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Evaluation of plant growth-promoting rhizobacteria for control of Phytophthora blight on squash under greenhouse conditions

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ABSTRACT

Phytophthora blight caused by *Phytophthora capsici* is a serious threat to vegetable production worldwide. Currently, no single method provides adequate control of *P. capsici*. Greenhouse studies were conducted to evaluate the potential of the use of bacilli plant growth-promoting rhizobacteria (PGPR) for control of Phytophthora blight on squash. PGPR strains were applied as a soil drench 1 and 2 weeks after planting (WAP), and *P. capsici* was applied to squash roots at 3 WAP. PGPR strains SE34 and SE49 applied at 1×10^8 CFU/ml significantly ($P < 0.05$) reduced disease severity in all three repeated greenhouse trials compared to the nontreated control. Treatments with PGPR strains SE52, SE76, INR7, IN937a, and IN937b demonstrated significantly lower disease in two of three trials when compared with the nontreated control. Certain PGPR strains applied as 2-, 3-, and 4- strain mixtures significantly reduced disease severity. Treatment with T4 + SE56 demonstrated significantly lower levels of disease than any individual PGPR strain, indicating either an additive or synergistic effect on disease reduction achieved by mixing PGPR strains. Others such as INR7 + T4 + SE56 and INR7 + IN937a + T4 + SE56 have a high potential to significantly improve the control efficacy. When applied as seed treatments, only PGPR strain1PC-11 at 1×10^5 CFU/seed resulted in significant reduction in Phytophthora blight disease in all trials, while PGPR strains SE56 at 1×10^5 and 1×10^6 CFU/seed, GB03 at 1×10^5 CFU/seed, 1PC-11 at 1×10^6 CFU/seed, and 1PN-19 at 1×10^4 CFU/seed significantly suppressed the disease in two of three trials. These results indicate that certain PGPR strains are effective against *P. capsici* on squash, and improved disease control can be achieved by multiplexing them.

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1. Introduction

Phytophthora blight, caused by the oomycete *Phytophthora capsici* Leonian, is one of the most devastating diseases affecting cucurbit production in the US and worldwide (Babadoost, 2004; Hausbek and Lamour, 2004). *P. capsici* is a soilborne pathogen and survives as oospores for many years in the soil or as mycelia in plant debris. Zoospores of *P. capsici* can be readily dispersed across a field by rain and irrigation. *P. capsici* infects more than 50 species belonging to a wide range of plant taxa (Tian and Babadoost, 2004), including major vegetable crops and weeds. Recently, the incidence of Phytophthora blight has dramatically increased in many cucurbit growing areas, causing up to 100% yield loss (Babadoost, 2004; Hausbek and Lamour, 2004). For instance, Phytophthora blight outbreaks have jeopardized the processing pumpkin and other cucurbit industries in Illinois, where 90% of processing pumpkins produced in the US are grown (Tian and Babadoost, 2004). In Michigan, the increased occurrence of Phytophthora blight threatens the sustainability of the pickling cucumber industry (Hausbek and Lamour, 2004). In south Florida, *P. capsici* is of concern to producers causing foliar blight and fruit rot in summer squash (Roberts et al., 2001) and winter squash, and it over-summers in the weed *Portulacca* (Ploetz and Haynes, 2000).

Practices for management of soilborne pathogens in the field include cultural practices (field sanitation and control of alternate hosts), crop rotation, fungicide applications, and the use of resistant (or tolerant) varieties. At present, no single method provides adequate control of *P. capsici* (Babadoost, 2004; Hausbek and Lamour, 2004). Although commercial cucurbit varieties vary with respect to their Phytophthora blight resistance, highly resistant varieties with ideal horticultural traits are not yet available to producers (Olson et al., 2007). Crop rotation is an important component of integrated disease management; however, the long-term survival of *P. capsici* oospores even in the absence of a host limits the effectiveness of this strategy (Hausbek and Lamour, 2004). A limited number of fungicides have been registered for use on cucurbits, but no fungicides are highly effective against *P. capsici*

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(Hausbek and Lamour, 2004). In addition, *P. capsici* has developed resistance to metalaxyl, mefanoxam, and some other fungicides used for Phytophthora blight control (Hausbek and Lamour, 2004; Ploetz et al., 2002).

The fumigant methyl bromide has been used extensively to control soilborne pathogens for several decades. It is effective against the mycelia and the long-term persistent oospores of *P. capsici* in the soil. However, agricultural emissions of methyl bromide have been shown to be a significant source of ozone depletion (Spreen et al., 1995). Therefore, the phase-out of the use of methyl bromide has been ongoing under an international treaty of 1989, known as the Montreal Protocol. Consequently, many tomato and pepper growers are replacing the use of a mixture of methyl bromide and chloropicrin with a combination of a nematicide, 1,3-dichloropropene, and herbicides. However, 1,3-dichloropropene cannot be used in some areas of Florida with karst geography such as Miami-Dade County. Metam sodium and chloropicrin have been registered for control of *P. capsici* (Hausbek and Lamour, 2004), but are less reliable than methyl bromide and chloropicrin mixtures. Methyl iodide and chloropicrin mixtures are highly effective against *P. capsici* and have undergone extensive trials for protection of tomato (Rosskopf et al., 2005). Currently a 50:50 mixture of methyl iodide (MIDAS®) is available; however, the high cost of methyl iodide is likely to be prohibitive for use of this product in cucurbit production. In addition, the U.S. Environmental Protection Agency will require buffer zones around all fields treated with a soil fumigant (USEPA, 2009) beginning in 2010. This will remove the option of fumigating many small fields in peri-urban areas where much vegetable production is practiced most profitably. Spreen et al. (1995) estimated that the loss of methyl bromide would result in a $1 billion impact on the US winter vegetable industry. Clearly alternative practical strategies and technologies for control of *P. capsici* in vegetable production are urgently needed.

