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Note

# Biocontrol of Korean Ginseng Root Rot Caused by *Phytophthora cactorum* Using Antagonistic Bacterial Strains ISE13 and KJ1R5

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**In this biocontrol research, we evaluated disease suppressive effects of antagonistic bacterial strains ISE13 and KJ1R5 against Korean ginseng root rot caused by *P. cactorum*. We also examined the effects of nutrient solution in the hydroponic culture system for Korean ginseng on biological activity of the bacterial strains. As results of dual culture tests of the bacterial strains on $V_8$ juice agar, the strain ISE13 showed antifungal activity against *P. cactorum* and other plant pathogenic fungi, but the strain KJ1R5 did not. When their inhibitory effects against infection of *P. cactorum* on the roots grown in either nutrient solution or water were tested, the strains ISE13 and KJ1R5 inhibited the disease severity of Korean ginseng roots only grown with water, compared to buffer-treated, inoculated controls. However, the nutrient solution used for hydroponic cultures of ginseng in pots caused higher levels of disease severity by the strains ISE13 and KJ1R5 from 18.8% to 40.0% and from 24.3% to 45.0%, respectively. In this study, the bacterial strains ISE13 and KJ1R5 could be potentially biocontrol agents to suppress Korean ginseng root rot caused by *P. cactorum*. However, more attention using nutrient solution in hydroponic cultures for Korean ginseng production should be applied in biocontrol of plant diseases using the antagonistic microorganisms.**

***Keywords*** : antagonistic bacteria, biocontrol, Korean ginseng, *Phytophthora cactorum*, root rot

Biocontrol of plant diseases using microorganisms has been attempted as an alternative measure to agricultural chemicals due to high cost of their application, and potential hazards to the environments, toxicity to crops, and the development of fungicide-resistant strains of plant pathogens (Haas and Defago, 2005; Handelsman and Stabb, 1996). A number of antagonistic bacteria from rhizosphere soils and interior of plants have been potential as biocontrol agents and plant growth-promoting rhizobacteria. Especially these antagonistic bacteria were effective to reduce development of soilborne pathogens such as *Phytophthora* spp., *Fusarium* spp., and *Rhizoctonia* spp. (Knudsen et al., 1997).

Korean ginseng (*Panax ginseng* C.A. Meyer) has usually been cultivated for a long period from 3 to 5 years in a field that could generate side-effects of the production. To avoid the effects from continuous cultivation of Korean ginseng, nutrient cultures for its production has also been searched (Park et al., 2002). The continuous cultivation caused many problems including plant diseases caused by *P. cactorum*, *Cylindrocarpon destructans*, *F. solani*, and *R. solani* (Reeleder and Brammall, 1994; Punja, 1997). Among these pathogens, *P. cactorum* caused root rot of ginseng for loss of its yield and reduction of its quality (Bobev et al., 2003). In addition, since Korean ginseng was used as a medicinal plant that caused to difficult to apply for chemicals, alternatively biocontrol with antagonists would be desirable to reduce root rot of ginseng by *P. cactorum* (Jeffers et al., 2004). In this study, we evaluated disease suppressive effects of potentially antagonistic bacterial strains ISE13 and KJ1R5 to Korean ginseng root rot by *P. cactorum*. We also examined the effects of nutrient solution in hydroponic cultures for Korean ginseng in the greenhouse on biological activity of the bacterial strains against root infection of *P. cactorum*.

Antifungal activity of bacterial strains ISE13 and KJ1R5 selected from our preliminary experiments (Kim et al., 2003) was tested against ginseng root rot fungus *P. cactorum* and other plant pathogenic fungi including *Alternaria mali*, *F. oxysporum* f. sp. *lycopersici*, and *Magnaporthe grisea*. The bacterial strains ISE13, KJ1R5 or sterile water as a control were streaked in a line down the centers of $V_8$ juice agar and incubated for 24 hours. Then, the mycelial plugs (5 mm in diameter) from the actively growing 5-day-old cultures of the fungi were put on both sides of the $V_8$ juice agar. Inhibition of mycelial growth of the fungi by the strains ISE13 and KJ1R5 was measured when fungal mycelia in the water controls reached the center of the media. The experiments were conducted with six repli-

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cations in which one replication indicated the mean of the two measurements of each test plate.

