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Suppression of pre- and postemergence damping-off in corn by *Burkholderia cepacia*

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Abstract

Burkholderia cepacia (syn. *Pseudomonas cepacia*) strain PHQM100 applied as a seed coating was tested in growth chamber experiments for its ability to suppress preemergence damping-off, and postemergence damping-off in corn induced by *Pythium* and *Fusarium* spp. The symptoms observed in bioassays with soils naturally infested with the fungal pathogens were seed rot with *Pythium* spp. and mesocotyl and root tissue necrosis in the presence of *Fusarium* spp. Three corn cultivars that differed in their susceptibility to damping-off pathogens were used. Cultivar L was susceptible to pre- and postemergence damping-off, whereas cv. LPDP and cv. LG11 were moderately resistant and resistant to the damping-off diseases respectively. In the presence of *Pythium* spp., seed treatment with *B. cepacia* reduced seed rot, as compared to the untreated seeds, and this reduction was more consistent in the cv. LPDP than in the resistant cv. LG11 or the susceptible cv. L. In soils infested with *Fusarium* spp., seed treatment significantly reduced root and mesocotyl necrosis as compared to the untreated seeds, and this reduction was more consistent in the resistant cultivars LG11 and LPDP than in the susceptible cv. L. Root colonization levels by *B. cepacia* were similar in the three corn cultivars tested. Biocontrol efficiency of *B. cepacia* varied among cultivars mainly due to the differences in their susceptibility to the fungal pathogens. In spite of variability and also irrespective of the soil characteristics, *B. cepacia* increased seedling emergence and decreased mesocotyl and root necrosis when used as a seed coating.

Introduction

Corn seedlings are attacked by various soilborne fungi, such as *Pythium* spp. and *Fusarium* spp., that cause either seed rots before germination or seedling rots after germination. These diseases are often termed pre- and postemergence damping-off, or seedling blights. Isolates of the antagonistic gram positive bacterium, *Bacillus subtilis*, and the fungus, *Chaetomium globosum*, have been reported to suppress corn seedling blights caused by *Fusarium roseum* f. sp. *cerealis* (Chang and Kommedahl, 1968; Kommedahl and Chang, 1975). In some cases, plant stand was comparable to that resulting from seed treatment with the fungicides captan and thiram. Few other reports exist on biological suppression of seedling blights of corn.

Previous growth chamber studies have shown that *Burkholderia cepacia* (syn. *Pseudomonas cepacia*) (Yaabuchi et al., 1992), a soil bacterium, was able to reduce early corn seedling infection by *Fusarium moniliforme*, a pathogen often associated with stalk rots (Hebbar et al., 1992a). Numerous reports have been published on the isolation, mode of action and utilization of *B. cepacia* for biological control of various soilborne fungal pathogens in different crops (Lumsden et al., 1987; Homma et al., 1989; Homma et al., 1990; DeFreitas and Germida, 1991; Hebbar et al., 1991; Hebbar et al., 1992a; McLoughlin et al., 1992; King and Parke, 1993; Burkhead et al.,

1994; Cartwright and Benson, 1995). Although the type strain of *B. cepacia* ATCC 25416 was described earlier as a phytopathogen causing sour skin of onion bulbs (Burkholder, 1950), the strains isolated from the rhizosphere of corn do not cause necrosis of onion tissue (Hebbar et al., 1992a). These strains isolated from corn produce an antifungal compound, pyrrolnitrin, which has broad-spectrum antifungal activity, whereas *B. cepacia* reference strain ATCC 25416 does not produce pyrrolnitrin (Homma et al., 1990). Hebbar et al. (1992a) determined in growth chamber studies that, a while the majority of soil strains of *B. cepacia* were unable to suppress early corn seedling infection by *Fusarium moniliforme*, those from corn roots could.

