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Note

Root-Dipping Application of Antagonistic Rhizobacteria for the Control of Phytophthora Blight of Pepper Under Field Conditions

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This study was to examine the efficacy of a root-dipping application of antagonistic bacterial strains for the control of Phytophthora blight of pepper caused by *P. capcisi*, and to evaluate their plant growth-promoting effects in the field in 2005 and 2006. The candidate antagonistic rhizobacterial strains CCR04, CCR80, GSE09, ISE13, and ISE14 were treated by dipping plant roots with bacterial suspensions prior to transplanting. The candidate rhizobacterial strains CCR04, CCR80, GSE09, and ISE14 significantly ($P = 0.05$) reduced the disease incidence and the area under the disease progress curves when compared to buffer-treated controls in at least a year test. The metalaxyl (fungicide-treated control) resulted in one of the lowest disease incidences among the treatments in both years. Moreover, the strains CCR04, CCR80, GSE09, and ISE13 significantly ($P = 0.05$) increased the fruit weights and/or numbers of peppers in at least a year test compared to the buffer-treated controls. These results suggest that the antagonistic rhizobacterial strains CCR04, CCR80, and GSE09 could be efficient biocontrol agents by controlling Phytophthora blight of pepper and promoting the plant growth when treated with root-dipping at transplanting.

Keywords : antagonistic bacteria, biocontrol, pepper, *Phytophthora capsici*, plant growth-promoting rhizobacteria

Pepper (*Capsicum annum* L.) is one of the most important fresh market vegetables in Korea but its yield and quality have been frequently limited by the soilborne disease, Phytophthora blight caused by the oomycete pathogen *Phytophthora capsici* worldwide (Hausbeck and Lamour, 2004; Hwang and Kim, 1995). The control of this disease has depended on the application of agro-chemicals such as soil fumigants and fungicides (Hausbeck and Lamour, 2004; Hwang and Kim, 1995). However, the agro-chemical application often causes harmful effects such as environmental hazards and the development of fungicide-resistant strains (Cohen and Coffey, 1986; Lamour and Hausbeck, 2000). Also, consumers tend to prefer environmentally safe products despite the higher costs. Biological control with root-associated microorganisms has been an alternative measure to control soilborne plant diseases (Whipps, 2001). Such rhizobacteria have been used as either biocontrol agents or plant growth-promoting agents (Jetiyanon et al., 2003). However, these rhizobacteria often fail to control disease in field conditions and may not persist long enough to exhibit their disease suppression ability. The reasons for this phenomenon may be attributed to the inappropriate application in the field conditions (Gerhardson, 2002). In our previous studies (Kim et al., 2001; 2002), we developed a selection procedure with pepper plant tests through radicle and seedling assays for bacterial strains antagonistic to *P. capsici*. Using this procedure, we have selected several rhizobacterial strains effective against *P. capsici* (Sang et al., 2004). Hence, the objectives of this study were to examine the efficacy of root-dipping application of the potentially antagonistic bacterial strains against the Phytophthora blight of pepper caused by *P. capsici*, and to evaluate their plant growth-promoting effects in the field.

The potentially antagonistic bacterial strains used in this study included bacterial strains CCR04, CCR80, GSE09, ISE13, and ISE14 which were isolated from rhizosphere soils and roots of cucumber (Sang et al., 2004). For field test treatments, the bacterial strains were streaked onto nutrient agar (Difco, Detroit, USA) and incubated at 28°C for 48 hr. The single bacterial colonies were inoculated in test tubes containing 5 ml of nutrient broth (NB, Difco, Detroit, USA) and incubated in a shaking incubator (160 rpm) at 28°C for 24 hr. These pre-cultured bacterial strains were transferred into 500 ml of NB and once again incubated in a shaking incubator (160 rpm) at 28°C for 48 hr. The bacterial cells were harvested with 10 mM $MgSO_4$ buffer by centrifuging at 5,000 g and 20°C for 15 min. After centrifugation, the pellets (bacterial cells) were washed twice with the same buffer following by centrifugation. Bacterial suspensions were adjusted to 10^8 bacterial cells/ml (OD_{600}=0.5) in 10 mM $MgSO_4$ buffer. For the preparation of the pathogen inoculum, *P. capsici* S197 was grown on an oatmeal agar for 7 days at 28°C; following by incubation under continuous fluorescent light for an