Suppressive effects of the bacterial strains ISE13 and KJ1R5 against *P. cactorum* were tested on ginseng *in vitro* experiments. Roots (1 year old) of Korean ginseng (var. Jagyungjong) were transplanted and grown in pots containing with potting mixtures [Peat moss (Acadian Peat Moss Ltd., Lamègue, New Brunswick, Canada), perlite, and vermiculite, 5:3:2, v/v/v] in the greenhouse. One hundred ml of nutrient solution [$Ca(NO_3)_2 \cdot 4H_2O$ 1.0g, $KNO_3$ 0.9g, $KH_2PO_4$ 0.3g, $MgSO_4 \cdot 7H_2O$ 0.3g, FeEDTA 42.0 mg, $MnSO_4 \cdot 5H_2O$ 1.8 mg, $CuSO_4 \cdot 7H_2O$ 0.1 mg, $ZnSO_4 \cdot 7H_2O$ 1.0 mg, $H_3BO_3$ 1.6 mg, $Na_2MoO_4 \cdot 2H_2O$ 0.1 mg, 1L $H_2O$] or water were drenched into pots at every 4 days for about 60 days since initial leaves from the roots were developed about 30 days after transplanting. For *in vitro* inoculation tests, Korean ginseng roots from the pots were obtained and adhesive soils of roots were removed by shaking manually in sterile water. Thereafter the roots were surface-sterilized with 1% hypochlorite for 90 sec, rinsed several times with sterile water and blotted on sterile filter papers.

The bacterial strains ISE13 and KJ1R5 stored in nutrient broth (NB, Difco, Detroit, MI, U.S.A.) with 20% glycerol at –70°C were streaked onto nutrient agar (NA) and incubated at 28°C for 48 h. Single colony from NA was inoculated in 5 ml of NB and incubated in a shaking incubator (160 rpm) at 28°C for 24 h. These pre-cultured bacterial strains were transferred to 500 ml of NB and incubated again in a shaking incubator (160 rpm) at 28°C for 48 h. The bacterial cells were harvested with 10 mM $MgSO_4$ buffer through centrifugation at 5,000g at 20°C for 15 min to eliminate culture media. After centrifugation, the supernatants were discarded and pellets (bacterial cells) were washed twice with the buffer by centrifugation. Bacterial suspensions were adjusted to $10^8$ cells per ml. The roots prepared as above were dipped into these bacterial suspensions for 30 minutes and then dried on sterile filter papers. The 10 mM $MgSO_4$ buffer served as untreated controls.

For preparation of zoospore inoculum, *P. cactorum* was grown on $V_8$ juice agar for 7 days at 28°C and incubated under fluorescent light for 4 days at 28°C for inducing sporangia. The cultures were then added with chilled sterile water and stored for 30 min at 4°C, following by 30 min at the room temperature to release zoospores from sporangia. Fifty μl of *P. cactorum* zoospores ($4 \times 10^5$ zoospores/ml) were drop-inoculated on the prepared Korean ginseng roots in petri dishes containing moist filter papers. The inoculated roots in the petri dishes were again put in a container at 28°C. Water served as uninoculated controls. Percent disease severity of each inoculated ginseng root was visually evaluated 4 days after drop-inoculation. The experiments were conducted with eight replications.

