Induction of Defense Response Against *Rhizoctonia solani* in Cucumber Plants by Endophytic Bacterium *Bacillus thuringiensis* GS1

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An endophytic bacterium, *Bacillus thuringiensis* GS1, was isolated from bracken (*Pteridium aquilinum*) and found to have maximal production of chitinase (4.3 units/ml) at 5 days after culture. This study investigated the ability of *B. thuringiensis* GS1 to induce resistance to *Rhizoctonia solani* KACC 40111 (RS) in cucumber plants. Chitinase activity was greatest in RS-treated plants at 4 days. β-1,3-Glucanase activity was highest in GS1-treated plants at 5 days. Guaiacol peroxidase (GPOD) activity increased continuously in all treated plants for 5 days. Ascorbate peroxidase (APX) activity in RS-treated plants was increased 1.5-fold compared with the control at 4 days. Polyphenol oxidase (PPO) activity in RS-treated plants was increased 1.5-fold compared with the control at 3 days. At 5 days after treatment, activity staining revealed three bands with chitinase activity (Ch1, Ch2, and Ch3) on SDS-PAGE of cucumber plants treated with GS1+RS, whereas only one band was observed for RS-treated plants (Ch2). One GPOD isozyme (Gp1) was also observed in response to treatment with RS and GS1+RS at 4 days. One APX band (Ap2) was present on the native-PAGE gel of the control, and GS1- and GS1+RS-treated plants at 1 day. PPO bands (P01 and P02) from RS- and GS1+RS-treated plants were stronger than in the control and GS1-treated plants upon native-PAGE at 5 days. Taken together, these results indicate that the induction of PR proteins and defense-related enzymes by *B. thuringiensis* GS1 might have suppressed the damping-off caused by *R. solani* KACC 40111 in cucumber plants.

**Keywords:** Endophytic bacteria, chitinase, β-1,3-glucanase, guaiacol peroxidase, *Rhizoctonia solani, Bacillus thuringiensis* GS1

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Biological control is defined as the use of biological processes to lower the inoculum density of phytopathogens, with the goal of reducing the activities of disease [3]. Endophytes have been defined as microorganisms that are found residing within a wide variety of surface-sterilized plant tissues. Endophytes offer a means of biological control for crops, such as the use of *Paenibacillus* sp. to reduce the severity of disease in soybean [46], inducing plant defense mechanisms using *Pseudomonas* sp. [4], and disease management in banana using *Bacillus subtilis* and *P. fluorescens* [25]. *Bacillus thuringiensis* has long been used as a biological control agent for insecticidal activity. *B. thuringiensis* primarily forms Cry proteins during the stationary phase of its growth cycle and also produces chitinase. Several studies have demonstrated that chitinase enhances the insecticidal activity of *B. thuringiensis* against *Spodoptera exigua* [29] and *Aedes aegypti* [51]. Chitinase produced by *B. thuringiensis* can also be used in the biological control of phytopathogens [43].

Pathogenesis-related (PR) proteins not only accumulate locally in infected leaves, but are also induced systemically and associated with the development of systemic acquired resistance against further infection by fungi, bacteria, and viruses [53]. PR proteins are assigned an important role in plant defense against pathogenic constraints and in general adaptation to stressful environments [15]. Chitinases are glycosyl hydrolases that catalyze the hydrolytic degradation of chitin and have a wide variety of functions in bacteria [7], fungi [35], insects [26], plants [1], and protozoa [54]. Chitinases play an important role in plant defense mechanisms against fungal pathogens [40].

In potato plants infected with *R. solani*, the tuber size distribution showed an increased proportion of small and large progenies, and an increase in the number of malformed tubers led to a reduction in the marketable yield [16, 19]. Chemical fungicides are often used when losses from *R.*
Endophytic bacteria were isolated from the stems of healthy bracken. 100 ml of broth medium containing 0.5% swollen chitin in a 250 ml Chitinase-producing endophytic bacteria were grown aerobically in Chitinase Activity Assay and Cell Growth. With high enzyme activity were selected as chitinolytic bacteria for the change in PR protein activation in host plants with respect to inoculation with B. thuringiensis GS1 and R. solani KACC 40111 in cucumber plants.