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additional 4 days at 28°C for induction of zoosporangia. Zoospores were released from zoosporangia when the cultures were added to chilled sterile water and stored for 30 min at 4°C, following by 30 min at room temperature. Mycelia and sporangial debris was removed from the zoospores by filtration through three layers of cheesecloth. One ml of zoospores was vortexed for 30 sec to make them encyst and allow for counting with a haemocytometer. The final zoospore concentration was adjusted to 1.0×10^4 zoospores/ml.

The field tests with artificial inoculation were conducted at the Deokso Experimental Farm of Korea University, Namyangju, Korea in 2005 and 2006. Raised beds (20 cm high × 50 cm wide), spaced 85 cm apart (center to center), were mechanically constructed and covered with black plastic mulch. Pepper plants (cv. Nockwang) were grown for 9 weeks in 2005 and 8 weeks in 2006 in pots containing commercial potting mixture (Sunshine® Mix #5 Plug, Sun Gro Horticulture Canada Ltd., Seba Beach, Canada). These plants were transplanted into the beds in rows (30 cm between plants) on June 1, 2005 and May 10, 2006. Tests were established in beds with 3.5-m-long plots and arranged in a randomized complete block with three replications (10 plants/replication) per treatment. Plots were treated with one of five candidate bacterial strains (CCR04, CCR80, GSE09, ISE13, and ISE14), 10 mM $MgSO_4$ buffer (untreated control) or metalaxyl (a.i. 7.5%, Ridomil MG®, Dongbu Hannong Chemicals Ltd., Seoul, Korea; fungicide-treated control). Plant roots were dipped in 10 mM $MgSO_4$ buffer or bacterial suspensions prepared as described above prior to transplanting for 30 min. In addition, plants were also treated with 100 ml of metalaxyl (1 g per liter of water) according to the supplier's recommendations on August 6, 2005 and August 2, 2006 for each experimental year.

Five days after the metalaxyl treatment, 10 ml of 10^4 zoospores/ml of *P. capsici* prepared as described above, were added to the soil around each plant. We commenced evaluation of disease incidence (%) when symptoms first appeared on the inoculated plants. Subsequently, the disease incidence was evaluated every 3 days for 27-30 days after inoculation. Areas under the disease progress curves (AUDPC) based on disease incidence were also determined. Fifteen and 10 plants for 2005 and 2006 tests, respectively, regardless plots were randomly selected and evaluated fruit production by the various bacterial strains. Marketable pepper fruits (over 8 cm long) were harvested twice on August 6 and 13 for the 2005 test, and August 6 and 14 for the 2006 test, respectively. Fresh weights and numbers of fruits per pepper plant were determined in all treatments except the metalaxyl treatment. In case of metalaxyl, fruit harvests were not possible since no differences were found between treatment and harvest dates in both years. Statistical analysis of data was conducted using the Statistical Analysis Systems Institute Inc (SAS Institute, Cary, USA, 1990). Percent data of disease incidence were statistically analyzed after arcsine square-root transformation. The analysis of variance (ANOVA) was determined using the general linear model (GLM) procedures and the means were separated with the least significant difference (LSD). AUDPC was determined using the formula described by Shaner and Finney (1977), in which $AUDPC = \sum_{i=1}^{n} (X_{i+1} + X_i)(t_{i+1} - t_i)/2$, where X_i = disease severity or incidence at the ith observation, t_i = time (day) at the ith observation, and n = total number of observations.