Studies of corn monoculture soils in mid-western USA (Hebbar et al., 1992b) and a recent one in France (Hebbar et al., 1994) showed that high populations of *B. cepacia* were associated with the rhizosphere and roots of corn. When applied as seed inoculants, *B. cepacia* strains isolated from corn colonized the rhizosphere and roots of corn extensively (Hebbar et al., 1992c). *B. cepacia* is also an efficient colonizer of roots and rhizosphere of radish (Homma et al., 1989), pea (Parke, 1990), and sunflower (Hebbar et al., 1991). In a preliminary study of corn seedling growth in two (from North east and from South west France) corn field soils, with differing characteristics, the disease symptoms observed were mainly preemergence damping off in the former, and post-emergence damping-off in the latter soil. This also varied with the corn cultivar used. The failure of biocontrol agents to establish at sufficient levels on the seed or in the rhizosphere has been attributed to unfavorable soil temperature, pH or moisture (Callan et al., 1990). There are few reports on the effect of seed exudation and plant species on rhizosphere microflora and disease suppression by biocontrol agents (Lemanceau et al., 1995; Nelson et al., 1988; Maurhofer et al., 1995), however only few reports exist on the effect of biological agents on disease suppression in different cultivars of the same species (Liu et al., 1995, Leeman et al., 1995, Meera et al., 1995). However, the mechanism of action in these reports were mainly through induction of systemic resistance. The objective of this study was to determine the pathogens involved and the ability of *B. cepacia* isolated from corn roots to suppress pre- and postemergence damping-off in three corn cultivars differing in their susceptibility to damping-off pathogens, in two soil types with differing characteristics.

Materials and methods

Bacterial strain and corn cultivars. *B. cepacia* strain PHQM100 used in this study was isolated from corn roots grown in corn monoculture soils (Hebbar et al., 1994). The strain PHQM100 was selected based on characteristics such as, being a predominant isolate in corn monoculture soils, a good colonizer of corn roots and also for exhibiting strong *in vitro* antifungal activity against species of *Pythium* and *Fusarium* (Hebbar et al., 1994). Corn cultivars LG11, LPDP and L, supplied by Limagrain (France) were considered variable in their susceptibility to damping-off diseases (personal communication, P. Gadille, Limagrain). Seeds used in bioassays were not treated with fungicides.

Soil and fungal pathogens. The two soil samples used for bioassays, which had been in corn cultivation for several years, were a sandy clay loam (SCL) soil (pH 7.9) from Alsace, N. E. France (Mr. Peterschmitt's farm in Rhein Felderhof) and a sandy loam (SL) soil (pH 6.0) from Chappes, S.W. France. In preliminary studies of corn seedling growth in SCL soil, disease symptoms observed were mainly seed rot (preemergence damping-off), and root necrosis of the seedlings that had emerged. On the contrary, in seedlings grown in the SL soil, mesocotyl and root necrosis were the disease symptoms observed with no seed rot. Populations of *Pythium* and *Fusarium* spp. in the two soils were estimated by plating serial dilutions of soil on semi-selective medium for *Pythium* (Jeffers and Martin, 1986) and *Fusarium* (Burgess and Liddell, 1983). The presence of fungal pathogens on the plant tissues were confirmed by plating the rotted seeds, necrosed mesocotyl and roots on media selective for *Pythium* and *Fusarium*.

Seed bacterization. Bacterization of seeds was performed using a peat-based inoculum of *B. cepacia*. Overnight nutrient broth (Difco) cultures of *B. cepacia* strain PHQM100 (30 ml of $5x10^9$ cells ml^{-1}) were inoculated into sterile packets containing 200 g of gamma-irradiated peat and incubated for 10 days at 23 °C. Bacterial counts in the peat, determined by plating serial dilutions on nutrient agar, were approximately $1x10^9$ colony forming units (CFU) g^{-1} peat. A slurry was prepared by adding 7 g of peat inoculum to 10 ml of 0.35% sticker solution made from a bacterial polysaccharide (Biolygel^{TM}, ARD, Pomacle, France) (Hebbar et al., 1992d). The slurry inoculum was then thoroughly mixed with 50 seeds in a beaker. Individual

seeds were spread out on a bed of sterile dry peat, lightly dusted with additional dry peat, and gently rotated to give an uniform and compact seed coating with the inoculum. The corn seeds, which had bacterial counts of approximately 10^7 CFU of *B. cepacia* per seed after coating, were sown immediately.

Bioassays. Conical black plastic tubes (6 × 25 cm, Tecu, France) filled with 200 g of soil were watered to saturation and incubated for 3 to 5 days at 15-20 °C before sowing seeds bacterized with strain PHQM100. A 16 h day and 8 h night cycle was used with corresponding temperature settings of 20-15 °C for bioassays with SCL soil (*Pythium* spp.) and 28–22 °C in bioassays with SL soil (*Fusarium* spp.). Observations were made on the percentage of seed rots, percentage of plants with necrotic mesocotyl and total length (cm) of necrotic root per plant. At the end of the bioassays (2 to 3 weeks) the population of seed-coated *B. cepacia* strain PHQM100 on corn roots was estimated by plating serial dilutions of seedling roots macerated in 10 ml of distilled water, on a semi-selective PCAT (*Pseudomonas cepacia* azelaic acid tryptamine) medium (Burbage et al., 1982). Root macerates of untreated control plants were also plated.