Plant growth-promoting rhizobacteria (PGPR) have been studied extensively for promoting plant growth and for inducing systemic resistance as well. PGPR-mediated induced systemic resistance (ISR) has been shown to effectively suppress several fungal, bacterial and viral pathogens in a number of crops both in greenhouse and field trials (Kloepper et al., 2004). Treatment with PGPR induces significant levels of resistance against oomycete pathogens including *Phytophthora*. Systemic protection of tomato against late blight, caused by *Phytophthora infestans* de Bary, was demonstrated with PGPR strain SE34 incorporated into the potting medium (Yan et al., 2002). The severity of blue mold of tobacco, caused by the oomycete *Peronospora tabacina* Adam, has been reduced by treatment with PGPR strains C-9 and SE34 and T4 (Zhang et al., 2002). Sporulation of this pathogen was also significantly decreased by treatment with all three of the bacterial strains in pot trials.

There are studies on bacterial suppression of *P. capsici* on pepper. However, little is known about bacilli PGPRs with the potential to be utilized to suppress Phytophthora blight on squash. Ahmed et al. (2003) isolated bacterial isolates from the aerial part and rhizosphere of sweet pepper and assayed *in vitro* against *P. capsici*. Four bacterial isolates including *B. subtilis* HS93 and *B. licheniformis* {(Weigmann, 1898) Chester 1901} LS234, LS523, and LS674 reduced *P. capsici* root rot on pepper by up to 80% relative to the control. Recently published research by Aravind et al. (2009) indicates that *B. megaterium* (de Bary, 1884) IISRBP 17, an endophytic bacterium isolated from black pepper stem and roots, was effective against *P. capsici* on black pepper in greenhouse assays. *B. subtilis* ME488 (Chung et al., 2008) and mixtures of two isolates of *Bacillus* (Jiang et al., 2006) suppressed *P. capsici* on pepper in greenhouse and field trials, respectively. The objective of this study was to evaluate the potential of bacilli PGPR for suppressing Phytophthora blight on squash (*Cucurbita pepo* L.) possibly through PGPR-mediated ISR, and to investigate if the joint use of two or more PGPR strains could improve the level of disease reduction.

2. Materials and methods

2.1. PGPR strains and inoculum preparation

Twelve bacilli PGPR strains were selected for inclusion in this research study based on results of previous experiments in which PGPR strains led to significant reductions in foliar or root diseases following their application as a seed treatment or soil drench to cucumber, cotton, pepper, peanut, tomato, and tobacco, etc. (Table 1). *Bacillus macauensis* (Zhang et al., 2006) 1PC-11 and *Bacillus subtilis* subsp. *subtilis* {(Ehrenberg 1835) Cohn 1872} 1PN-19 were originally isolated by Joseph W. Kloepper (personal communication) by pasteurizing dilutions of soybean seedling roots grown in field soil, and subsequently by selecting them for inhibition of *Pythium ultimum* (Trow, 1901). The PGPR strains tested in this study and relevant information on induced systemic resistance are listed in Table 1. The identity of all strains was determined using 16S rDNA sequencing with comparison to sequences of type strains. The original species name for each strain, based on fatty acid analysis, is listed in Table 1. All PGPR strains used in this study were stored in tryptic soy broth (TSB) amended with 15% glycerol at −80 °C prior to use.

For greenhouse assays in which PGPR were applied as a soil drench, bacterial cell suspensions were prepared first by streaking each PGPR strain taken from ultracold storage onto Luria–Bertani (LB) agar plates, then incubating the plates at 28 °C for 24 h to check for purity, and finally by transferring single colonies to fresh LB agar plates for 2 days. Bacteria were washed off the plates with 10–15 ml of sterilized distilled water. For use in our experiments, the bacterial suspensions were adjusted to 1×10^8 CFU/ml with sterilized distilled water. For seed treatment, spores of PGPR strains were prepared using a medium called Spore Preparation Medium (SPM; 3.3 g of peptone, 1.0 g of beef extract, 5.0 g of NaCl, 2.0 g of K_2HPO_4, 1.0 g of KCl, 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $MnSO_4$, 5.0 g of lactose, and 18 g of agar for 1 L). PGPR strains were streaked onto Luria–Bertani (LB) agar and incubated at 28 °C for 24 h to check for purity. Single colonies were transferred to SPM agar plates for 7–10 days to yield nearly 100% sporulation, and spores were washed off the plates with 10–15 ml of sterilized distilled water. Spore suspensions used in experiments were adjusted to appropriate concentrations in sterilized distilled water with the help of a hemacytometer and a compound microscope.

2.2. P. capsici isolates and inoculum preparation

Isolates of *P. capsici* were generously provided by Dr. Pamela D. Roberts, Southwest Florida Research and Education Center, Immokalee, Florida. To ensure successful infection, three isolates (#121, #146 and #151) were used in a ratio of 1:1:1 (i.e., a "cocktail") in all experiments in this study. The isolates were cultured separately and then combined to form a mixed population for inoculating squash plants that had been treated with one or more PGPR strains to evaluate the responses of the squash plants to *P. capsici* infection.

P. capsici inoculum for the greenhouse assays was prepared as described by Ploetz et al. (2002). Briefly, a 5-mm-diameter plug with mycelia of an isolate on cornmeal agar was transferred to a V8 agar plate. After one week of incubation at 25 °C, ten 5-mm-diameter V8 agar plugs with mycelia were each placed into a Petri dish containing V8 broth, and allowed to grow for another week at 28 °C. The V8 broth was then drained and each plate was washed twice with sterile distilled water. Sterile water was added to cover

Table 1
PGPR strains tested in this study against Phytophthora blight of squash.