This study describes the control efficacy of the potenti-

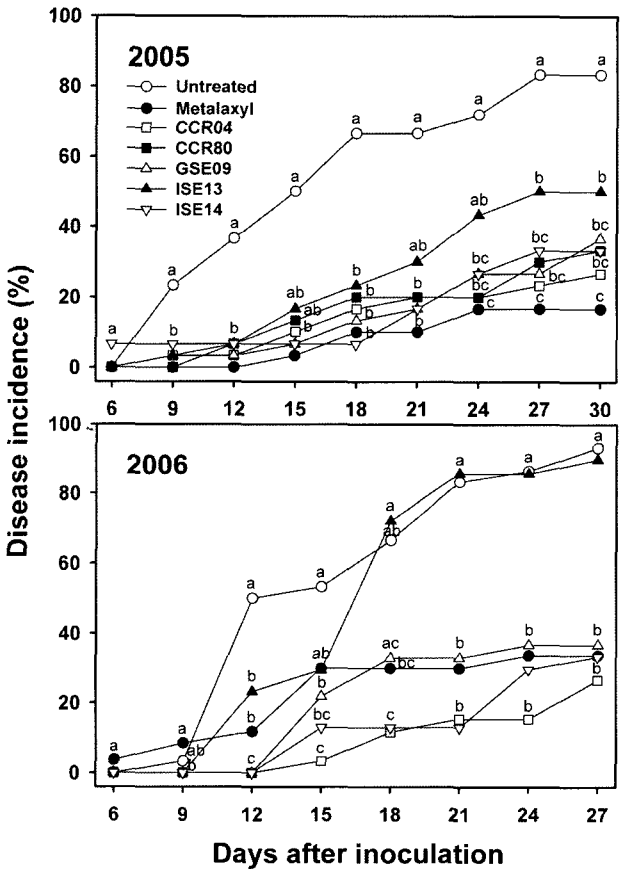

Fig. 1. Progress curves for incidence of Phytophthora blight of pepper plants (cv. Nockwang) treated with five potentially antagonistic bacterial strains (CCR04, CCR80, GSE09, ISE13, and ISE14), 10 mM $MgSO_4$ (untreated control), and metalaxyl (fungicide-treated control) in the field at the Deokso Experimental Farm of Korea University, Namyangju, Korea in 2005 and 2006. The bacterial strain CCR80 was not included in the 2006 test. These experiments were conducted with three replications (10 plants/replication) per treatment in both years. Means given by same letters are not significantly (P=0.05) different according to least significant difference (LSD) test. For disease incidence, arcsine square-root transformed data were used for statistical analysis; however, untransformed data are presented.

ally antagonistic bacterial strains CCR04, CCR80, GSE09, ISE13, and ISE14 against Phytophthora blight of pepper plants. Phytophthora blight of pepper plants occurred severely in the fields in 2005 and 2006 (Fig. 1). Final disease incidence ranged from 17-83% in 2005 and 27-93% in 2006, respectively. In the 2005 test, all the tested strains significantly (P=0.05) reduced disease incidence compared to a buffer-treated control (Fig. 1). In the 2006 test, three strains CCR04, GSE09, and ISE14 significantly (P=0.05) reduced disease incidence compared to the buffer-treated control 12-27 days after inoculation; however, the strain ISE13 failed to reduce the disease. In contrast, metalaxyl produced one of the lowest disease incidences among the treatments in both years (Fig. 1). Similar results were observed from AUDPC analysis, in which all tested bacterial strains significantly (P=0.05) reduced AUDPC in 2005, as do strains CCR04, GSE09, and ISE14 in 2006 compared with those of untreated buffer control (Table 1). Metalaxyl also significantly (P=0.05) reduced AUDPC in both year tests (Table 1). The bacterial strains, CCR04, CCR80, GSE09, and ISE13 significantly (P=0.05) increased both pepper fruit weights and/or numbers in at least one of years compared with the buffer-treated controls (Table 2).