The objectives of this study were (i) to investigate the contribution of the chitinase-producing endophytic bacterium B. thuringiensis GS1 to the growth inhibition of R. solani and (ii) to establish the induction of pathogenesis-related proteins for defense mechanisms by inoculation of cucumber plants with B. thuringiensis GS1.

**MATERIALS AND METHODS**

**Isolation of Endophytic Bacteria**

Endophytic bacteria were isolated from the stems of healthy bracken [Pteridium aquilinum (L.) Kuhn] collected from Gurye, Korea. Plant samples were washed with tap water, followed by three rinses with deionized water, and then separated into roots and stems. Samples were sterilized by sequential immersion in 20% (v/v) H$_2$O$_2$ for 3 min, and then transferred to 70% (v/v) ethanol for 90 s and then 0.5% HgCl$_2$ for 1 min. This treatment was followed by three thorough rinses with sterile distilled water. To confirm that the surface disinfection process was successful, plant impressions were taken and water from the final rinse was plated on Petri plates containing Luria–Bertani (LB) agar. No contamination was found. The tissue samples were then cut into segments and incubated on tryptic soy agar (TSA) or LB for 48 h. Bacteria that appeared on the plates at this stage were purified by subculturing individual colonies three times, after which they were stored in a 50% glycerol solution at -80°C.

Chitinase-producing endophytic bacteria were screened for the production of a clear zone on swollen chitin mineral medium (SCM) plates [0.5% swollen chitin, 0.1% K$_2$HPO$_4$, 0.05% MgSO$_4$·7H$_2$O, 0.05% yeast extract, 0.1% trace elements (1% FeSO$_4$·7H$_2$O, 1% MnSO$_4$·H$_2$O, and 1% ZnSO$_4$·7H$_2$O, plus 1–2 drops cone, H$_2$SO$_4$)] based on the amount of reducing sugar in the broth medium and chitinase activity. Bacteria producing a clear zone on swollen chitin mineral medium were selected and purified by subculturing individual colonies three times, after which they were stored in a 50% glycerol solution at -80°C.

Chitinase Activity Assay and Cell Growth

Chitinase-producing endophytic bacteria were grown aerobically in 100 ml of broth medium containing 0.5% swollen chitin in a 250 ml Erlenmeyer flask at 30°C with shaking at 150 rpm for 6 days. The culture supernatant was collected after centrifugation at 10,000 g for 15 min. The chitinase activity was then assayed by measuring the amount of the reducing end group, GlcNAc (N-acetyl-β-D-glucosamine), produced from swollen chitin [30]. One unit of chitinase activity was defined as the amount of enzyme that liberated 1 µmol of GlcNAc per hour. SDS-PAGE was performed according to the method described by Laemmli [28] using a Bio-Rad Mini Protein II Apparatus (50–100×1.5 mm) and 12% (w/v) polyacrylamide separating gels. Chitinase active staining was conducted according to the method described by Trudel and Asselin [52]. Cell growth was measured at 600 nm using a spectrophotometer.

**Antifungal Activity**

Rhizoctonia solani AG-1(B) Kuhn KACC 40111 was used to screen the antifungal activity of the endophytic bacteria and plant extracts. The fungal strains were obtained from the Korean Agricultural Culture Collection (KACC), Suwon, Korea. Mycelial culture discs with a diameter of approximately 6 mm were cut and then transferred to the center of a new petri plate containing potato dextrose agar (PDA) medium.

To screen for antifungal activity, 8-mm-diameter paper discs were placed so that their edge was 10 mm from the edge of the hyphal colony. About 20 and 50 µl of culture broth from the endophytic bacteria were then applied to the paper discs, after which the samples were incubated at 25°C.

**Treatment with B. thuringiensis GS1**

B. thuringiensis GS1 was transferred onto TSA and incubated at 29°C for 24 h. The bacterial cells were then suspended in 10 ml of 0.1 M sodium phosphate buffer (pH 7.0), after which the suspensions (1×10$^8$ CFU/ml) were spotted onto cucumber seeds at 100 µl per seed. R. solani KACC 40111 was incubated at 27°C for 3 days on PDA plates, which were then cut into pieces and mixed with 800 ml of sterile soil. Subsequently, the mixture of bacteria and fungi was incubated at 24°C for 2 days, after which it was used as the challenge inoculum.

