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Biocontrol Potential of *Streptomyces griseus* H7602 Against Root Rot Disease (*Phytophthora capsici*) in Pepper

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The root rot of pepper (*Capsicum annuum* L.) caused by *Phytophthora capsici* is one of the most important diseases affecting this crop worldwide. This work presents the evaluation of the capacity of *Streptomyces griseus* H7602 to protect pepper plants against *Phytophthora capsici* and establishes its role as a biocontrol agent. In this study, we isolated an actinomycete strain H7602 from rhizosphere soil, identified it as *Streptomyces griseus* by 16S rRNA analysis and demonstrated its antifungal activity against various plant pathogens including *P. capsici*. H7602 produced lytic enzymes such as chitinase, β -1,3-glucanase, lipase and protease. In addition, crude extract from H7602 also exhibited destructive activity toward *P. capsici* hyphae. In the pot trial, results showed the protective effect of H7602 against pepper from *P. capsici*. Application of H7602 culture suspension reduced 47.35% of root mortality and enhanced growth of pepper plants for 56.37% in fresh root and 17.56% g in fresh shoot as compared to control, resulting in greater protection to pepper plants against *P. capsici* infestation. Additionally, the enzymatic activities, chitinase and β -1,3-glucanase, were higher in rhizosphere soil and roots of pepper plants treated with H7602 than other treated plants. Therefore, our results indicated a clear potential of *S. griseus* H7602 to be used for biocontrol of root rot disease caused by *P. capsici* in pepper.

Keywords : antifungal activity, biocontrol, *Phytophthora capsici*, *Streptomyces griseus*

Based on increasing public concern about residues of fungicides in food products, as well as about their soil-

degrading effects, availability of a sustainable and environmentally friendly method for disease control in pepper is highly desirable. Technical, economical and environmental factors are forcing to adopt new sustainable methods, such as use of microbial antagonists for the control of soilborne pathogens. Recently, the interest in biological control by beneficial microorganisms has increased consistently as an alternative disease control to substitute for various chemical controls against airborne or soilborne plant pathogens (An et al., 2010; Mukherjee and Sen, 2006; Sang et al., 2011), and antagonistic microorganisms have been shown to inhibit the growth and proliferation of various phytopathogens with little or no side effects (Arthurs et al., 2009).

Phytophthora root rot (PRR), caused by *Phytophthora capsici*, is one of the most devastating soilborne diseases in the world. Due to a lack of resistant cultivars, control of soilborne pathogens of pepper is mainly aimed at *P. capsici* and involves numerous applications of fungicides both before and after transplanting in the field (Hwan and Kim, 1995). To combat wilt caused by *P. capsici* in pepper, cultural practices based on the biology and ecology of the pathogen have been suggested (Ristaino and Johnston, 1999). One of the strategies is use of biocontrol agents in order to reduce the number of applications of fungicides and the pathogen population resistant to the fungicides (Sang et al., 2008).

There have been considerable efforts to find biological control agents against PRR of pepper and several potential candidates have been reported including: *Penicillium striatisporum* (Ma et al., 2008), *Pseudomonas fluorescens* (Paul and Sarma, 2006), and *Streptomyces rochei* (Ezziyyani et al., 2007). The mechanisms of disease suppression by antagonistic bacteria have been extensively studied and they involved the production of antibiotics, extracellular enzymes, biosurfactant or cyanide production and/or the

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preoccupation of infection courts (Ma et al., 2008; Sang et al., 2008) and growth promotion in host (Compant et al., 2005). Management of the agricultural crops by inoculation with antagonistic microorganism has shown considerable promise for biocontrol of plant pathogens worldwide (Raupach and Kloepper, 1998).

Streptomyces griseus is a gram positive, aerobic, filamentous, soil dwelling actinomycete. The first person to isolate *S. griseus* was Krinsky in 1914 during the outbreak of World War I from Russian soil. It could produce many useful secondary metabolites such as enzyme inhibitors and contribute 70% of naturally occurring antibiotics (Waksman et al., 1948). The aim of this work was to isolate antagonistic microorganism against various fungal pathogens especially against *P. capsici*. Here, we reported the characterization of *S. griseus* H7602 against *P. capsici* *in vitro* and *in vivo*, and to demonstrate H7602 as a high potential biocontrol agent against PRR in pepper.

Materials and Methods

Isolation and identification of antagonistic microorganism.