Table 1. Areas under the disease progress curves (AUDPC) of Phytophthora blight of pepper plants (cv. Nockwang) treated with five potentially antagonistic bacterial strains (CCR04, CCR80, GSE09, ISE13, and ISE14), metalaxyl (fungicide-treated control), and 10 mM $MgSO_4$ buffer (untreated control) in the field at the Deokso Experimental Farm of Korea University, Namyangju, Korea in 2005 and 2006

Treatment	AUDPC[a]	
	2005	2006
Untreated[b]	1325 a[c]	1170 a
Metalaxyl[d]	195 b	465 b
CCR04	330 b	177 b
CCR80	380 b	ND[e]
GSE09	345 b	428 b
ISE13	595 b	1025 a
ISE14	370 b	256 b

[a] AUDPC was determined based on disease incidence (%) for 10 and nine observations over 30 and 27 days after inoculation in 2005 and 2006, respectively.

[b] Plants were root-dipped in 10 mM $MgSO_4$ buffer or bacterial suspensions just before transplanting on June 1, 2005 and May 10, 2006. These experiments were conducted with three replicates (10 plants/replication) per treatment in both years.

[c] Means designated with the same letters are not significantly (P=0.05) different according to least significant difference (LSD) test.

[d] Plants were treated with 100 ml of metalaxyl (1 g per liter of water) prepared at rates recommended by the supplier on August 6, 2005 and August 2, 2006.

[e] ND = not detected.

Table 2. Fresh weights and numbers of fruits of pepper plants (cv. Nockwang) treated with five potentially antagonistic bacterial strains (CCR04, CCR80, GSE09, ISE13, and ISE14) and 10 mM $MgSO_4$ buffer (untreated control) in the field at the Deokso Experimental Farm of Korea University, Namyangju, Korea in 2005 and 2006

Treatment	Fruit fresh weight (g)[a] /plant		Fruit number/plant	
	2005	2006	2005	2006
Untreated[b]	227 c[c]	73 c	20 b	8 c
CCR04	249 bc	192 a	23 ab	15 a
CCR80	280 ab	123 b	23 ab	12 ab
GSE09	298 a	71 c	26 a	7 c
ISE13	256 abc	134 b	25 b	11 b
ISE14	256 abc	104 bc	23 ab	10 bc

[a] Fresh weights (g) and numbers of fruits per pepper plant were determined with two harvests on August 6 and 13, 2005, and August 6 and 14, 2006. These experiments were conducted with 15 and 10 replicate plants in 2005 and 2006, respectively.

[b] Plants were root-dipped in 10 mM $MgSO_4$ buffer or bacterial suspensions just before transplanting on June 1, 2005 and May 10, 2006. In case of metalaxyl treatment, fruit harvests were not possible since no differences were found between treatment and harvest dates in both years.

[c] Means designated with the same letters are not significantly (P = 0.05) different according to least significant difference (LSD) test.

Researches have demonstrated that rhizobacteria have a relatively consistent ability to control plant pathogens and to promote plant growth as treated for transplant amendments and seed treatments (Çakmakçi et al., 2006; Jetiyanon et al., 2003; Kokalis-Burelle et al., 2006). The disease control efficacy developed from induced systemic resistance by activating defense-related enzymes against plant pathogens (Jetiyanon et al., 2003; Kokalis-Burelle et al., 2006). The colonization by antagonistic rhizobacteria on elongating roots to protect infection courts by plant pathogens could be another effective mechanism of the biological control (Bolwerk et al., 2003; Lucas García et al., 2003). However, the plant growth promotion may be influenced by bacteria dissolving phosphate, fixing nitrogen or releasing potassium (Çakmakçi et al., 2006; Sheng, 2005). These factors may explain the decreased Phytophthora blight and increased pepper yields in the fields, as a result of the colonization of antagonistic rhizobacteria by root-dipping prior to transplanting. The root-dipping application proved to be a simple and effective treatment for controlling Phytophthora blight of pepper. In conclusion, the antagonistic rhizobacterial strains, CCR04, CCR80, and GSE09 proved to be effective biocontrol agents in the control of Phytophthora blight of pepper and in the promotion of the plant growth when treated by root-dipping at transplanting.

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