The rows were 76 cm (30 in.) apart and the seeds were planted at 23 cm (9 in.) spacings in the rows. Each treatment was replicated five times. Cultivar White Rose was used in field 1 and cultivar Netted Gem in fields 2 and 3.

Populations of *Ec* were determined 2 wk prior to harvest by sampling 50 cm of root per plant as previously described (15). Three plants were randomly selected per replication. Roots were agitated in 10 ml of sterile distilled water and serial 10-fold dilutions were prepared to 10^{-3}. Aliquots (0.1 ml) of each dilution were plated onto the medium described below.

Erwinia medium. The medium used was a modification of Miller-Schroth medium for *Erwinia amylovora* (21) with the following changes: 0.17 g cobalt chloride added to improve storage, 10.0 g lactose substituted for mannitol, 5 g Oxoid bile salts substituted for sodium taurocholate, 6.3 g calcium chloride dihydrate added, and nicotinic acid was deleted. The pH was adjusted to 7.2 making the medium blue-green prior to autoclaving. The medium may be stored up to 3 mo and melted prior to pouring plates. The medium in the plates was allowed to set 12–24 hr after pouring and then was overlayed with sodium polypectate containing EDTA. The polypectate-EDTA medium was prepared by dissolving 1 g of disodium EDTA in 1 L of distilled water and mixing with 20 g sodium polypectate (manufactured by Sunkist Growers Inc., Ontario, CA, and available at WARF Institute, Inc., Madison, WI 53707) suspended in 60 ml of 95% ethanol. The pH was adjusted with 1N NaOH while stirring and straining out lumps that formed when the NaOH was added.

The efficiency of the modified Miller-Schroth (MMS) medium for recovering low populations of *Ec* was compared with McConkey-pectate medium. Individual potato tubers (cultivars White Rose and Netted Gem) were gently washed to remove soil, wrapped in wet paper towels, tightly wrapped in Saran Wrap, and incubated at 24 C until rot pockets formed (3–5 days). Soft rot tissue was suspended in 10 ml of sterile distilled water, serial 10-fold

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dilutions were prepared, and 0.1-ml aliquots of each tube were plated on the MMS and McConkey-pectate medium. The number of *Ec* colonies that developed on each medium was compared for each sample tuber after incubation for up to 4 days at 28 C. Pectolytic colonies, which were presumed to be *Ec*, were transferred to King's medium B and the presence or absence of fluorescence was noted. Purified strains were identified as *Ec* as described by Molina and Harrison (22).

Effect of PGPR on infection of daughter tubers by *Erwinia carotovora*. PGPR inocula were prepared as before and used in two fields (fields 4 and 5) in 1980 and one field (field 6) in 1981. Fields 4 and 5 were on sandy loams, pH 7.2, near Shafter, and cultivar White Rose was used at both sites. Field 6 was on a silt loam, pH 5.2, near Gold, PA, and cultivar Katahdin was used. PGPR strains tested were BK1 and a mixture of BK1, TL3B1, and B10 in field 5; and A1, E6, and a mixture of A1, B10, BK1, TL3A, TL3B1, TL3B2, and E6 in field 6. Controls were included as previously described; all treatments were replicated six times in four-row plots as previously described.

After harvest, 20–30 tubers were randomly selected from each replication of each treatment in each field and were individually wrapped as previously described to induce formation of soft rot pockets. Pectolytic bacteria detected on the Erwinia medium were periodically removed and tested as described above to confirm identification as *Ec*. The percentage of daughter tubers from which *Ec* was recovered was recorded for each treatment.

RESULTS

Erwinia medium. MMS medium consistently yielded higher populations of *Ec* from potato tubers than did McConkey-pectate medium; up to 1.8 log units more *Ec* colonies were detected from the same sample on MMS than on McConkey medium. Bacterial growth was slower on MMS than on McConkey. *Ec* colonies developed after incubation for 3 days (24 C) on MMS and after 2 days on McConkey. Pectolytic colonies from McConkey medium frequently included fluorescent *Pseudomonas* spp.; however, no pectolytic pseudomonads grew on MMS in this study.