Statistical analysis

Statistical analysis on the data were performed using the general linear models procedures of SAS (SAS Institute, Cary, NC), and analysis of variance was conducted using a factorial treatment structure with interactions. In each bioassay, twenty replicate plants were used per treatment which were completely randomized. Bioassays were conducted three times for each soil type and data from all bioassays were combined for analyses.

Results

Damping-off pathogens in Sandy Clay Loam (SCL) and Sandy Loam (SL) soils

Approximately 10^2 CFU g^{-1} dry weight soil of *Pythium* spp. was estimated in the SCL soil from Alsace and 10^3CFU g^{-1} dry weight soil of *Fusarium* spp. was estimated in the SL soil from Chappes. Soil populations of *Fusarium* spp. in SCL soil and *Pythium* spp. in SL soil, were below detectable levels ($<10^2$ CFU g^{-1} soil). The symptoms observed in bioassays with the SCL soil were seed rot and root tissue necrosis in emerged seedlings. The symptoms observed in bioassays with the SL soil were root and mesocotyl tissue necrosis with subsequent damping-off. Mesocotyl necrosis and post-emergence damping-off was not observed in plants grown in SCL soil (for 2 weeks) and seed rots were not observed in any of the replicated experiments with SL soil (for 3 weeks). Based on microscopic observation of morphological characteristics, the fungi isolated from the rotted seed and necrosed roots from SCL soil were identified as *Pythium arrhenomanes*, and *P. spinosum*. At least three *Fusarium* spp. were isolated from necrotic roots and mesocotyl tissues of corn grown in SL soil, and they were identified as *Fusarium roseum* var. *culmorum*, *F. acuminatum*, and *F. sambucinum*.

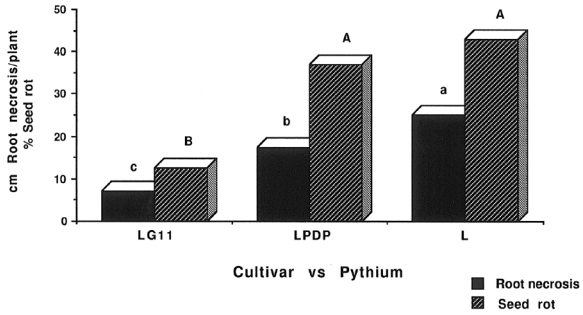

Figure 1. Effect of cultivar on the percentage (%) of seed rot and root necrosis (cm/plant) in corn seedlings grown in sandy clay loam (SCL) soil in the presence of *Pythium* spp. Bioassays were conducted in growth chambers. Seeds were bacterized with $\sim 10^7$ *B. cepacia* strain PHQM100 per seed. Bar graphs for each variable (seed rot and root necrosis), which represent mean values from three replicate bioassays, followed by the same letter are not significantly different to each other according to Duncan's multiple range test ($P \leq 0.05$).

Effect of cultivars on pre and post emergence damping-off of corn

Effect of cultivar was significant ($P<0.05$) on seed rot and root necrosis in the presence of *Pythium* spp. and on mesocotyl and root necrosis, in the presence of *Fusarium* spp. (Table 1). Mean values from the three repeated experiments in SCL soil infested with *Pythium* indicated that percentage seed rot was lowest (12.5%) in cv. LG11, followed by cv. LPDP (37%) and the cv. L (43%) (Figure 1). The effect of cultivar on root necrosis was also highly significant. The cultivars LG11 and LPDP, exhibited lower amounts (7.2 and 17.4 cm plant respectively) of root necrosis per plant

Table 1. Effect of corn cultivar, seed inoculation with *B. cepacia* and their interaction on damping-off in the presence of *Pythium* spp. and *Fusarium* spp.

Source	SCL soil with *Pythium*		SL soil with *Fusarium*	
	SR	RN	RN	MN
	F values			
Cultivar	18.8**	77.9**	51.3**	1.1 ns
Seed treatment[b]	7.8**	1.0 ns	34.7**	9.6**
Interaction	2.4 ns	1.0 ns	4.1**	1.6 ns

[a] Resistant cv. LG11, moderately resistant cv. LPDP, and susceptible cultivar cv. L were used; [b] Seeds were bacterized with *B. cepacia* strain PHQM100. SR seed rot, RN root necrosis, MN mesocotyl necrosis, SCL sandy clay loam soil, SL sandy loam soil. (** P $\leq$ 0.05, ns not significant).