PGPR	Identity[a]	Plant species	Target pathogen	References
SE 34	*Bacillus safensis*	Cucumber	*Colletotrichum orbiculare*	Jetiyanon (1997)
	(*B. pumilus*)	Tobacco	*Peronospora tabacina*	Zhang et al. (2002)
		Tomato	CMV	Zehnder et al. (2000a)
			ToMoV	Murphy et al. (2000)
			Phytophthora infestans	Yan et al. (2002)
SE49	*Bacillus safensis* (*B. pumilus*)	Cucumber	*Colletotrichum orbiculare*	Jetiyanon (1997)
SE52	*Bacillus safensis* (*B. pumilus*)	Cucumber	*Colletotrichum orbiculare*	Jetiyanon (1997)
SE56	*Lysinibacillus boronitolerans* (*Bacillus sphaericus*)	Loblolly pine	*Cronartium quercuum* f. sp. *fusiforme*	Enebak and Carey (2000)
SE76	*Bacillus safensis* (*B. pumilus*)	Cucumber	*Colletotrichum orbiculare*	Jetiyanon (1997)
IN937a	*Bacillus subtilis* subsp. *subtilis*	Cucumber	CMV	Jetiyanon et al. (2003)
	(*B. amyloliquefaciens*)			Jetiyanon et al. (2003)
		Pepper	*Colletotrichum gloeosporioides*	Zehnder et al. (2000a)
				Jetiyanon et al. (2003)
		Tomato	CMV	Jetiyanon et al. (2003)
			ToMoV	Murphy et al. (2000)
			Ralstonia solanacearum	Jetiyanon and Kloepper (2002)
			Sclerotium rolfsii	Jetiyanon et al. (2003)
IN937b	*Bacillus subtilis* subsp. *subtilis*	Tomato	CMV ToMoV	Zehnder et al. (2000a)
	(*B. amyloliquefaciens*)			Murphy et al. (2000)
INR7	*Bacillus pumilus*	Cucumber	*Erwinia tracheiphila*	Zehnder et al. (2001)
	(*B. pumilus*)		*Pseudomonas syringae* pv. *lachrymans*	Raupach and Kloepper (1998)
			Colletotrichum orbiculare	Wei et al. (1996)
T4	*Bacillus safensis*	Cucumber	*Erwinia tracheiphila*	Zehnder et al. (2000b)
	(*B. pumilus*)	Tobacco	*Peronospora tabacina*	Zhang et al. (2002)
			Pseudomonas syringae pv. *tabaci*	Park and Kloepper (2000)
GB03	*Bacillus subtilis* (*B. amyloliquefaciens*)	Cucumber	*Pseudomonas syringae* pv. lachrymans	Raupach and Kloepper (1998)
1PC-11	*Bacillus macauensis*	Soybean	*Pythium ultimum*	Kloepper (personal communication)
1PN-19	*Bacillus subtilis* subsp. *subtilis*	Cotton	*Rhizoctonia* sp.	Kloepper (personal communication)
		Peanut	*Cercosporidium personatum*	
		Soybean	*Pythium ultimum*	

[a] The ID is based on sequencing of 16S rDNA, and the original ID as reported in past publications based on fatty acid analysis is in parentheses.

the mycelia on each plate, and then the plates were placed under wide-spectrum light at room temperature for 24–48 h to induce sporangial development. The sporangia were chilled at 4 °C for 45 min to induce the release of zoospores. The inoculum from each plate was strained through four layers of cheesecloth. Counts of zoospores were obtained using a hemacytometer and a compound microscope.

2.3. In vitro test for antagonistic activity between PGPR strains and P. capsici

Tests for antibiosis between individual PGPR strains and *P. capsici* were conducted on PDA plates. A 6-mm-diameter plug of *P. capsici* mycelia taken from the edge of a colony on V8 agar was placed at the center of each PDA plate. PGPR bacterial cells were streaked in a straight line 3 cm from the *P. capsici* inoculation plug. The plates were incubated at 25 °C for 7 days, and then checked for inhibition zones and any changes in morphology of the *P. capsici* mycelia. Each *P. capsici* isolate (#121, #146 and #151) was individually tested as a target pathogen of the 12 selected PGPR strains. Four PGPR strains were tested on each plate, and the experiment was conducted twice.

2.4. Squash plants culture, inoculation with P. capsici, and disease rating

Seeds of squash (HMX 5703 F1 hybrid) were planted to a depth of approximately 1–2 cm in 10-cm-diameter plastic pots containing soilless pro-mix growing medium (Miracle-Gro Lawn Products, Inc., Marysville, OH) in a greenhouse, and watered daily. The squash plants were allowed to grow for 2–3 weeks after planting (WAP), and then they were inoculated by soil drench with *P. capsici* to create conditions for root infection. Five milliliters of the "cocktail" inoculum of *P. capsici* (2×10^4 zoospores/ml), which contains an equal number of zoospores of each of the three isolates, was applied by pipette to the soil around the stem of each plant. Inoculated plants were placed on greenhouse benches for 1–2 weeks before being rated for disease. Disease severity of Phytophthora blight was rated according to a rating scale of 0–5, where 0 = no visible symptom, 1 = small brownish lesion at the base of stem, 2 = stem lesions extend to cotyledons or the lesion has girdled the stem causing plant collapse, 3 = plant has collapsed with all leaves wilted or turned yellow except for the young leaves, 4 = plant has completely collapsed, and 5 = plant is dead.

2.5. Evaluation of PGPR strains applied as a soil drench against Phytophthora blight of squash

An experiment was conducted to test the efficacy of each PGPR strain to suppress Phytophthora blight of squash by applying the PGPR strain alone as a soil drench. Eight bacilli PGPR strains (SE34, SE49, SE52, SE76, T4, INR7, IN937a, and IN937b) were evaluated in the greenhouse at the concentrations of 1×10^8 CFU/ml, prepared as described above. Squash plants grown in soilless potting pro-mix were treated by pipetting twenty milliliters of the PGPR suspension into the soilless pro-mix at the base of the stem of each squash plant at 1 and 2 WAP. This was followed by inoculating the squash plants with *P. capsici* at 3 WAP. The squash plants treated with Actigard® 50WG, a commercial product of acibenzolar-*S*-methyl (ASM; Syngenta Crop Protection Inc., Greensboro, NC), served as a positive SAR control as previously reported (Zhang et al., 2009), and those which received only water were included as

a nontreated control. Treatments were arranged as a randomized complete block (RCB) with 10 replications each consisting of a single plant per treatment. The experiment was conducted three times.