Rhizosphere soils were collected from crop fields (rice, bean or vegetables) in Gunsan, Korea in March, 2011. Soil samples were serially diluted with sterile distilled water and inoculated on chitin agar plates containing [colloidal chitin 0.5%; Na₂HPO₄ 0.2%; KH₂PO₄ 0.1%; NaCl 0.5%; NH₄Cl 0.1%; MgSO₄·7H₂O 0.05%; CaCl₂·2H₂O 0.05%; yeast extract 0.05%; Agar 2%; and pH 7.0]. The plates were incubated at 30°C for 3 days, after that several colonies possessing strong chitin clearance zones were selected and sub-cultured on the same medium for more purification. All isolates were tested by a dual culture assay against *P. capsici* on chitin potato dextrose (CP) agar medium containing [colloidal chitin 0.25%; Na₂HPO₄ 0.1%; KH₂PO₄ 0.05%; NaCl 0.25%; NH₄Cl 0.05%; MgSO₄·7H₂O 0.025%; CaCl₂·2H₂O 0.025%; yeast extract 0.025%; Agar 2%; and potato dextrose broth 0.5%]. One isolate having the strongest activity was selected and stored in glycerol solution 25% at -70°C for further experiments. This isolate was later identified by 16S rRNA gene sequence analysis and matching sequences using BLAST search at gene bank data base of NCBI (Bethesda, MD).

Antagonism of H7602 to various plant pathogenic fungi.

H7602 was tested for antagonism against several fungal pathogens namely: *Phytophthora capsici* KACC 40483, *Fusarium oxysporum* f. sp. *lycopesci* KACC 40032 and *Rhizoctonia solani* AG-2-2 (IV) KACC 40132 purchased from KACC (Korea Agriculture Culture Collection, 225 Seodun-dong, Suwon, Gyeonggi Province, Korea). H7602 was inoculated on CP agar medium one day before

inoculation of the fungal pathogens. The antagonist and the test pathogen were placed 4 cm apart on the same CP plate, and all cultures were incubated at 26°C. All tests were carried out in five replications. Growth of fungal pathogens was evaluated at 7 days after inoculation by measuring colony radius from the original point of inoculation in the direction of the antagonist using the following formula:

$$[(R - r)/R \times 100]$$

where, r is the distance of fungal growth from the point of inoculation to the colony margin

R is the distance between the points of inoculation of fungal colony and bacterial colony.

Antifungal activity was defined in the terms of size of the inhibition zone and denoted as follows: – no inhibition zone; + (very weak), 0-5 mm; ++ (weak), > 5-10 mm; +++ (moderate), > 10-15 mm; ++++ (strong), > 15-20 mm; +++++ (very strong), > 20 mm as the distance between the fungal pathogen and the area of the antagonist growth after 7 days.

Lytic enzyme assay. To examine chitinase and β-1,3-glucanase activities, H7602 was cultured on medium containing [Na₂HPO₄ 0.2%; KH₂PO₄ 0.1%; NaCl 0.05%; NH₄Cl 0.1%; MgSO₄·7H₂O 0.05%; CaCl₂·2H₂O 0.05%; and yeast extract 0.01%] supplemented with 1.0% of *P. capsici* hyphae powder [made from the fungal mycelium grown in PDB at 30°C for 15 days, filtered through Whatman no. 1 filter paper, thoroughly washed with distilled water, autoclaved at 121°C for 15min, dried at 70°C for 3 days, and then ground using mixer (SMB-S20HSI, SPN Corporation, Korea)] at 30°C on a rotary shaker at 170 rpm for 7 days. The supernatant was daily collected and assayed by method of Tabatabai (1982) and Yedidia et al. (2000), separately. Also, protease and lipase activities were determined by using skim milk agar (MA) plate and Luria-Bertani (LB) plate supplemented with 1% Tween 80, respectively (Folman et al., 2003).

Effect of crude extract from H7602 on *P. capsici* hyphae morphology.

H7602 was cultured in gelatin chitin (GC) medium containing [gelatin 0.5 g; chitin powder 0.5 g; complex fertilizer 3 g (21-17-17; N 0.63 g; P₂O₅ 0.51 g; K₂O 0.51 g; Dongbu Hitek company, Korea); dry grass powder 6.0 g; rice bran 0.5 g; yeast extract 0.03 g; water 1 L; and pH 7.0] at 30°C on a rotary shaker at 170 rpm for 5 days. The culture broth (4 L) was centrifuged at 6,000 rpm and 4°C for 15 min (Supra 21 K; Hanil Science Industrial, Korea). The supernatant was acidified with concentrated HCl to pH 3.0 and extracted with an equal volume of n-Hexane (4 L, two times). The n-Hexane soluble organic fraction was concentrated by a rotary evaporator (Büchi,

Switzerland) to obtain crude extract. To examine the effect of this crude extract on hyphae of *P. capsici*, one ml of *P. capsici* culture grown on potato dextrose broth (PDB) medium at 30°C for 5 days was put in test tube, and the crude extract dissolved in methanol was added to the test tubes to a final concentration of 500 and 1,000 ppm, respectively. The same volume of methanol was used as control. The mixtures of crude extract and *P. capsici* were incubated at 30°C for 72 h and the mycelia were observed under the light microscope (Olympus BX41TF, Japan). All tests for observations of morphological mycelia were done in triplicate.