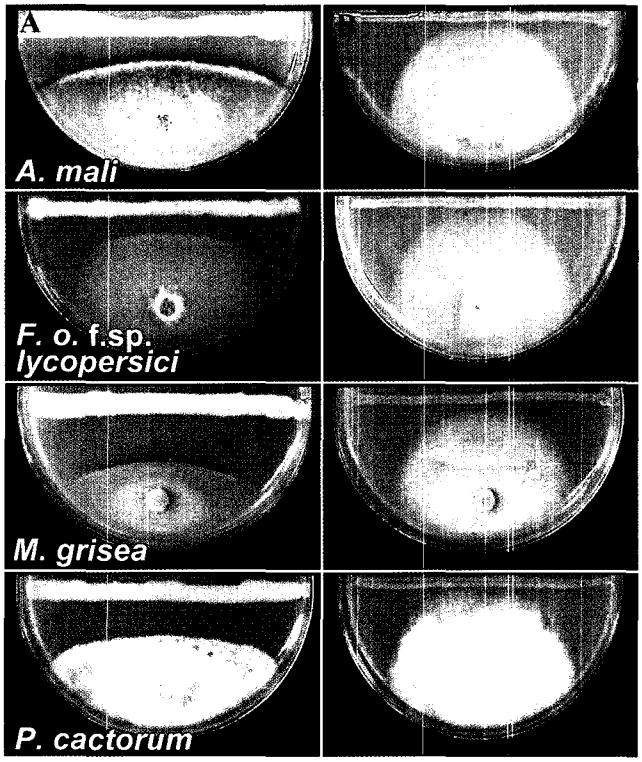


**Fig. 1.** Inhibition of mycelial growth of four plant pathogenic fungi, *Alternaria mali*, *Fusarium oxysporum* f.sp. *lycopersici*, *Magnaporthe grisea*, *Phytophthora cactorum* by antagonistic bacterial strains (A) ISE13 and (B) KJ1R5. Pictures were taken when fungal mycelia in water controls reached the centers of the $V_8$ juice agar plates.

**Table 1.** Antifungal activity of antagonistic bacterial strains ISE13 and KJ1R5 against plant pathogenic fungi on $V_8$ juice agar

| Bacterial strain | Inhibition zone (mm)[a] of mycelial growth | | | |
|---|---|---|---|---|
| | AM[b] | FOL | MG | PC |
| ISE13 | 9.0 ± 1.7[c] | 4.3 ± 0.6 | 12.7 ± 0.6 | 8.1 ± 0.2 |
| KJ1R5 | 1.7 ± 0.6 | 1.7 ± 1.0 | 1.3 ± 0.6 | 2.3 ± 0.3 |

[a]Inhibition of mycelial growth was determined when mycelia in sterile water controls reached the centers of the $V_8$ juice agar plates.
[b]AM, *Alternaria mali*; FOL, *Fusarium oxysporum* f.sp. *lycopersici*; MG, *Magnaporthe grisea*; PC, *Phytophthora cactorum*.
[c]Values are means and standard deviations of six replications.

As results of dual culture tests of bacterial strains, strain ISE13 had antifungal activity against *P. cactorum* and other plant pathogenic fungi on $V_8$ juice agar, but strain KJ1R5 had slight activity to the test fungi (Fig. 1 and Table 1). The bacterial strain ISE13 inhibited effectively mycelial growth of *A. mali*, *M. grisea*, and *P. cactorum* in the range of about 8-13 mm. However, strain KJ1R5 inhibited mycelial growth of the test fungi with only about 1-2 mm (Table 1). These results would suggest that strain ISE13, but not strain