2.6. Effect of mixing PGPR on Phytophthora blight on squash

In order to increase the reliability of control efficacy against Phytophthora blight on squash, mixtures of certain PGPR strains were evaluated by applying them as a soil drench in the greenhouse. Experiments were designed as randomized complete blocks comprising various PGPR treatments, Actigard® at 30 mg/L as a positive control, and a nontreated control. Two separate experiments were conducted in which the PGPR strains were tested individually or in combination of two or more strains. In the first experiment, treatments included (i) single PGPR strains (INR7, T4 and IN937a), (ii) these same strains in all possible combinations of 2- or 3-strain PGPR mixtures, (iii) Actigard®, and (iv) the nontreated control. This experiment was conducted four times. In another experiment, PGPR strains INR7, T4, SE56, and IN937a were tested individually or in all possible combinations except for those mixtures that had been tested in the first experiment. The experiment was performed three times.

2.7. Testing PGPR strains applied as a seed treatment for efficacy against Phytophthora blight of squash

To evaluate the potential of bacilli PGPR applied as a seed treatment for suppressing Phytophthora blight on squash, another experiment was simultaneously conducted in the greenhouse to test PGPR strains individually for control efficacy against Phytophthora blight of squash. The selection of the strains in this experiment was based on results from previous testing (data not shown). Bacterial spores were prepared on SPM agar as described above. The squash seeds (HMX 5703 F1 hybrid) were mixed with the bacterial suspension resulting in 1×10^4, 1×10^5, and 1×10^6 CFU per seed. The coated seeds were air-dried overnight in a laminar flow hood. Seeds treated with the PGPR were planted in transplant trays containing soilless pro-mix medium and watered daily. Squash plants were inoculated with *P. capsici* at 3 WAP. The treatments included three PGPR strains, Actigard® at 30 mg/L as a SAR control, and a nontreated control. The experiment was conducted three times.

2.8. Statistical analysis

Data of Phytophthora blight disease collected from greenhouse experiments were analyzed separately for each repeated experiment and were submitted to analysis of variance (ANOVA) or PROC GLM procedures using the Statistical Analysis System software (SAS Institute Inc., Cary, NC). One-way ANOVAs were performed for treatments including individual PGPR strain and strain combinations. The significance of treatment effects was determined by the magnitude of the F value ($P \leqslant 0.05$). When a significant F test for treatments was obtained, separation of disease severity means was accomplished using Fisher's protected least significant difference (LSD) at $P = 0.05$.

3. Results

3.1. In vitro testing for antagonism between PGPR strains and P. capsici

Most PGPR strains tested in this study exhibited no or weak antagonistic activity against the tested isolates of *P. capsici* on PDA plates (Table 2). The exceptions include T4 and 1PN-19 which produced an inhibition zone larger than 1 cm between the line of the bacteria and the mycelia of the *P. capsici* isolates #121 and #146. Also, strains SE49 and IN937a were strongly antagonistic to *P. capsici* isolate #146. PGPR strains INR7, SE56 SE76, and IN937b demonstrated no apparent antibiosis against any of the three isolates of *P. capsici*. 1PC-11 had no apparent effect against isolates #121 and #151, while it showed weak antibiosis against #146. The antagonistic activities of the tested PGPR strains varied with respect to the target isolates of *P. capsici*, but generally they were weaker against isolate #151 than against isolates #121 and #146.

3.2. Effects of individual PGPR strains applied as a soil drench to suppress Phytophthora blight on squash

The PGPR strains SE34 and SE49 applied as a soil drench significantly ($P < 0.05$) reduced disease severity of Phytophthora blight on squash compared to the nontreated control (Table 3) in all three replicated greenhouse assays. The treatments with PGPR strains SE52, SE76, INR7, and IN937 each had a significant effect on disease reduction, when compared with the nontreated control, in two of the three assays. T4 and IN937b provided significant protection against *P. capsici* only in one of the three assays. Actigard® applied as a soil drench at 30 mg/L consistently suppressed Phytophthora blight in all three assays, just as it had done in an earlier study (Zhang et al., 2009).

3.3. Effect of mixtures of PGPR strains applied as a soil drench against Phytophthora blight on squash

In the first experiment, PGPR strains INR7, IN937a, and T4 were evaluated either separately or together for the efficacy against *P. capsici* on squash under greenhouse conditions. The treatments with the single PGPR strain IN937a consistently resulted in significantly ($P < 0.05$) lower disease severity than the nontreated control in all four assays (Table 4). PGPR strains INR7 and T4 each applied individually as a soil drench significantly reduced the disease severity in three of four assays. Among the treatments with combinations of PGPR strains, the 2-strain (IN937a + T4) and 3-strain combinations (INR7 + IN937a + T4) significantly reduced disease in all assays. Squash plants treated with two 2-strain combi-

Table 2
In vitro antagonistic activity between individual PGPR strains and each of three *Phytophthora capsici* isolates[a].

PGPR strain	*P. capsici* isolate		
	#121	#146	#151
SE34	+[b]	+	–
SE49	+	++	–
SE52	+	+	–
SE56	–	–	–
SE76	–	–	–
INR7	–	–	–
T4	++	++	+
IN937a	+	++	+
IN937b	–	–	–
GB03	+	+	–
1PC-11	–	+	–
1PN-19	++	++	+

[a] A plug (6-mm in diameter) with *P. capsici* mycelia from the edge of a colony on V8 agar was placed at the center of PDA plates. Bacterial cells of PGPR were streaked in a straight line 3 cm from the inoculation site of *P. capsici*. Plates were incubated at 25 °C for 7 days when checked for inhibition zones and any morphological change of *P. capsici* mycelia.

[b] Reaction of *P. capsici* mycelia to PGPR on PDA plates. – indicates no inhibition between PGPR and *P. capsici*, + represents inhibition zone is less than 1 cm or the tip of mycelia altered, and ++ represents inhibition zone is greater than 1 cm.

Table 3
Effect of PGPR applied as a soil drench on Phytophthora blight of squash.

