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Bacillus spp. as Biocontrol Agents of Root Rot and Phytophthora Blight on Ginseng

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Ginseng (*Panax ginseng*) is one of the most widely cultivated medicinal herbs in Korea. However, yield losses reached up to 30-60% due to various diseases during 3 or 5 years of ginseng cultivation in the country. Therefore, successful production of ginseng roots depends primarily on the control of diseases. The objective of this study was to select potential biocontrol agents from rhizobacteria isolated from various plant internal root tissues for the control of multiple ginseng diseases as an alternative to fungicides. Among 106 *Bacillus* strains, two promising biocontrol agents, *Bacillus pumilus* strain B1141 and *Paenibacillus lentimobus* strain B1146, were selected by screening against root rot of ginseng caused by *Cylindrocarpon destructans* in a greenhouse. Pre-inoculation of selected isolates to seed or 1-year-old root of ginseng resulted in stimulation of shoot and/or root growth of seedlings, and successfully controlled root rot caused by *C. destructans* ($P<0.05$). Furthermore, drenching of cell suspension of the selected isolates on seedling-growing pots reduced the incidence of Phytophthora blight after the seedlings were challenged with zoospores of *Phytophthora cactorum* ($P<0.05$). *P. lentimorbus* strain B1146 showed antifungal activity against various soil-borne pathogens *in vitro*, while *B. pumilus* strain B1141 did not show any. Results of this study suggest that some rhizobacteria can induce resistance against various plant diseases on ginseng.

Keywords : Biological control, ginseng root rot, Phytophthora blight, rhizobacteria

In Korea, the production of harvestable ginseng (*Panax ginseng*, Araliaceae) roots requires a 3- to 5-year cultivation period after transplanting of 1-year-old roots. During this period, ginseng is susceptible to various diseases caused by soil- and air-borne pathogens which can reduce yield up to 30-60% (Ohh, 1986). Therefore, successful production of ginseng roots depends on the control of diseases. Commercial cultivation of ginseng mainly requires fungicide application during the cultivation period. However, growers and consumers are increasingly concerned about fungicide residues. To address this concern, new strategies for disease control are continually explored. Development of resistant breeds could be one of the best approaches. However, there are no known resistant types of Korean ginseng so far (personal communication, Dr. K.T. Choi). Another promising approach is biological control as an alternative to fungicides. Joy and Parke (1995) reported the biological control potential of *Burkholderia cepacia* strain AMMD against Alternaria leaf blight.

Root rot caused by *Cylindrocarpon destructans* is one of the most important diseases in both mature ginseng plants and seedlings. The pathogen is also known as a major causal agent of replant failure in Korea (Ohh et al., 1992). *Phytophthora cactorum* also causes root rot and leaf blight showing water-soaked and wilted symptoms on infected leaves (Ohh et al., 1992).

The objective of this study was to select potential biocontrol agents from rhizobacteria toward developing a biological method against multiple ginseng diseases.

Materials and Methods

Microorganisms. A total of 106 *Bacillus* isolates were isolated from various plant roots heated at 80°C for 30 min after being surface-sterilized with 75% ethanol and 1% sodium hypochlorite solution for 1 min. Each isolate was maintained on tryptic soy agar (TSA). All pathogens (*Rhizoctonia solani*, *C. destructans*, *Fusarium* sp., *Sclerotinia sclerotiorum*, and *P. cactorum*) except *Pythium ultimum* used in this experiment were isolated from naturally infected ginseng tissues and properly maintained on potato dextrose agar (PDA) or V8 juice agar. *P. ultimum* was obtained from the fungal and bacterial taxonomical laboratory of the Plant Pathology Division, National Institute of Agricultural Science and Technology (NIAST), Rural Development Administration (RDA), Korea.

Biocontrol screens on ginseng seeds. Field soil heavily infested with *C. destructans* was obtained from an experimental field of KT&G in Suwon, Korea. The soil was sieved through a 2-mm mesh and stored in 4°C before use. A plastic pot (20 × 30 × 20 cm, W × L × D) was filled with about 15-cm thick layer of steamed

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peat moss followed by 1-cm of field soil. Finally, the soil was covered with another layer of steamed peat moss adjusting the total layer of the preparation to 20-cm thick. Ginseng seeds (10 seeds per isolate) were soaked in each bacterial suspension (1×10^8 cfu/ml) prepared from 2-day-old culture on TSA. After 1 hour of soaking, the seeds were sown in pots. All pots were placed in plastic containers containing sterile water for watering. Pots were then removed from the plastic container and placed in a growth room (20-23°C) after saturating with water. The same method was used to water the pots once a week. The experiment was repeated at least twice. Seedling stands and root lengths for each treatment were evaluated 30 days after treatment.