Effect of PGPR on root zone populations and daughter tuber infestations of *Erwinia*. Populations of *Ec* on roots from PGPR-treated seed pieces were 95–100% less than on roots of control plants ($P = 0.05$) at harvest time in all three fields (Table 1). Neither soil type nor soil pH seemed to affect efficacy of the PGPR in displacing or preventing root colonization by *Ec*. PGPR strain

TABLE 1. Reduction in *Erwinia carotovora* populations on roots of potato treated with plant growth-promoting rhizobacteria (PGPR) in field tests

Field number, location, year, and soil type	Seed piece treatment[a]	*Erwinia carotovora* population (cfu/cm)[b]	Reduction (%)
1. Shafter, CA; 1979; sandy loam	Control	41	...
	A_1	1*[c]	97
	B10	0*	100
	TL13B2	16	61
	BK1	0.8*	98
LSD ($P = 0.05$) = 32			
Tulelake, CA; 1979; peat	Control	4,660	...
	B10	52*	99
	E6	188*	96
LSD ($P = 0.05$) = 932			
3. Tulelake, CA; 1979; peat	Control	312	...
	A_1	31*	90
	B10	16*	96
LSD ($P = 0.05$) = 58			

[a] Strains of PGPR fluorescent pseudomonads were applied to seed pieces in a dried powder formulation (16) prior to planting.
[b] Colony-forming units per centimeter of root; average of 50 cm root per sample with five replications.
[c] Asterisk (*) indicates statistically significant reduction compared to control at the indicated level of significance.

TABLE 2. Reduction in the percentage of daughter tubers infested with *Erwinia carotovora* following seed piece treatments with plant growth-promoting rhizobacteria (PGPR) in field tests

Field number, location, year and soil type	Seed piece treatment[a]	Daughter tubers with *E. carotovora*[b]	Reduction (%)
4. Shafter, CA; 1980; sandy loam	Control	32	...
	BK1	14*[c]	56
	B10	15*	53
	B10 + TL3B1	15*	53
LSD ($P = 0.10$) = 16			
5. Shafter, CA; 1980; sandy loam	Control	18	
	BK1	10*	44
	BK1 + TL3B1 + B10	6*	67
LSD ($P = 0.07$) = 8			
6. Gold, PA; 1981; silt loam	Control	25	...
	A1	18	28
	E6	2*	92
	Mixture[d]	3*	88
LSD ($P = 0.01$) = 11			

[a] Strains of PGPR fluorescent pseudomonads applied to seed pieces in a dry powder formulation (16) prior to planting.
[b] Average of six replications with 20–30 tubers per replication.
[c] (*) Asterisk indicates statistically significant reductions relative to the control at the indicated level of significance.
[d] Mixture in field 6 contained PGPR strains A1, B10, BK1, TL3A, TL3B1, TL3B2, and E6.

TL3B2 was the only strain that did not cause a significant reduction in root-zone populations of *Ec*.

Root colonization by PGPR resulted in reductions in the percentage of daughter tubers infested with *Ec* (Table 2) ranging from 28 to 92% compared to control plants. The level of significance varied in each of the three fields from $P = 0.10$ to $P = 0.01$. One treatment in each field consisted of a mixture of two or more PGPR strains, and the resulting reductions in daughter tuber infestation by *Ec* were similar to those obtained by using treatments of single PGPR strains.

DISCUSSION

The MMS medium was an effective selective and differential medium for the detection of *Ec* from both roots and tubers. The medium supported higher *Ec* populations than McConkey pectate medium, which is similar in sensitivity (M. D. Harrison, *unpublished*) to crystal violet pectate (CVP) medium (7). MMS prevented growth of pectolytic pseudomonads that grow on both McConkey-pectate and CVP media; and therefore appears to be a useful alternative to McConkey and CVP for certain studies.

The interactions between PGPR and *Ec* were encouraging since PGPR reduced natural populations of *Ec* on root and daughter tubers using five different potato seed lots in three distinct geographic areas. However, there was considerable varation among replications, and it is desirable to extensively test PGPR under widely differing conditions to fully assess their reliability as seed treatments to control *Ec*. The reduction in *Ec* on potato root zones following inoculation of potato seed pieces with PGPR is in line with previous findings showing that PGPR cause shifts in populations of root zone microorganisms (18,28). PGPR are inhibitory to *Ec* in vitro (17,18), which is related to siderophore production (12,13). However, fluorescent pseudomonads also produce antibiotics and other metabolites that may play an additional role in suppressing *Ec* populations.

The major inoculum source of *Ec* presumably consists of strains from seed pieces infested with *Ec* (8,9), although *Ec* may also be present in field soils (4,5,20,25). The reduction of *Ec* on daughter tubers reflects the aggressive root colonization capacity of PGPR. While it is possible that naturally occurring strains of *Ec* exist that are resistant to the PGPR used in this study, there are likely to be additional PGPR that would inhibit such strains of *Ec*. PGPR should prove useful in management of blackleg and soft rot by reducing both the incidence of infection of daughter tubers by *Ec* and the population of *Ec* in infested tubers. This is important since the incidence of seed piece decay and blackleg are proportional to inoculum density in seed pieces under certain environmental conditions (1). PGPR may also prove useful in reducing the rate of recontamination of potatoes with *Erwinia* after release from Erwinia-free stem cutting stock, which has been recently documented (6,10).

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