Treatment[a]	Disease severity[b]		
	Trial 1	Trial 2	Trial 3
SE 34	0.8 bcd[c]	0.8 b	1.5 d
SE49	0.7 cd	0.8 b	1.6 d
SE52	0.7 cd	1.4 a	2.5 bc
SE76	1.1 bc	1.6 a	2.0 cd
IN937a	1.0 bc	1.4 a	2.0 cd
IN937b	1.1 bc	1.5 a	2.9 ab
INR7	1.0 bc	1.8 a	2.6 bc
T4	1.5 ab	1.3 ab	2.5 abc
Actigard® 30 mg/L	0.2 d	0 c	0 d
Nontreated control	2.1 a	1.5 a	3.5 a

[a] PGPR strains were applied as a root drench into a soilless potting medium in which squash plants were grown. Actigard® 50WG was included as a positive SAR control.
[b] Squash plants were inoculated with *P. capsici* by applying 5 ml of inoculum (2×10^4 zoospores/ml) into soilless pro-mix per plant. Phytophthora blight was rated based on a scale of 0–5 as described in Section 2.
[c] Disease parameter represents mean value of disease rating from 10 replications per treatment and one plant per replication. Means in columns followed by different letters are significantly different at $P = 0.05$ according to the LSD test.

nations (INR7 + IN937a and INR7 + T4) demonstrated significantly lower levels of Phytophthora blight in three of the four assays when compared with the nontreated plants. Treatment with Actigard® at 30 mg/L consistently reduced the disease severity in all four assays.

In a second experiment, PGPR strains INR7, IN937a, T4, and SE56 were tested separately or in combination against *P. capsici*-inoculated squash in the greenhouse (Table 5). The combined treatments did not include any of those evaluated in the first experiment due to the large number of the treatments. In treatments with a single PGPR strain, IN937a significantly ($P < 0.05$) reduced the disease severity in two of three replicated assays, compared to the nontreated control (Table 5). The combined treatments IN937a + SE56, T4 + SE56, INR7 + T4 + SE56, IN937a + T4 + SE56, and INR7 + IN937a + T4 + SE56 each had a significant effect on disease reduction in all three assays. Importantly, treatments with T4 + SE56 demonstrated significantly greater disease suppression than did each individual PGPR strain, indicating either an additive or synergistic effect on disease reduction achieved by the PGPR mixtures. Other PGPR mixtures such as

Table 4
Effect of PGPR applied as a soil drench separately or in combination on Phytophthora blight of squash.

Treatment[a]	Disease severity[b]			
	Trial 1	Trial 2	Trial 3	Trial 4
INR7	3.2 a[c]	2.0 b	1.1 b	1.1 b
IN937a	1.9 b	1.9 b	1.0 bc	0.2 bc
T4	0.6 c	1.0 bc	1.6 ab	0 c
INR7 + IN937a	0.3 c	0.8 bc	1.4 ab	0.4 bc
INR7 + T4	0.5 c	0.1 c	1.5 ab	0.8 bc
IN937a + T4	0 c	1.5 bc	1.2b	0.6 bc
INR7 + IN937a + T4	0.2 c	0.4 bc	0.2cd	0.2 bc
Actigard® 30 mg/L	0 c	0.9 bc	0 d	0 c
Nontreated CK	3.4 a	3.7 a	2.1 a	2.5 a

[a] PGPR strains were applied as a root drench into a soilless potting medium in which squash plants were grown. Actigard® 50WG was included as a positive SAR control.
[b] Squash plants were inoculated with *P. capsici* by applying 5 ml of inoculum (2×10^4 zoospores/ml) into soilless pro-mix per plant. Phytophthora blight was rated based on a scale of 0–5 as described in Section 2.
[c] Disease parameter represents mean value of disease rating from 10 replications per treatment and one plant per replication. Means in columns followed by different letters are significantly different at $P = 0.05$ according to the LSD test.

Table 5
Effect of PGPR strains applied as a soil drench separately or in combinations against Phytophthora blight of squash.

Treatment[a]	Disease severity[b]		
	Trial 1	Trial 2	Trial 3
INR7	2.3 cd[c]	3.9 a	4.5 a
IN937a	3.4 ab	1.7 de	2.1 f
T4	3.9 a	2.9 bc	3.3 bcde
SE56	2.6 bcd	3.8 a	4.3 ab
INR7 + SE56	1.7 de	3.3 ab	3.7 abcd
IN937a + SE56	0.7 ef	2.3 cd	3.0 def
T4 + SE56	0.4 f	1.2 e	2.0 fg
INR7 + IN937a + SE56	0.7 ef	3.8 a	4.3 ab
INR7 + T4 + SE56	0.9 ef	2.2 cd	2.9 def
IN937a + T4 + SE56	0.3 f	2.0 de	2.9 def
INR7 + IN937a + T4 + SE56	0.1 f	1.5 de	2.3 ef
Actigard® 30 mg/L	0.1 f	0.3 f	1.0 g
Nontreated CK	3.2 abc	3.5 ab	4.0 abc

[a] PGPR strains were applied as a root drench into a soilless potting medium in which squash plants were grown. Actigard® 50WG was included as a positive SAR control.
[b] Squash plants were inoculated with *P. capsici* by applying 5 ml of inoculum (2×104 zoospores/ml) into soilless pro-mix per plant. Phytophthora blight was rated based on a scale of 0–5 as described in Section 2.
[c] Disease parameter represents mean value of disease rating from 10 replications per treatment and one plant per replication. Means in columns followed by different letters are significantly different at $P = 0.05$ according to the LSD test.

INR7 + T4 + SE56 and INR7 + IN937a + T4 + SE56 have a high potential to significantly improve the control efficacy, even though the effect of combined strains on disease severity was not significant in all repeated experiments compared to individual strain IN937a and T4.

3.4. Effect of PGPR applied as a seed treatment against Phytophthora blight of squash

The PGPR strains GB03, SE56, 1PC-11, and 1PN-19 each applied as a seed treatment (1×10^4, 1×10^5, and 1×10^6 CFU per seed) were tested for their potential to reduce Phytophthora blight in squash. In all three greenhouse assays, PGPR strain 1PC-11 at 1×10^5 CFU/seed significantly ($P < 0.05$) reduced the disease severity of Phytophthora blight compared to the nontreated control (Table 6). Seed treatment with GB03 at 1×10^5 CFU/seed, SE56 at 1×10^5 and 1×10^6 CFU/seed, 1PC-11 at 1×10^4 and 1×10^6 CFU/seed, and 1PN-19 at 1×10^4 CFU/seed demonstrated greater levels of disease reduction than the nontreated control in two of the three assays. Actigard® at 30 mg/L, applied as a seed treatment, significantly suppressed Phytophthora blight in only one out of three assays.