Biocontrol of ginseng root rot. The field soil described above was evenly mixed with peat moss (1:3, w/w) and placed in round plastic pots (20 x 20 cm). One-year-old ginseng roots were socked in cell suspension (1×10^8 cfu/ml) of each selected isolate or water as control. After 1 hour of soaking, 10 roots were planted in a pot containing the pathogen mixture or peat moss. Treatments include the following: 1) negative control without bacteria and the pathogen; 2) positive control with the pathogen; 3) *B. pumilus* B1141 with the pathogen; and 4) *Paenibacillus lentimorbus* strain B1146 with the pathogen. All pots were placed in a growth room at 20-23°C and watered once a week for 30 days. Shoot stands and healthy roots were recorded at 30 days. There were three replicates per treatment in a completely randomized design. The experiment was repeated at least once.

Biocontrol of Phytophthora blight. Five ginseng seeds were sown in a round plastic pot (10 x 10 cm) containing peat moss and grown in a growth room at 20-23°C for over 2 months. Cell suspension (1×10^8 cfu/ml) of each selected isolate was prepared from 2-day-old TSA cultures. Five milliliter of each bacterial suspension or water as control was drenched on each ginseng seedling with 5 replicates per treatment before 5 days of challenge inoculation with *P. cactorum*.

To produce sporangia of *P. cactorum*, the pathogen was grown on V8 juice agar plates at 23°C. After 5 days of incubation, the plates were filled with sterile distilled water and incubated for another 2 days with light. Then, the plates were incubated at 4°C for 1 hour and returned at 25°C for another 1 hour to induce zoospores (Mitchell and Kannwischer-Mitchell, 1992). Zoospores were collected through four layers of cheesecloth and adjusted to a final concentration of approximately 3×10^4 zoospores/ml. Then, zoospore suspension was evenly sprayed on the seedlings. The seedlings were incubated in a humid chamber for 12 hours and placed in a growth room. Disease incidence was recorded at 10 days after challenge inoculation. All data of pot experiments were subjected to one-way analysis of variance (ANOVA) and means comparisons using Students t (P=0.05).

Antifungal activity of selected *Bacillus*. Antifungal activity of selected *Bacillus* strains was examined against ginseng plant pathogens, *C. destructans*, *Rhizoctonia solani*, *Sclerotinia* sp., *Botrytis cinerea*, *P. ultimum*, and *P. cactorum*, on half strength of potato dextrose agar. Each fungal pathogen was cultured on PDA for 5 days and 7-mm mycelial disk of each pathogen was placed on the center of half strength of PDA. Then, 100 µl of cell suspension (approximately 1×10^8 cfu/ml) of each *Bacillus* isolate was dropped on the medium 2-cm apart from the pathogen. All plates were incubated at 25°C and antifungal activity of each isolate was measured at 7 days after incubation.

Results and Discussion

A total of 106 *Bacillus* isolates were examined as potential biocontrol agents against ginseng diseases in a preliminary screening with ginseng seeds. In this experiment, all *Bacillus* spp. were isolated from surface-sterilized roots of various plants, indicating that most *Bacillus* isolates are possibly endophytic bacteria. Some endophytes involved in plant growth promotion and disease control are caused by soil- or air-borne pathogens (Kempe and Sequira, 1983; Mahaffee and Kloepper, 1994; Nejad and Johnson, 2000). Based on their performance, two isolates (B1141 and B1146) were selected for further experiments, identified as *B. pumilus* and *Paenibacillus lentimorbus*, respectively, by fatty acid analysis (data not shown, Sherlock microbial identification system, MIDI, Inc.).

Treatment of selected isolates to ginseng seeds showed significantly higher seedling stand than that of untreated control in the layer of infested soil (Fig. 1, P<0.05). Treatment of B1141 or B1146 resulted in significant root growth of seedlings compared with those of untreated controls in steamed peat moss or infested peat moss with *C. destructans* (Fig. 2, P<0.05). Furthermore, B1141 and B1146 stimulated the root growth of seedlings when compared with that of control in steamed peat moss (P<0.05). Compared with that of control in peat moss, the shoot development of 1-year-old ginseng was significantly inhibited in infested peat moss with *C. destructans* (Fig. 3,