Preparation of H7602 culture and pathogen inoculum.

For the pot trial, H7602 was grown in GC media (as mentioned above) for 5 days at 30°C. Zoospores were prepared by growing *P. capsici* (KACC 40483) on the V8 juice agar medium containing [V8 juice 10%; CaCO₃ 0.1%; and Agar 2%] at 30°C. After incubation for 5 days, the fungal media were cut and moved to an empty plate (9 × 9 cm). All fungal media were flooded with sterile water and replaced daily with the same amount of sterile water. The continuous incubation was performed under fluorescent light for 5 days at 30°C to produce sporangia and then chilled at 4°C for 30 min to release zoospores. Zoospore suspension was filtrated by sterile cheesecloth and diluted with sterile water to a concentration of 1×10^6 zoospore ml⁻¹ (Kim et al., 1997).

Plant growth condition and sampling. Pepper seeds (*Capsicum annuum* L., Chungok) were sown in 3 × 3 cm plastic cell plug tray filled with commercial grade bedding soil. At 4 weeks after sowing, pepper seedlings were transplanted to plastic pots containing 600 g of non-sterilized soil mixture (soil:sand:vermiculite, 2:1:1, v:v:v). Pepper plants were grown at 24°C in an artificially illuminated room (12,000 lux at plant height) with a 16 h photoperiod. At 2, 3, 4 and 5 weeks after transplanting, each pot was amended with 50 ml of H7602 culture (GC+H7602), GC medium only (GC medium) or commercial fungicide 1% (Fungicide) named as Kkae Kkeu Tan with composition of carbendazim 25%, diethofencarb 25% and supplements 50% (Dong Bang Agro company, Korea) which has been popularly used in Korea. The same volume of tap water was used as (Control). Treatments were poured into five deep holes (1 × 4 cm) in pepper rhizosphere. At the time of 6 weeks after transplanting, 50 ml of zoospore suspension of *P. capsici* (1×10^6 zoospore ml⁻¹) were poured into five deep holes (1 × 4 cm) in the rhizosphere of peppers. Pots were randomly distributed with the position changed weekly to avoid any positional effects.

Plants and rhizosphere soils were harvested at 0, 3, 6, 9

and 12 days after infection with *P. capsici* zoospores. The samples of roots were washed under running tap water and airdried gently. Plants were divided into shoots and roots, and then weighted. Root samples ground under liquid nitrogen were extracted with 3 ml of 100 mM KH₂PO₄ buffer (pH 7.0). The extracted samples were centrifuged at 12,000 rpm for 10 min at 4°C and then the supernatant was kept at -20°C until enzyme analysis.

Root mortality assay. Root mortality was measured by modified method of Knievel (1973). Two hundred and fifty mg of fresh roots were incubated with 10 ml of 0.6% 2, 3, 5-triphenyltetrazolium chloride in 0.05 M sodium phosphate buffer (pH 7.4) for 24 h in the incubator at 30°C. Roots were rinsed twice with distilled water and then extracted twice with 95% ethanol at 70°C for 4 h. Combined extracts were adjusted to a final volume of 20 ml with 95% ethanol. Absorbance was measured using spectrophotometer at 490 nm. A standard curve was made using different proportions of living roots and killed roots to calculate root mortality. Root mortality was expressed as percentage dead root dry weight (D.W) of the total root D.W.

Enzyme activity assay in soil and root. Chitinase and β -1,3-glucanase activities in the rhizosphere soil samples and roots were determined using the modified method of Tabatabai (1982) and Yedidia et al. (2000), respectively.

Statistical analysis. The data were subjected to analysis of variance using SAS 9.1 software (SAS Institute, 2003). Mean values among treatments were compared by the least significant difference (LSD) test at 5% level ($p = 0.05$) of significance and presented as the mean values $\pm$ standard deviation (SD).

Results

Isolation and identification of antagonistic microorganism.

Thirty isolates showing clear zones on chitin agar medium were isolated from rhizosphere samples of Gunsan area in Korea. Dual culture assay was performed for the selection of antagonistic microorganism against various fungal pathogens on chitin potato dextrose (CP) agar plates. One isolate exhibited antifungal activities against *P. capsici*, *F. oxysporum* and *R. solani*. When this isolate and fungal pathogens were simultaneously inoculated for 7 days, the growth inhibitions were found to be 53.33% against *P. capsici*, 41.67% against *F. oxysporum* and 30.00% against *R. solani* (Table 1).