4. Discussion

Results from our study indicate that various individual PGPR strains applied as a soil drench or seed treatment each significantly reduced disease severity of Phytophthora blight on squash under greenhouse conditions, and that certain combinations of PGPR strains applied as a soil drench further increased the efficacy of disease control against *P. capsici*. ISR is likely to be, at least partly, a mechanism by which PGPR suppress the disease severity of Phytophthora blight on squash. Our studies add squash to the range of crops that can be protected with PGPR against *P. capsici*.

The reliability of efficacious disease suppression can be increased by the combined use of PGPR strains has been reported by Kloepper et al. (2004). Enhanced consistency of inducing systemic disease resistance by the use of mixtures of PGPR strains was achieved and reported by Raupach and Kloepper (1998).

Table 6
Evaluation of PGPR as a seed treatment at planting for suppressing Phytophthora blight of squash.

Treatment[a]	Root disease severity[b]		
	Trial 1	Trial 2	Trial 3
GB03 10^6	1.6 bcd[c]	2.9 ab	2.9 abcd
GB03 10^5	0.5 def	0.9 d	2.3 abcd
GB03 10^4	1.7 abc	2.2 bc	3.6 a
SE56 10^6	0.2 f	0.7 d	2.1 bcd
SE56 10^5	0.3 ef	2.3 bc	1.7 de
SE56 10^4	1.6 bcd	2.8 bc	2.4 abcd
1PN-19 10^6	1.6 bcd	3.0 ab	3.4 ab
1PN-19 10^5	2.8 a	4.0 a	3.1 abc
1PN-19 10^4	0.3 ef	0.8 d	1.9 cde
1PC-11 10^6	0.8 cdef	1.7 cd	3.4 ab
1PC-11 10^5	0 f	0.8 d	1.8 de
1PC-11 10^4	1.0 cdef	3.2 ab	0.6 e
Actigard® 30 mg/L	1.4 bcde	3.0 ab	0.7 e
Nontreated CK	2.2 ab	3.2 ab	3.1 abc

[a] PGPR in the form of bacterial suspensions were coated onto the squash seeds. Bacterial spores were prepared on SPM agar and the squash seeds were stirred in the bacterial suspensions resulting in 1×10^4, 1×10^5, and 1×10^6 CFU per seed, and air-dried overnight in a laminar flow hood prior to planting. Actigard® 50WG applied as a seed treatment was included as a control.
[b] Squash plants were inoculated with *P. capsici* by applying 5 ml of inoculum (2×10^4 zoospores/ml) into soilless pro-mix per plant. Phytophthora blight was rated based on a scale of 0–5 as described in Section 2.
[c] Disease parameter represents mean value of disease rating from 10 replications per treatment and one plant per replication. Means in columns followed by different letters are significantly different at $P = 0.05$ according to the LSD test.

Jetiyanon and Kloepper (2002) discovered that the use of mixtures of PGPR strains has a high potential for inducing systemic resistance against diseases of several different plant hosts in the greenhouse. Jetiyanon et al. (2003) confirmed this finding in the field and reported that some mixtures of two PGPR strains more consistently protected several different crop species against multiple diseases in field tests in Thailand than did a single strain. These experiments were conducted under the multi- or inter-cropping agricultural conditions prevalent in Thai agriculture. During both rainy and dry seasons, mixtures of *B. amyloliquefaciens* {(ex Fukumoto, 1943) Priest et al., 1987} IN937a and *B. subtilis* IN937b significantly protected against all the tested diseases including southern blight of tomato (caused by *Sclerotium rolfsii* Sacc.), mosaic viral disease of cucumber (caused by CMV), and anthracnose of long cayenne pepper (caused by *Colletotrichum gloeosporioides* Penz.). In our studies, we have demonstrated that PGPR strains used separately or in combinations have the potential to suppress Phytophthora blight on squash in the greenhouse. The mixture of T4 + SE56 significantly improved control efficacy compared to the individual strain (Table 5). Others such as INR7 + T4 + SE56 and INR7 + IN937a + T4 + SE56 tended to induce higher levels of disease reduction compared to individual PGPR strains.

Results from our study are in agreement with those of Duffy and Weller (1995) and Pierson and Weller (1994), who reported that certain mixtures of *Pseudomonas fluorescens* {(Trevisan) Migula} strains were significantly more effective against take-all disease of wheat than treatment with only one strain. A synergistic effect on the suppression of rice sheath blight was reported by Sung and Chung (1997), who used chitinase-producing *Streptomyces* spp. and *Bacillus cereus* (Frankland and Frankland, 1887) isolates in combination with antibiotic-producing *P. fluorescens* and *Burkholderia cepacia* {(Palleroni and Holmes, 1981) Yabuuchi et al., 1993} isolates. In our experiments with combinations of PGPR strains to suppress Phytophthora blight of squash, T4 + SE56 demonstrated either an additive or synergistic effect when compared with the effects of treatments with a single PGPR strain. The mechanism(s) involved in suppression of Phytophthora disease on squash may be different for each PGPR strain. The performance of mixing bacilli PGPR cannot be predicted just by the individual strains as shown in the results from present research. Certain mixtures of two or more strains of PGPR (INR7 + T4, IN937a + T4, and INR7 + IN937a + T4), whether or not individually showing antagonistic activities to *P. capsici*, demonstrated comparable levels of disease suppression (Table 4). More research will be required to elucidate the mode(s) of action by which PGPR strains elicit Phytophthora disease reduction on squash.

Extensive studies have been conducted to assess the potential of systemic acquired resistance (SAR) inducers for disease control. However, little is done against Phytophthora blight on squash. Recently, Koné et al. (2009) reported induced resistance in squash against *P. capsici* by treating plants with SAR inducers including acibenzolar-*S*-methyl (ASM), which is consistent with results from our studies (Zhang et al., 2009). Therefore, Actigard® (ASM) was included in all experiments as a positive check for testing PGPR against Phytophthora blight on squash since squash plants treated with Actigard® at 30 mg/L as a soil drench consistently had significantly lower disease than nontreated plants. In general, levels of disease reduction by Actigard® were superior to those by individual PGPR strains, indicating that alternative strategies are required to achieve improvement of control efficacy by combining PGPR strains or PGPR with SAR inducers.