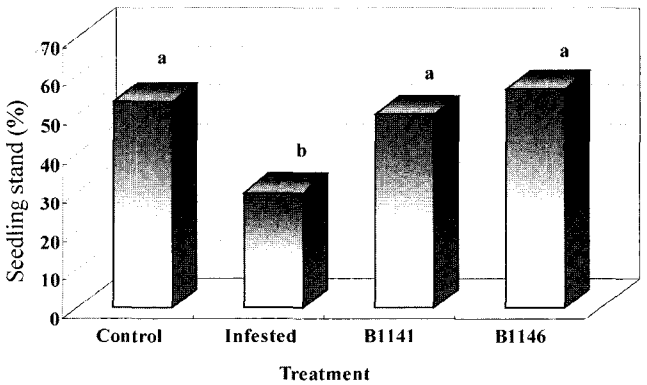

Fig. 1. Effect of selected *Bacillus* sp. B1141 and B1146 on seedling stand of ginseng in infested soil with *Cylindrocarpon destructans* when the isolates were applied to ginseng seeds. Control represents negative control without bacteria and the pathogen, and infested represents positive control with the pathogen. Shoot stands were recorded 30 days after treatment. Values with the same letters on the bar are not significantly different (P<0.05).

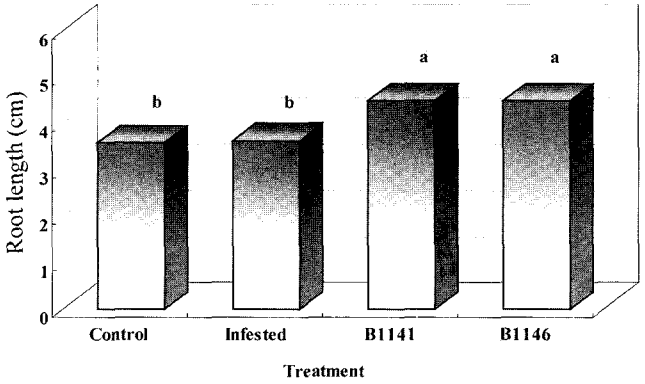

Fig. 2. Effect of selected *Bacillus* sp. B1141 and B1146 on the root growth of ginseng seedlings in the layer of infested soil with *Cylindrocarpon destructans*. Control represents negative control without bacteria and the pathogen, and infested represents positive control with the pathogen. Shoot stands were recorded 30 days after treatment. Values with the same letters on the bar are not significantly different ($P<0.05$).

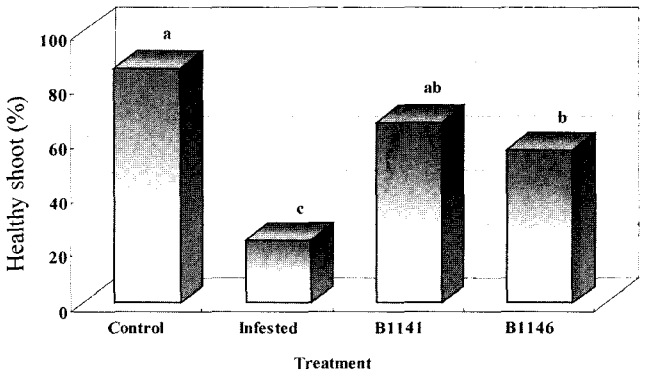

Fig. 3. Effect of selected *Bacillus* sp. B1141 and B1146 on the shoot development and stand of 1-year-old ginseng roots in peat moss infested with *Cylindrocarpon destructans*. Untreated or treated 1-year-old ginseng roots with *Bacillus* isolates were planted in peat moss with or without the pathogen. Values with the same letters on the bar are not significantly different ($P<0.05$).

$P<0.05$). Treatment of selected *Bacillus* isolates to 1-year-old ginseng roots resulted in significantly higher healthy shoot and root than those of untreated control in infested peat moss with *C. destructans* during the experimental period (Fig. 3, 4, 6A, $P<0.05$). *P. cactorum* infected stem, petiole, and leaf of the seedlings resulting to water-soaked and wilted symptoms when inoculated with zoospores by spraying (Fig. 6B). The infected seedlings were finally blighted within 5 days after inoculation. Seedlings having symptoms on any part were counted as infected in this experiment. Soil drenching of selected *Bacillus* isolates before challenging with *P. cactorum* significantly reduced disease incidence during the experimental period (Fig. 5 & 6B, $P<0.05$).

Selected *Bacillus* isolates were dual-cultured with various

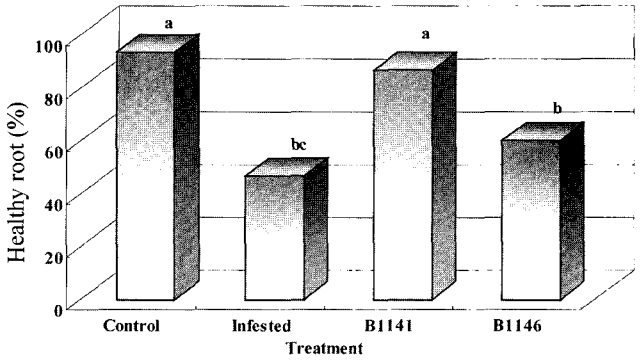

Fig. 4. Effect of selected *Bacillus* sp. B1141 and B1146 on the control of root rot caused by *Cylindrocarpon destructans*. Untreated or treated 1-year-old ginseng roots with *Bacillus* isolates were planted in peat moss with or without the pathogen. Data were collected 30 days after treatment. Values with the same letters on the bar are not significantly different ($P<0.05$).