This isolate was identified as *Streptomyces griseus* based on 16S rRNA gene sequence analysis. The septate isolate

Table 1. Antifungal activity of *S. griseus* H7602 against various fungal pathogens

Fungi	Inhibition (%)	Antifungal activity
<i>Phytophthora capsici</i> KACC 40483	53.33 ± 1.44	+++++
<i>Fusarium oxysporum</i> f. sp <i>lycopesci</i> KACC 40032	41.67 ± 3.82	++++
<i>Rhizoctonia solani</i> AG-2-2 (IV) KACC 40132	30.00 ± 2.50	+++

Antifungal activity (inhibition zone): – no inhibition zone; + (very weak), 0-5 mm; ++ (weak), > 5-10 mm; +++ (moderate), > 10-15 mm; ++++ (strong), > 15-20 mm; +++++ (very strong), > 20 mm as the distance between the fungal pathogen and the area of antagonist growth after 7 days. Calculated mean values are from five replicates.

Fig. 1. Neighbor-Joining tree showing the position of *S. griseus* H7602 (bold, accession No. JN827310) compared to related organisms in a 16S rRNA gene tree. Numbers at nodes indicate levels of bootstrap support (%) based on 1000 resampled datasets, and only those branches with greater than 50% bootstrap support are labeled. The scale bar at the bottom indicates genetic distance units based on Nei's genetic distance.

sequence showed high identity when compared with other matching sequences (97-100%), and designated as *Streptomyces griseus* H7602 with GenBank accession No. JN827310 and BLAST analysis revealed strong homology with *Streptomyces griseus* EF687741.1 (Fig. 1).

Production of lytic enzymes. Production of lytic enzymes, such as chitinase and β -1,3-glucanase, was examined from the culture supernatant of H7602. Chitinase and β -1,3-glucanase activities rapidly increased in the time period of 4

Fig. 2. Changes in chitinase and β -1,3-glucanase activity of H7602 at 30°C for 7 days in the culture. Calculated mean values are from three replicates. Error bars represent standard error of the mean.

days, and eventually reached a maximum value of 4.12 unit ml^{-1} and 5.20 unit ml^{-1} , respectively. Thereafter, they gradually decreased from 4 to 7 days (Fig. 2). In addition, H7602 showed lipase and protease activity as evidenced by the formation of precipitation zones on LB agar supplemented with 1% of Tween 80 and a clear zone on MA medium, respectively (data were not shown).

Hyphae morphology of *P. capsici* affected by crude extract of H7602. To determine the effect of antifungal substance on fungal pathogen, *P. capsici* was grown on PDB medium with the presence of active crude extract.

Fig. 3. Light microscopy examination on effect of crude extract from H7602 on hyphal morphology of *P. capsici* grown at 30°C for 72 h. Treated with methanol as control (A); treated with 500 ppm of crude extract (B); treated with 1,000 ppm of crude extract (C). All bar = 100 μm .

Fig. 4. Changes in root mortality of pepper plants of treatments infected with *P. capsici*. Calculated mean values are from three replicates. Means with the same letter are not significantly different at $P = 0.05$ when compared by LSD.

After 72 h, *P. capsici* hyphae incubated with methanol showed normal morphology under the light microscope (Fig. 3A). However, Some *P. capsici* hyphae incubated with 500 ppm of crude extract revealed abnormal mycelia such as degradation, deformation, and lysis (Fig. 3B), and most of *P. capsici* hyphae were degraded in incubation with 1000 ppm (Fig. 3C).

Growth promotion and biocontrol effect of H7602. To examine the effect of H7602 on pepper growth and disease control, pepper rhizosphere was inoculated with H7602 culture (GC+H7602), GC medium only (GC medium), commercial fungicide 1% (Fungicide) or tap water (Control) and then infected with *P. capsici* zoospores. There were increases in root mortality percentages of all treatments during observation time, but they were different in each treatment. Pepper plants treated with tap water, GC medium and fungicide showed wilting and rotting of the roots at 6 days after *P. capsici* infection, and progressive development of disease led to a high root mortality percentage of 78.56, 70.62 and 53.99%, respectively, at 12 days after *P. capsici* infection. On the other hand, root mortality percentage of GC+H7602 treatment slowly increased, had the lowest value of 31.21% at 12 days after *P. capsici* infection (Fig. 4), and reduced 47.35% in comparison with control. There were not significant differences among treatments at 0 and 3 days, but they were found at 6, 9 and 12 days after *P. capsici* infection between treatments (GC+H7602 and fungicide) and others.