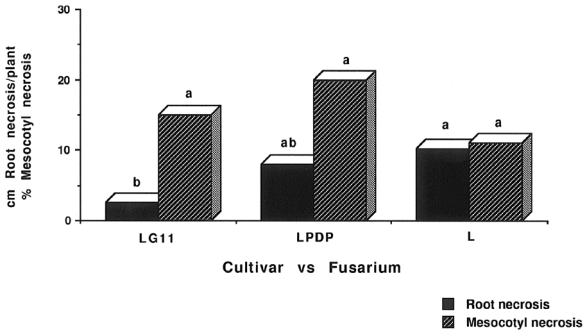

Figure 2. Effect of cultivar on root necrosis (cm/plant) and percentage (%) of plants with mesocotyl necrosis in corn seedlings grown in sandy loam (SL) soil in the presence of *Fusarium* spp. Resistant cv. LG11, moderately resistant cv. LPDP, and sensitive cv. L were used. Seeds were bacterized with $\sim 10^7$ *B. cepacia* strain PHQM100 per seed. Bar graphs for each variable root necrosis, and mesocotyl necrosis, which represent mean values from two replicate, bioassays, followed by the same letter are not significantly different to each other according to Duncan's multiple range test (P $\leq$ 0.05).

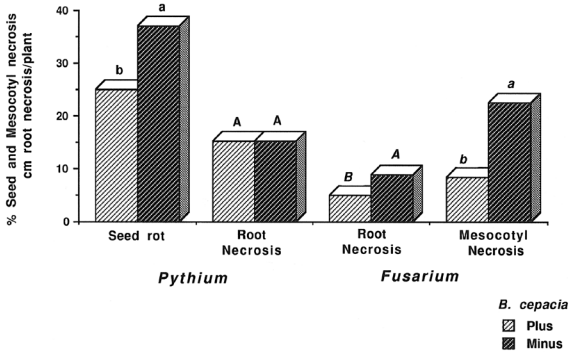

Figure 3. Effect of seed treatment with *B. cepacia* on seed rot, root necrosis and mesocotyl necrosis in the presence of *Pythium* spp. or *Fusarium* spp. Corn seeds bacterized with $\sim 10^7$ CFU of *B. cepacia* strain PHQM100 per seed were grown in sandy clay loam (SCL) soil in the presence of *Pythium* spp. and in sandy loam (SL) soil in the presence of *Fusarium* spp. Bar graphs for each variable (seed rot, root necrosis and mesocotyl necrosis), which represent combined mean values from three cultivars, followed by the same letter are not significantly different to each other according to Duncan's multiple range test (P $\leq$ 0.05).

than cultivar L (25.1 cm) (Figure 1). Mean values from the two experiments in SL soil infested with *Fusarium* indicated that root necrosis per plant was significantly lower in cv. LG11 (2.5 cm) than cv. LPDP (8.0 cm) and L (10.3 cm) (Figure 2). However, mean differences in mesocotyl necrosis were not significant between cultivars (15% in cv. L11, 20% in cv. LPDP and 11% in cv. L (Figure 2)).

Suppression of pre and postemergence damping-off by B. cepacia

Seed treatment with *B. cepacia* had a significant effect (P<0.05) on seed rot in the presence of *Pythium* spp. and on mesocotyl and root necrosis in the presence of *Fusarium* spp. (Table 1 and Figure 3). Overall means from the three cultivars indicated that in presence of *Pythium* spp., maize seed treated with *B. cepacia* had significantly less seed rot, than untreated seed, but showed no difference in root necrosis (Figure 3). Similarly, seed treatment with *B. cepacia* also significantly reduced mesocotyl necrosis and root necrosis in presence of *Fusarium* spp. (Figure 3).

There was no significant interaction between cultivar and seed treatment for most of the symptoms observed (except for root necrosis in SL soil), which indicated that the overall effect of seed treatment did not depend on the cultivar tested (Table 1). When compared by cultivars individually, reduction of seed rot and root necrosis due to *Pythium* spp. was not statisti-

Table 2. Effect of corn cultivar and seed inoculation with *B. cepacia* on seed rot (SR), root necrosis (RN), and mesocotyl necrosis (MN) in sandy clay loam (SCL) soil in the presence of *Pythium* spp. and in sandy loam (SL) soil in the presence of *Fusarium* spp.