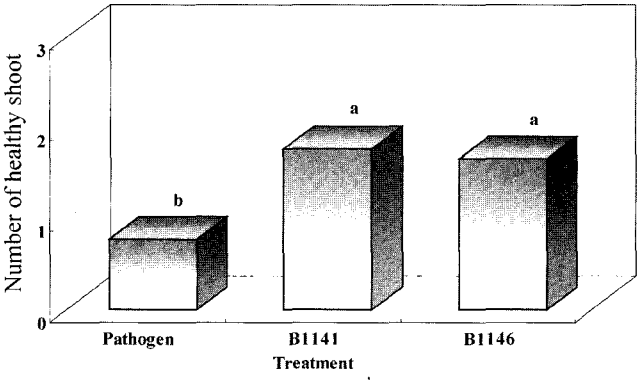

Fig. 5. Effect of selected *Bacillus* sp. B1141 and B1146 on Phytophthora blight on ginseng caused by *Phytophthora cactorum*. Number of healthy shoot was recorded 10 days after challenge inoculation by spraying zoospore suspension (3×10^4 zoospore/ml) on ginseng seedlings. Values with the same letters on the bar are not significantly different ($P<0.05$).

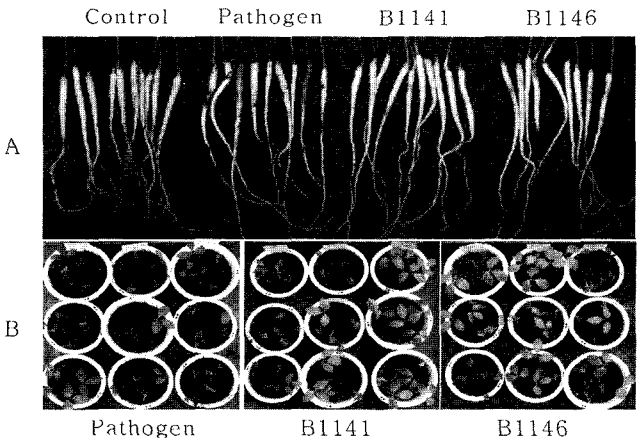

Fig. 6. Effect of selected *Bacillus* sp. B1141 and B1146 on the control of root rot and Phytophthora blight on ginseng. A) root rot caused by *Cylindrocarpon destructans*; B) Phytophthora blight caused by *Phytophthora cactorum*.

Table 1. Antifungal activity of selected *Bacillus* sp. B1141 and B1146 against various ginseng fungal pathogens

Pathogen	*Bacillus pumilus* B1141	*Paenibacillus lentimorbus* B1146
Rhizoctonia solani	–	+
Cylindrocarpon destructans	–	++
Fusarium sp.	–	++
Sclerotinia sclerotiorum	–	++
Pythium ultimum	–	–

*Antifungal activity was measured on half strength of potato dextrose agar for 7 days.
**– = no activity; + = 1-5 mm; ++ = > 6 mm of inhibition zone.

ginseng pathogens on half strength of PDA. *B. pumilus* strain B1141 did not show any inhibitory activity against the pathogens used. However, *P. lentimorbus* strain B1146 had strong antifungal activity against *C. destructans*, *Rhizoctonia solani*, *Sclerotinia* sp., *Botrytis cinerea*, and *P. cactorum*, except *P. ultimum* (Table 1).

The results indicate the potential of *Bacillus* strains as biological control agents against multiple ginseng diseases including soil- and air-borne pathogens. Similar results were reported for other pathosystems (Jetiyanon and Kloepper, 2002; Liu et al., 1995a, 1995b). In the experiments, specific mechanisms were not intended to identify which bacteria promoted ginseng seedling growth and which ones prevented ginseng diseases caused by *C. destructans* and *P. cactorum*. In general, *B. pumilus* strain B1141 might be involved in induced systemic resistance, but not antibiosis, on ginseng plant as a mechanism. Therefore, further studies are needed to test the physiological responses of ginseng plant induced to resistance by these bacteria, and to evaluate the ability of these bacteria to control ginseng diseases in the fields.

Acknowledgments

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