For measurement of plant growth, fresh root and shoot weights confirmed that pepper plants treated with H7602 were still increased from the *P. capsici* challenge. The fresh shoot/root weights of control, GC medium and fungicide treatments gradually increased from 0 to 6 days after *P. capsici* infection and then slightly decreased at 9 and 12

Table 2. Changes of fresh root and shoot weight as infected with *P. capsici*

Days after Challenge	Treatments	Fresh shoot weight (g)	Fresh root weight (g)
0	Control	28.39 ± 2.87 ^a	3.43 ± 0.40 ^a
	GC media	30.75 ± 1.73 ^a	4.21 ± 0.53 ^a
	Fungicide	28.37 ± 2.18 ^a	3.40 ± 0.20 ^a
	GC+H7602	31.55 ± 3.41 ^a	4.23 ± 0.94 ^a
3	Control	28.83 ± 2.15 ^b	3.93 ± 0.32 ^b
	GC media	32.85 ± 0.81 ^a	4.42 ± 0.39 ^{ab}
	Fungicide	29.42 ± 2.82 ^{ab}	3.97 ± 0.27 ^b
	GC+H7602	32.97 ± 1.70 ^a	4.92 ± 0.73 ^a
6	Control	34.13 ± 1.37 ^a	5.02 ± 0.18 ^b
	GC media	35.76 ± 0.87 ^a	5.63 ± 0.17 ^{ab}
	Fungicide	31.18 ± 2.69 ^a	5.66 ± 0.35 ^{ab}
	GC+H7602	35.02 ± 4.10 ^a	6.01 ± 0.55 ^a
9	Control	33.99 ± 1.81 ^{ab}	4.51 ± 0.41 ^c
	GC media	35.62 ± 0.93 ^a	5.23 ± 0.34 ^b
	Fungicide	31.25 ± 2.16 ^b	5.33 ± 0.39 ^b
	GC+H7602	37.72 ± 2.88 ^a	6.31 ± 0.19 ^a
12	Control	32.70 ± 0.86 ^{bc}	4.08 ± 0.53 ^c
	GC media	34.72 ± 0.80 ^b	4.69 ± 0.30 ^{bc}
	Fungicide	30.86 ± 0.96 ^c	5.23 ± 0.39 ^b
	GC+H7602	38.31 ± 2.99 ^a	6.38 ± 0.38 ^a
LSD (0.05)		3.16	0.77

Calculated mean values are from three replicates. ± SD (standard deviation). Means with the same letter are not significantly different at $P = 0.05$ when compared by LSD.

days due to reduced growth of pepper by the pathogen. However, the fresh root and shoot weights of H7602 treated pepper plants continuously increased during infection period (Table 2), and they had higher values of 56.37% and 17.56% than control at 12 days after *P. capsici* infection, respectively. Hence, pepper plants inoculated with H7602 showed better significant growth than others from *P. capsici* attack.

Enzyme activity in soil and root. Enzyme activities in rhizosphere of pepper plants were presented in Fig. 5A and 5B. During experiment period, chitinase activity in soil treated with H7602 was always higher than that of soil treated with others, and β -1,3-glucanase activity also presented a similar pattern with chitinase activity in all treatments. In pepper roots, chitinase activity gradually increased in H7602 treated pepper roots at 6, 9 and 12 days after *P. capsici* infection. In contrast, this activity gradually decreased at the same time in other treated pepper roots (Fig. 5C and

Fig. 5. Changes of chitinase activity in rhizosphere soils (A) and in roots (B), and β -1,3-glucanase in rhizosphere soils (C) and in roots (D) of peppers as infected with *P. capsici*. These changes were noted after treatments of Control (◆), GC medium (■), Fungicide (▲) and GC + H7602 (●). Calculated mean values are from three replicates. Error bars represent standard error of the mean.

5D). β -1,3-glucanase activity indicated little fluctuation in GC medium treatment during observation, while it gradually increased in pepper roots treated with H7602 and gradually decreased in others treated with tap water or fungicide.

Discussion

The need to produce innocuous food crops and reduce the pollution generated by synthetic chemicals has led to a search for biocontrol agents against plant pathogens which are safe for both the crops and human consumption. Several streptomyces species have been described as biocontrol agents effective against numerous plant pathogens, and have shown the potential to produce bioactive compounds as well as to reduce or inhibit mycelial growth of several fungi (Bressan and Figueiredo, 2008; Ezziyani et al., 2007; Mukherjee and Sen, 2006). In present study, H7602 strain was isolated from rhizosphere and demonstrated a broad spectrum of strong antifungal activity against various fungal pathogens including *P. capsici* (Table 1). It was identified as *S. griseus* (Accession No. JN827310) using 16S rRNA analysis (Fig. 1).