Cultivar[a]	Seed treatment *B. cepacia*[b]	SCL soil *Pythium*		SL soil *Fusarium*	
		SR (%)	RN (cm plant^{-1})	RN (cm plant^{-1})	MN (%)
LG11	minus	13a	7.3a	3.7a	25a
	plus	11a	6.9a	1.3b	5b
LPDP	minus	50A	12.9A	11.2A	30A
	plus	25A	13.3A	4.8B	10B
L	minus	48*a*	24.4*a*	11.6*a*	12.5*a*
	plus	38*a*	20.0*a*	9.0*b*	10*a*

[a] Resistant cv. LG11, moderately resistant cv. LPDP, and susceptible cultivar cv. L were used; [b] Seeds were bacterized with $\sim 10^7$ *B. cepacia* strain PHQM100 per seed; Values for each cultivar in a column followed by the same letter are not significantly different to each other according to Duncan's multiple range test at $P \leq 0.05$.

Table 3. Root colonisation by *B. cepacia* strain PHQM100 in two different field soils

Soil[a]	Pathogen[b]	Cultivar[c]	$\log_{10}$ CFU g^{-1} root $\pm$ SD[d]
SCL	*Pythium*	LG11	6.2 ± 1.16
		LPDP	6.1 ± 0.83
		L	6.2 ± 1.40
SL	*Fusarium*	LG11	7.6 ± 0.32
		LPDP	7.6 ± 0.11
		L	7.5 ± 0.10

[a] SCL and SL soil were prehumidified, and when plated on PCAT medium did not contain background *B. cepacia*.[b] Soils were naturally infested with *Pythium* spp. and *Fusarium* spp. [c] Seeds of resistant cv. LG11, moderately resistant cv. LPDP, and sensitive cv. L were bacterized with $\sim 10^7$ of the *B. cepacia* strains per seed and sown in the pre-humidified soil. Root macerate dilutions from roots of three replicate plants were plated on PCAT medium, *B. cepacia* were not detected from roots of control plants. [d] Values, log10 CFU of *B. cepacia* g^{-1} dry wt. root + standard deviation, within columns for each soil type were not significantly different for any cultivar according to LSD test.

cally significant when seeds were treated with *B. cepacia* (Table 2). However, *B. cepacia* treatment reduced seed rot significantly in cv. LPDP in two out of the three experiments which had 25 to 50% seed rots in the control treatment (data not presented). In soils infested with *Fusarium* spp., seed treatment as compared to the untreated seeds, significantly reduced root necrosis in all the three cultivars tested, and mesocotyl necrosis in cv. LG11 and LPDP but not in cv. L (Table 2).

Effect of corn cultivars on root-colonizing ability of B. cepacia

The effect of corn cultivars on the root-colonizing ability of *B. cepacia* strain PHQM100 was studied in conjunction with bioassays in SCL soil (*Pythium* spp.) and SL soil (*Fusarium* spp.) by plating the serial dilutions of root macerates on PCAT medium at the end of each bioassay. In experiments with both the SCL and SL soil, *B. cepacia* colonized the three cultivars equally well (Table 3). However, the root colonization levels were ten fold higher in the SL soil incubated at 22-28 °C than in SCL soil incubated at a lower temperature (15-20 °C). *B. cepacia* was not isolated from roots of the untreated control plants.

Discussion

Some of the factors that affect disease severity in plants include environmental characteristics, the pathogens present and their populations, the quality of the seed, and the genetic resistance of the plant to the pathogen. Fungal pathogens associated with pre-emergence (seed rots) and post-emergence damping-off include various species of *Fusarium* and *Pythium* (Compendium of Corn Diseases, 1992). Common symptoms observed during postemergence damping-off, are rotting of mesocotyl and root tissue resulting in yellowing and wilting of the above ground parts.

In the present study, the primary *Pythium* spp. isolated from the rotted seed and necrosed roots from SCL soil from Alsace were identified as *Pythium arrheno-*

manes and *P. spinosum*. The former, *P. arrhenomanes* has been reported to be an economically important pathogen and widespread in corn growing areas of France (Rouhani, 1984). According to the above author, cultivars completely resistant to this pathogen are not available. Results obtained in the present study indicate that among the three cultivars tested, cv. LG is resistant to *Pythium* seed rot, while cv. LPDP and cv. L are susceptible. Of the three *Fusarium* species isolated from necrotic roots and mesocotyl tissues of corn grown in SL soil from Chappes, *Fusarium roseum* var. *culmorum* has been implicated in seedling blights of corn, especially in the North and North western regions of France (Rouhani, 1984). Our results suggest that it is also prevalent in the South western region.