Since induced systemic resistance is horizontal (Lyon and Newton, 1997), PGPR strains that induce systemic resistance may be more likely to achieve disease suppression against a wider range of pathogens than antagonists which suppress pathogens primarily by producing antibiotics (Kloepper et al., 1996). Jetiyanon et al. (2003) have shown the success of using combinations of PGPR strains for control of multiple diseases on different host plants. Based on our tests shown in Table 2, antibiosis is not the main mechanism whereby the PGPR strains used in this study suppress Phytophthora blight on squash. This warrants more experiments to determine whether these PGPR strains can suppress multiple diseases of squash and other vegetable crops.

Levels of disease biocontrol may vary with different parameters of the environment, and this to some extent explains why some biocontrol agents do not work under field conditions whereas they are effective in the greenhouse. Inconsistent performance of biocontrol agents against white mold of dry bean in field testing was believed to be caused by environmental differences (Huang et al., 2000). Biocontrol efficiency by nonpathogenic *Fusarium oxysporum* was significantly affected by both temperature and light (Larkin and Fravel, 2002). Mendoza Garcia et al. (2003) demonstrated that high organic matter in the soil favored root rot pathogen of cocoa more than the biocontrol agents and that biocontrol was most efficient at higher pH values. Therefore, it is important to determine to what extent environmental factors such as temperature, moisture, soil type, and other parameters affect biocontrol performance. Unlike biological control mediated through bacterial antagonism, disease suppression through PGPR-mediated induced systemic resistance can be sustained. Once activated, the natural resistance mechanisms of the host maintain an enhanced defensive capacity for prolonged periods and are effective against multiple pathogens. The effect of PGPR on Phytophthora blight of squash needs to be confirmed under field conditions.

In addition, the degree of disease biocontrol by a biocontrol agent depends on the population density of the agent and pathogen, the efficacy of individual units of the agent rendering the pathogen ineffective, and on the proportion of the pathogen population that is potentially affected by the agent (Johnson, 1994). Larkin and Fravel (1999) conducted an experiment in which three isolates of nonpathogenic *Fusarium* spp. (CS-1, CS-20 and Fo47), previously shown reduction in the incidence of Fusarium wilt diseases of multiple crops, were evaluated to determine

antagonist-pathogen inoculum density relationships. They concluded that variations in dose–response relationships were attributed to differences in their mechanisms of action with CS-20 and CS-1 functioning primarily by induced resistance, whereas Fo47 functioning primarily by competition for nutrients. In our studies, unconventional dose–responses were observed in the seed treatment experiment (Table 6), i.e. the strains tested were effective at the mid-dose in most cases. It is understandable that certain populations of the test strains have to meet for biocontrol efficacy. However, the reason that high doses did not significantly reduced disease severity remains unknown. Other mode(s) of action other than induced resistance may be also involved in the disease reduction by tested PGPR strains. Further research needs to be done on mechanisms by which PGPR strains reduce Phytophthora blight on squash in order to elucidate whether other mechanisms are also involved.

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References

Ahmed, A., Ezziyyani, M., Egea-Gilabert, C., Candela, M.E., 2003. Selecting bacterial strains for use in the biocontrol of diseases caused by *Phytophthora capsici* and *Alternaria alternata* in sweet pepper plants. Biologia Plantarum 47, 569–574.

Aravind, R., Kumar, A., Eapen, S.J., Ramana, K.V., 2009. Endophytic bacterial flora in root and stem tissues of black pepper (*Piper nigrum* L.) genotype: isolation, identification and evaluation against *Phytophthora capsici*. Letters in Applied Microbiology 48, 58–64.

Babadoost, M., 2004. Phytophthora blight: a serious threat to cucurbit industries. Available at: <http://www.apsnet.org/online/feature/cucurbit/links.asp>.

Chung, S., Kong, H., Buyer, J.S., Lakshman, D.K., Lydon, J., Kim, S.-D., Roberts, D., 2008. Isolation and partial characterization of *Bacillus subtilis* ME488 for suppression of soilborne pathogen of cucumber and pepper. Applied Microbial and Cell Physiology 80, 115–123.

Duffy, B.K., Weller, D.M., 1995. Use of *Gaeumannomyces graminis* var. *graminis* alone and in combination with fluorescent *Pseudomonas* spp. to suppress take-all of wheat. Plant Disease 79, 907–911.

Enebak, S.A., Carey, W.A., 2000. Evidence for induced systemic protection to fusiform rust in loblolly pine by plant growth-promoting rhizobacteria. Plant Disease 84, 306–308.

Hausbek, M.K., Lamour, K.H., 2004. *Phytophthora capsici* on vegetable crops: research progress and management challenges. Plant Disease 88, 1292–1303.

Huang, H.C., Bremer, E., Hynes, R.K., Erickson, R.S., 2000. Foliar application of fungal biocontrol agents for the control of white mold of dry bean caused by *Sclerotinia sclerotiorum*. Biological Control 18, 270–276.

Jetiyanon, K., 1997. Interaction between PGPR and cucumber during induced systemic resistance: recognition and early host defense responses. Ph.D. Dissertation, Auburn University, Auburn, AL, 134p.

Jetiyanon, K., Fowler, W.D., Kloepper, J.W., 2003. Broad-spectrum protection against several pathogens by PGPR mixtures under field conditions in Thailand. Plant Disease 87, 1390–1394.

Jetiyanon, K., Kloepper, J.W., 2002. Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. Biological Control 24, 285–291.

Jiang, Z., Guo, Y., Li, S., Qi, H., Guo, J., 2006. Evaluation of biocontrol efficiency of different *Bacillus* preparations and field application methods against Phytophthora blight of bell pepper. Biological Control 36, 216–223.

Johnson, K., 1994. Dose-response relationships and inundative biological control. Phytopathology 84, 780–784.

Kloepper, J.W., Ryu, C.-M., Zhang, S., 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology 94, 1259–1266.