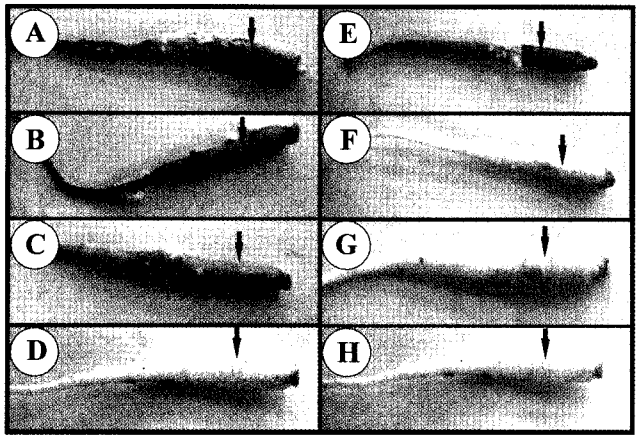


**Fig. 2.** Disease symptoms of Korean ginseng roots 4 days after drop-inoculation with zoospores of *Phytophthroa cactorum*. Sites of drop-inoculations on Korean ginseng roots were indicated by arrows. A and E; inoculated controls treated with $MgSO_4$ buffer solution, B and F; treated with the antagonistic bacterial strain ISE13, C and G; treated with the antagonistic bacterial strain KJ1R5, D and H; uninoculated controls treated with the buffer solution. Korean ginseng roots (A, B, C, and D) for inoculation experiments were initially grown in pots applied with nutrient solution or water (E, F, G, and H), respectively.

**Table 2.** Effects of antagonistic bacterial strains ISE13 and KJ1R5 on disease severity of Korean ginseng roots inoculated with *Phytophthora cactorum*

| Treatment | Disease severity (%)[a] | |
|---|---|---|
| | Nutrient solution[b] | Water |
| ISE13 | 40.0[c] ± 9.8 | 18.8 ± 4.4 |
| KJ1R5 | 45.0 ± 12.5 | 24.3 ± 10.0 |
| Inoculated control[d] | 46.3 ± 10.5 | 43.3 ± 10.2 |
| Uninoculated control | 0.0 ± 0.0 | 0.0 ± 0.0 |

[a] Disease severity (%) of each inoculated ginseng root was assessed 4 days after drop-inoculation with zoospores of *Phytophtora cactorum*.
[b] Korean ginseng roots for inoculation were initially grown in pots applied with nutrient solution or water, respectively.
[c] Values are means and standard deviations of eight replications.
[d] Korean ginseng roots inoculated with *P. cactorum* or water served as inoculated and uninoculated controls, respectively.

KJ1R5, produced antifungal compounds associated with disease suppression as observed in other researches (Chang et al., 2000; Rodriquez and Pfender, 1997).

When inhibitory effects of bacterial strains ISE13 and KJ1R5 against infection of *P. cactorum* were tested using Korean ginseng roots grown with either nutrient solution or water, inhibitory effects of the strains were observed only on Korean ginseng roots grown with water (Fig. 2 and Table 2). Both strains ISE13 and KJ1R5 significantly reduced disease severity of Korean ginseng root rot caused by *P. cactorum* compared to buffer-treated, inoculated controls. However, nutrient solution used for hydroponic cultures of ginseng in pots caused higher levels of disease severity by the antagonistic strains ISE13 and KJ1R5 from 18.8% to 40.0% and from 24.3% to 45.0%, respectively (Table 2). As observed in our study, Shaukat and Siddiqui (2003) also observed this phenomenon that mineral and carbon sources influenced the biocontrol performance of *Psudomonas aeruginosa* and *P. fluorescens* to *Macrophomina phaseolina*. Thus they suggested that components of nutrient solutions in hydropnic cultures could either increase or decrease the biocontrol efficacy of microbial antagonists.

In this study, the bacterial strains ISE13 and KJ1R5 could be potential biocontrol agents to suppress Korean ginseng root rot caused by *P. cactorum*. The suppression of Korean ginseng root rot by the strains ISE13 and KJ1R5 might be achieved through at least one of mechanisms among antibiosis, colonization, competition, and/or inducing host resistance (Haas and Defago, 2005; Handelsman and Stabb, 1996). In addition, more attention using nutrient solution in hydroponic cultures for Korean ginseng production should be applied in biocontrol of plant diseases.

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