In addition to the above enumeration of the pathogens, the symptoms observed in bioassays with the SCL soil (seed rot and root tissue necrosis) and the SL soil (root and mesocotyl tissue necrosis with subsquent damping-off) confirm the importance of *Pythium* and *Fusarium* spp. in the former and latter soil respectively. In bioassays with the SCL soil from Alsace, where *Pythium* seed rot was the main symptom, root necrosis of emerged plants did not result in seedling wilts even after 3 weeks of growth, and mesocotyl necrosis was not observed. This indicates that the window of opportunity for *Pythium* spp. to reduce plant growth is short. In contrast, in the SL soil from Chappes, mesocotyl and root necrosis caused by *Fusarium* spp. were the main symptoms observed. The plants which had severe root and mesocotyl necrosis had started to show symptoms of wilting after 3 weeks of growth. It has been reported that root rots due to *Fusarium* spp. apparently do not cause severe disease initially. However, they predispose seedlings and mature plants to post-emergence damping-off and late season stalk rots (Compendium of Corn Diseases, 1992). The distinct differences in symptom development observed in the two soils indicates that *Pythium* spp. were the predominant pathogens in the SCL soil from Alsace and *Fusarium* spp. in SL soil from Chappes. Although the involvement in the disease symptoms of *Pythium* spp. in the SCL soil and *Fusarium* spp. in SL soil cannot be completely ruled out, soil population estimates were below detectable levels ($<10^2$ CFU g^{-1} soil) and therefore not considered to be of major importance.

In the present study, the effect of cultivar on severity of both seed rots and symptoms leading to postemergence damping-off, was apparent in two different soil types. Cultivar LG11 was more resistant, cv. LPDP moderately resistant and cv. L was susceptible to damping-off. Results from the present study also indicated that the overall effect of seed treatment did not depend on the cultivar tested. The similarity in the root colonization levels of *B. cepacia* in three different corn cultivars indicates that differences observed in the effect of seed bacterization on cultivars was not due to their root colonization levels *per se*, but probably due to the resistance level of the cultivars to the pathogens. This is in contrast to earlier observations in cucumber (Meera et al., 1995) and potato (Azad et al., 1985), wherein cultivar-specificity were noticed in the root colonizing ability of rhizosphere bacteria.

An observation that seed treatment can reduce root necrosis in SL soil (incubated at 22-28 °C) but only seed rot and not root necrosis in SCL soil (15-20 °C) could be related to the ability of *B. cepacia* to a) colonize the roots better and produce more antifungal compounds at higher than at lower temperatures thereby reducing root necrosis, b) suppress *Pythium* seed rot but not root necrosis through a mechanism other than the production of antifungal compounds, such as competition for nutrients. Recent work by Mao et al. (1997) showed that suppression of damping-off in a field corn cultivar, in the presence of a pathogen complex (*F. graminearum*, *P. arrhenomanes* and *P. ultimum*) by seed treated *B. cepacia* was better when bioassays were performed at 25 °C than at 18 °C. The production of the antifungal compounds by a biocontrol strain of *B. cepacia* was shown to be better at higher (37 °C) than at lower temperatures (18 °C) (Upadhyay et al., 1991). Previous studies have shown that severity of *Pythium* damping-off in sweet corn (Callan et al., 1990) and its suppression in cotton by *Enterobacter cloacae* (Nelson and Craft, 1989) was related to the amount of seed exudation. Therefore, competition for nutrients rather than the production of antifungal compounds has been postulated as possible mode of action to suppress *Pythium* damping-off. However, this has not been verified in the present study using mutants which have lost the capacity to produce antifungal compounds.

Seed bacterization not only reduced necrosis of below-ground plant parts such as seed and root tissue, but also the sub-soil mesocotyl region. Seed-coated *B. cepacia* is already known for its ability to proliferate and colonize corn root and rhizosphere (Hebbar et al., 1992c), but how far it proliferates into the subterranean mesocotyl and shoot regions is yet to be established. The extensive use of root-associated or rhizobacteria for biological control of soilborne fungal diseases is often restricted by their ability to control only a par-

ticular plant disease in a specific soil type (Papavizas, 1985; Callan et al., 1990). We can conclude from this study that the overall impact of a one time seed bacterization on disease suppression, irrespective of soil pH or soil type, was positive and *B. cepacia* was able to suppress or reduce both pre- and postemergence damping-off in corn.

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