Kloepper, J.W., Zehnder, G.W., Tuzun, S., Murphy, J.F., Wei, G., Yao, C., Raupach, G.S., 1996. Toward agricultural implementation of PGPR-mediated induced systemic resistance against crop pests. In: Wenhua, T., Cook, R.J., Rovira, A. (Eds.), Advances in Biological Control of Plant Diseases. China Agricultural University Press, Beijing, pp. 165–174.

Koné, D., Csinos, A.S., Jackson, K.L., Ji, P., 2009. Evaluation of systemic acquired inducers for control of *Phytophthora capsici* on squash. Crop Protection 28, 533–538.

Larkin, R.P., Fravel, D.R., 1999. Mechanisms of action and dose-response relationships governing biological control of Fusarium wilt of tomato by nonpathogenic *Fusarium* spp. Phytopathology 89, 1152–1161.

Larkin, R.P., Fravel, D.R., 2002. Effects of varying environmental conditions on biological control of *Fusarium* wilt of tomato by nonpathogenic *Fusarium* spp. Phytopathology 92, 1160–1166.

Lyon, G.D., Newton, A.C., 1997. Do resistance elicitors offer new opportunities in integrated disease control strategies? Plant Pathology 46, 636–641.

Mendoza Garcia, R.A., Martijn ten Hoopen, G., Kass, D.C.J., Sanchez Garita, V.A., Krauss, U., 2003. Evaluation of mycoparasites as biocontrol agents of *Rosellinia* root rot in cocoa. Biological Control 27, 210–227.

Murphy, J.F., Zehnder, G.W., Schuster, D.J., Sikora, E.J., Polston, J.E., Kloepper, J.W., 2000. Plant growth-promoting rhizobacterial mediated protection in tomato against tomato mottle virus. Plant Disease 84, 779–784.

Olson, S.M., Simonne, E.H., Stall, W.M., Roberts, P.D., Webb, S.E., Taylor, T.G., Smith, S.A., Freeman, J.H., 2007. Cucurbit production in Florida. Available at: <http://edis.ifas.ufl.edu/pdffiles/CV/CV12300.pdf>.

Park, K.S., Kloepper, J.W., 2000. Activation of PR-1a promoter by rhizobacteria that induce systemic resistance in tobacco against *Pseudomonas syringae* pv. *tabaci*.. Biological Control 18, 2–9.

Pierson, E.A., Weller, D.M., 1994. Use of mixtures of fluorescent pseudomonads to suppress take-all and improve the growth of wheat. Phytopathology 84, 940–947.

Ploetz, R.C., Haynes, J.L., 2000. How does *Phytophthora capsici* survive in squash fields during the off-season? Proceedings of the Florida State Horticultural Society 113, 211–215.

Ploetz, R.C., Heine, G., Haynes, J.L., Watson, M., 2002. Investigating biological attributes that may contribute to *Phytophthora capsici*'s importance as a vegetable pathogen in Florida. Annals of Applied Biology 140, 61–67.

Raupach, G.S., Kloepper, J.W., 1998. Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88, 1158–1164.

Roberts, P.D., McGovern, R.J., Kucharek, T.A., Mitchell, D.J., 2001. Vegetable Disease Caused by *Phytophthora capsici* in Florida. University of Florida, EDIS SP-159, pp. 1–4.

Rosskopf, E.N., Chellemi, D.O., Kokalis-Burelle, N., Church, G.T., 2005. Alternatives to Methyl Bromide: A Florida Perspective. Available at: <http://www.apsnet.org/online/feature/methylbromide>.

Spreen, T.H., VanSickle, A.E., Moseley, M.S., Deepak, M.S., Mathers, L., 1995. Use of Methyl Bromide and the Economic Impact of its Proposed Ban on the Florida Fresh Fruit and Vegetable Industry. University of Florida, Bulletin, p. 898.

Sung, K.C., Chung, Y.R., 1997. Enhanced suppression of rice sheath blight using combination of bacteria which produce chitinases or antibiotics. In: Ogoshi, A., Kobayashi, K., Homma, Y., Kodama, F., Kondo, N., Akino, S. (Eds.), Plant Growth-Promoting Rhizobacteria – Present Status and Future Prospects. Proc. Int. Workshop on Plant Growth-Promoting Rhizobacteria, 4th ed. Nakanishi Printing, Sapporo, Japan, pp. 370–372.

Tian, D., Babadoost, M., 2004. Host range of *Phytophthora capsici* from pumpkin and pathogenicity of isolates. Plant Disease 88, 485–489.

U.S. Environmental Protection Agency, 2009. Buffer zones fact sheet. Available at: <http://www.epa.gov/opp00001/reregistration/soil_fumigants/buffer-zones-fs.htm>.

Wei, G., Kloepper, J.W., Tuzun, S., 1996. Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. Phytopathology 86, 221–224.

Yan, Z., Reddy, M.S., Ryu, C.-M., McInroy, J.A., Wilson, M., Kloepper, J.W., 2002. Induced systemic resistance against tomato late blight elicited by plant growth-promoting rhizobacteria. Phytopathology 92, 1329–1333.

Zehnder, G., Murphy, J.F., Sikora, E.J., Kloepper, J.W., 2001. Application of rhizobacteria for induced resistance. European Journal of Plant Pathology 107, 39–50.

Zehnder, G.W., Yao, C., Murphy, J.F., Sikora, E.J., Kloepper, J.W., 2000a. Induction of resistance in tomato against cucumber mosaic cucumovirus by plant growth-promoting rhizobacteria. BioControl 45, 127–137.

Zehnder, G.W., Yao, C., Wei, G., Kloepper, J.W., 2000b. Influence of methyl bromide fumigation on microbe-induced resistance in cucumber. Biocontrol Science and Technology 10, 687–693.

Zhang, S., Reddy, M.S., Kloepper, J.W., 2002. Development of assays for assessing induced systemic resistance by plant growth-promoting rhizobacteria against blue mold of tobacco. Biological Control 23, 79–86.

Zhang, S., White, T.L., Martinez, M.C., Klassen, W., 2009. Application of acibenzolar-*S*-methyl and silicic acid for suppressing Phytophthora blight of squash under greenhouse conditions. Phytopathology 99, S149.