A primary mechanism of pathogen inhibition used by plant growth promoting rhizobacteria (PGPR) includes the

production of antibiotics, lytic enzymes, volatile compounds, and siderophores (Mao et al., 2006). Antibiotics and lytic enzymes in particular are well known antifungal compounds that can directly or indirectly protect plants from pathogenic attack. Previous results of Mukherjee and Sen (2006) indicated that lytic enzymes such as chitinase and β -1,3-glucanase were produced by strains of *Streptomyces* sp. and they would be used in controlling plant pathogens. Kim et al. (2003) also reported that the purified chitinase from *Streptomyces* sp. M-20 showed antifungal activity against *Botrytis cinerea*, and lysozyme activity against the cell wall of *B. cinerea*. In our study, lytic enzyme (chitinase and β -1,3-glucanase) activities had high values in mineral nutrient medium supplemented with mycelium powder of *P. capsici* (Fig. 2). Similarly, Trejo-Estrada et al. (1998) reported that chitinase and β -1,3-glucanase production from *S. violaceusniger* YCED-9 was induced by fungal cell wall from *Fusarium oxysporum*.

Particular *Streptomyces* species have exhibited potential in the biological control of plant fungal pathogens thorough production of antibiotics (Ezra et al., 2004). Lee et al. (2005) reported 4-phenyl-3-butenic acid produced by *Streptomyces koyangensis* sp. nov., which inhibited the mycelial growth of several plant pathogenic fungi. In

present study, crude extract of H7602 destroyed mycelia of *P. capsici* where abnormal structures of *P. capsici* hyphae were observed (Fig. 3). The inhibition that occurred here is considered to be antibiosis, as defined by Baker and Cook (1982). Furthermore, the capacity of the crude extract of H7602 to degrade mycelia of *P. capsici* makes it an excellent candidate for further study in order to identify metabolites responsible for the inhibition, and to develop their use for combating *P. capsici* in the field. Igarashi et al. (2000) also reported when treated with 1000 ppm of fistupyrone from *Streptomyces* sp. TP-A0569, spore germination rate and infection hyphae formation of *Alternaria brassicicola* were suppressed.

The antagonistic activity of biocontrol microorganisms is often demonstrated by the inhibition of mycelial growth or a reduction in symptoms of infected plants (Khan et al., 2005). In the pot trial, our results demonstrated that the strain H7602 had a high potential to suppress PRR and enhance growth of pepper plants. Normally, when zoospores of *P. capsici* were inoculated in rhizosphere of pepper plants, root rot disease rapidly developed and reached high disease index. This is the reason why H7602 was inoculated before *P. capsici* infection. Moreover, it is important to stress the survival percentage of pepper plants inoculated with *P. capsici* after the pre-inoculation with H7602. The inoculation of pepper plants with H7602 significantly reduced the root mortality percentage, and improved pepper growth from the pathogen attack (Fig. 4 and Table 2). In this case, pepper plants were protected from root rot symptoms by the colonization of H7602. Similar conclusion was also made by Shen et al. (2002), when roots of pepper plants were treated with a suspension of *Serratia plymuthica* A21-4 and then inoculated with zoospores suspension of *P. capsici*, the number of diseased plants and disease severity were significantly reduced. Also, El-abyad et al. (1993) reported that plant growth was improved by the seed coating treated with *Streptomyces* spp.

Among pathogenesis-related proteins (PRs), primarily chitinase and glucanase possess potential antifungal activities through degradation of fungal cell walls (Dumas-Gaudot, 1996). In addition, chitinase and β -1,3-glucanase are a structurally and functionally diverse group of hydrolytic enzymes involved in defense reactions of plants against pathogens (Jackson and Taylor, 1996). Our investigation in pot trial studies demonstrated the chitinase and β -1,3-glucanase activities in soils and roots due to H7602 application (Fig. 5). Promotion of these enzymes in pepper roots and soils as the result of H7602 colonization could play a role in bioprotection against *P. capsici*. This is consistent with previous findings by Jung et al. (2005) who reported that the activities of β -1,3-glucanase and chitinase in *Paenibacillus illinoisensis* and *P. capsici* treated roots

had increased by 54.8, and 52.8%, respectively, compared to *P. capsici* treated roots at seven days after inoculation. Yedidia et al. (2000) reported that cucumber roots treated with *T. harzianum* T-203 exhibited higher activities of chitinase (EC 3.2.1.14), β -1,3-glucanase (EC 3.2.1.6) up to 72 h post-inoculation compared to untreated control in cucumber. *Pseudomonas fluorescens* isolate Pf1 induced β -1,3-glucanase and chitinase in tomato roots inoculated with the pathogen (Ramamoorthy et al., 2002). Also, colonization of bean roots by rhizobacteria correlated with induction of PR proteins resulting in induced systemic resistance against *B. cinerea* (Zdor and Anderson, 1992). This clearly indicated that application of the antagonistic microorganisms induced PR proteins in roots of host plants.

Based on these results, we have found that, the antagonistic actinomycete, *S. griseus* H7602, showed strong antifungal activity against several pathogens consisting of *P. capsici*, and produced active substance as well as several lytic enzymes including chitinase and β -1,3-glucanase *in vitro*. In the pot trial, application of H7602 for pepper plants greatly suppressed PRR, enhanced pepper plant growth, and promoted enzyme activities in soil and roots *in vivo*. Thus, our results clearly demonstrated the potential of H7602 to be used for biocontrol of root rot disease caused by *P. capsici* in pepper. Further studies are required to confirm the efficacy of H7602 under field conditions, and more work is needed to purify and characterize the secondary metabolites produced by H7602 responsible for disease suppression.

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References

- An, Y., Kang, S., Kim, K. D., Hwang, B. K. and Jeun, Y. 2010. Enhanced defense responses of tomato plants against late blight pathogen *Phytophthora infestans* by pre-inoculation with rhizobacteria. *Crop Prot.* 29:1406–1412.
- Arthurs, S. C., McKenzie, L., Chen, J., Dogramaci, M., Brennan, M., Houben, K. and Osborne, L. 2009. Evaluation of *Neoseiulus cucumeris* and *Amblyseius swirskii* (Acari: Phytoseiidae) as biological control agents of chilli thrips, *Scirtothrips dorsalis* (Thysanoptera: Thripidae) on pepper. *Biol. Control.* 49:91–96.
- Baker, K. F. and Cook, R. J. 1982. Biological control of plant pathogens. *The American Phytopathological Society*, Saint Paul, Minnesota. 433.

- Bressan, W. and Figueiredo, J. E. F. 2008. Efficacy and dose-response relationship in biocontrol of Fusarium disease in maize by *Streptomyces* spp.. *Eur. J. Plant Pathol.* 120:311–316.
- Compant, S., Duffy, B., Nowak, J., Clement, C. and Barka, E. A. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Appl. Environ. Microbiol.* 71:4951–4959.
- Dumas-Gaudot, E. 1996. Plant hydrolytic enzymes (chitinases and β -1, 3-glucanases) in root reactions to pathogenic and symbiotic microorganisms. *Plant Soil.* 185:211–221.
- El-abyad, M. S., El-sayed, M. A., El-shanshoury, A. R. and El-sabbagh, S. M. 1993. Towards the biological control of fungal and bacterial diseases of tomato using antagonistic *Streptomyces* spp. *Plant Soil.* 149:185–195.
- Ezra, D., Hess, W. M. and Strobel, G. A. 2004. New endophytic isolates of *Muscodor albus*, a volatile-antibiotic-producing fungus. *Microbiology* 150:4023–4031.
- Ezziyiani, M., Requena, E., Egea-Gilabert, C. and Candela, M. E. 2007. Biological control of Phytophthora root rot of pepper using *Trichoderma harzianum* and *Streptomyces rochei* in combination. *J. Phytopathol.* 155:342–349.
- Folman, L. B., Postma, J. and van Veen, J. A. 2003. Characterization of *Lysobacter enzymogenes* (Christensen and Cook 1978) strain 3.IT8, a powerful antagonist of fungal diseases of cucumber. *Microbiol. Res.* 158:107–115.
- Hwan, B. K. and Kim, A. H. 1995. Phytophthora blight of pepper and its control in Korea. *Plant Disease* 79:221–227.
- Igarashi, Y., Ogawa, M., Sato, Y., Saito, N., Yoshida, R., Kunoh, H., Onaka, H. and Furumai, T. 2000. Fistupyrone, a novel inhibitor of the infection of Chinese cabbage by *Alternaria brassicicola*, from *Streptomyces* sp. TP-A0569. *J. Antibiot.* 53:1117–1122.
- Jackson, A. O. and Taylor, C. B. 1996. Plant-microbe interactions: life and death at the interface. *Plant Cell* 8:1651–1680.
- Jung, W. J., Jin, Y. L., Kim, K. Y., Park, R. D. and Kim, T. H. 2005. Changes in pathogenesis-related proteins in pepper plants with regard to biological control of Phytophthora blight with *Paenibacillus illinoisensis*. *BioControl.* 50:165–178.
- Khan, F. U., Nelson, B. D. and Helms, T. C. 2005. Greenhouse evaluation of binucleate Rhizoctonia for control of *R. solani* in soybean. *Plant Dis.* 89:373–379.
- Kim, K. D., Nemec, S. and Musson, G. 1997. Control of Phytophthora root and crown rot of bell pepper with composts and soil amendments in the greenhouse. *Appl. Soil Ecol.* 5:169–179.
- Kim, K. J., Yang, Y. J. and Kim, J. G. 2003. Purification and characterization of chitinase from *Streptomyces* sp. M-20. *J. Biochem. Mol. Biol.* 36:185–189.
- Knievel, D. P. 1973. Procedure for estimating ratio of live to dead root dry matter in root core samples. *Crop Sci.* 13:124–126.
- Lee, J. Y., Jung, H. W. and Hwang, B. K. 2005. *Streptomyces koyangensis* sp. nov., a novel actinomycete that produces 4-phenyl-3-butenic acid. *International J. Syst. Evol. Microbiol.* 55:257–262.
- Ma, Y., Chang, Z. Z., Zhao, J. T. and Zhou, M. G. 2008. Antifungal activity of *Penicillium striatisporum* Pst10 and its biocontrol effect on Phytophthora root rot of chilli pepper. *Biol. Control.* 44:24–31.
- Mao, S., Lee, S. J., Hwangbo, H., Kim, Y. W., Park, K. H., Cha, G. S., Park, R. D. and Kim, K. Y. 2006. Isolation and characterization of antifungal substances from *Burkholderia* sp. culture broth. *Curr. Microbiol.* 53:358–364.
- Mukherjee, G. and Sen, S. K. 2006. Purification, characterization, and antifungal activity of chitinase from *Streptomyces venezuelae* P10. *Curr. Microbiol.* 53:265–269.
- Paul, D. and Sarma, Y. 2006. Antagonistic effects of metabolites of *Pseudomonas fluorescens* strains on the different growth phases of *Phytophthora capsici*, foot rot pathogen of black pepper (*Piper nigrum* L.). *Arch. Phytopathol. Plant Protect.* 39:311–314.
- Ramamoorthy, V., Raguchander, T. and Samiyappan, R. 2002. Induction of defense-related proteins in tomato roots treated with *Pseudomonas fluorescens* Pfl and *Fusarium oxysporum* f. sp. *lycopersici*. *Plant Soil.* 239:55–68.
- Raupach, G. S. and Kloepper, J. W. 1998. Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. *Phytopathology* 88:1158–1164.
- Ristaino, J. B. and Johnston, S. A. 1999. Ecologically based approaches to management of Phytophthora blight on bell pepper. *Plant Dis.* 83:1080–1089.
- Sang, M. K., Chun, S. C. and Kim, K. D. 2008. Biological control of Phytophthora blight of pepper by antagonistic rhizobacteria selected from a sequential screening procedure. *Biol. Control.* 46:424–433.
- Sang, M. K., Kim, J. D., Kim, B. S. and Kim, K. D. 2011. Root treatment with rhizobacteria antagonistic to Phytophthora blight affects anthracnose occurrence, ripening, and yield of pepper fruit in the plastic house and field. *Phytopathology* 101:666–678.
- Shen, S. S., Choi, O. H., Lee, S. M. and Park, C. S. 2002. *In vitro* and *in vivo* activities of a biocontrol agent, *Serratia plymuthica* A21–4, against *Phytophthora capsici*. *Korean J. Plant Pathol.* 18:221–224.
- Tabatabai, M. A. 1982. Soil enzymes. In: Page AL, Miller RH, Keeney DR (eds) *Methods of soil analyses, Part 2, chemical and microbiological properties. American Society of Agronomy*, Madison, WI, USA, 2nd edn:903–947.
- Trejo-Estrada, S. R., Sepulveda, I. R. and Crawford, D. L. 1998. *In vitro* and *in vivo* antagonism of *Streptomyces violaceusniger* YCED9 against fungal pathogens of turf grass. *World J. Microbiol. Biotechnol.* 14:865–872.
- Waksman, S. A., Reilly, H. C. and Harris Dale, A. 1948. *Streptomyces griseus* (Krinsky) Waksman and Henrici. *J. Bacteriol.* 56:259–269.
- Yedidia, I., Benhamou, N., Kapulnik, Y. and Chet, I. 2000. Induction and accumulation of PR proteins activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T-203. *Plant Physiol. Biochem.* 38:863–873.
- Zdor, R. E. and Anderson, A. J. 1992. Influence of root colonizing bacteria on the defense responses in bean. *Plant Soil.* 140:99–107.