Cucumber seeds were surface sterilized in a solution of 10% bleach, rinsed 3 times with distilled water, and then germinated and grown in pots (3 seeds per pot; pot size: 5×5 cm) containing perlite in a growth chamber at 26°C (day/night) for 14 days. Four inoculation treatments were imposed: 1, control (distilled water [D.W.]); 2, B. thuringiensis GS1 (GS1); 3, R. solani KACC 40111 (RS); and 4, B. thuringiensis GS1 + R. solani KACC 40111 (GS1+RS). The bacterial culture suspension (15 ml) was then soaked onto each seed in the GS1 and GS1+RS treatments as described above.

**Activity Assay of Pathogenesis-Related (PR) Proteins**

Plants were sampled at 0, 1, 2, 3, 4, 5, and 14 days after seeding. Samples [fresh weight (FW), 200 mg] were powdered with liquid nitrogen and then homogenized in 1 ml of homogenization buffer (100 mM potassium phosphate buffer at pH 7.0 containing 2 mM EDTA, 1% PVP, and 1 mM PMSE). The protein concentration was then measured according to the method described by Bradford [5] using bovine serum albumin (BSA) as a standard.

Chitinase activity was assayed by measuring the amount of the reducing end group, GlcNAc, produced from swollen chitin [30]. β-1,3-Glucanase activity was assayed by measuring the amount of the reducing end group, glucose, produced from laminarin [56]. One
unit of β-1,3-glucanase activity was defined as the amount of enzyme that liberated 1 µmol of glucose per hour. The guaiacol peroxidase (GPOD) activity was assayed to determine the amount of the reducing end group produced, using the method described by Fu and Huang [17]. One unit of enzymatic activity was defined as the amount of enzyme that catalyzed the conversion of 1 µmol of hydrogen peroxide per minute at 25°C. The ascorbate peroxidase (APX) activity was determined according to the method described by Chen and Asada [9]. One unit of APX activity was defined as the amount of the enzyme that oxidized 1 µmol of ascorbate per minute. Polyphenol oxidase (PPO) activity was determined according to the method described by Mohammadi and Kazemi [33]. One unit of PPO activity was defined as an increase in absorbance of 0.01 per minute per milligram of fresh weight.

Activity Staining of Pathogenesis-Related (PR) Proteins
Chitinase activity staining was done according to the method described by Trudel and Asselin [52]. β-1,3-Glucanase activity staining was performed according to the method described by Pan et al. [37]. GPOD activity staining was done according to the method described by Caruso et al. [8]. APX activity staining was conducted according to the method described by Chen and Asada [9]. PPO activity staining was conducted according to the method described by Mohammadi and Kazemi [33].

Statistical Analysis
Treatment effects were determined by analysis of variance (one-way ANOVA) conducted according to the general linear model procedure of the Statistical Analysis System 9.1. Means were separated with Tukey’s Studentized Range Test at p=0.05. All data are presented as the mean value ± the standard deviation.

RESULTS

Cell Growth and Chitinase Activity
B. thuringiensis GS1 cell growth and chitinase production in broth medium containing swollen chitin were measured for 6 days (Fig. 1). Cell growth increased rapidly to a maximum level within 4 days, and was then maintained at the same density. The trend in chitinase activity was similar to that of bacterial growth for the first 5 days of incubation, after which it decreased to 3.7 units/ml. The maximal extracellular chitinase production (4.3 units/ml) by B. thuringiensis GS1 was observed when the culture reached a cell density of 1.8 (OD 600 nm) at 5 days after culture.

Activity Staining of Chitinase Produced by B. thuringiensis GS1
The chitinase activity of GS1 was detected by modified 12% SDS-PAGE containing 0.01% (w/v) glycol chitin as a substrate. The chitinase isozymes were revealed on the gel as three active bands with molecular masses of about 50 kDa, 40 kDa, and 36 kDa (Fig. 2).

Antifungal Activity Against R. solani
Antifungal activity was determined on chitin/PDA (1:1) plates treated with 50 µl of culture supernatant obtained from B. thuringiensis GS1 at 5 days after incubation in SCM. As shown in Fig. 3, the bacterial culture supernatant...
strongly inhibited the mycelial growth of *R. solani* at 2 days after inoculation.

### Biological Control of Damping-Off Caused by *R. solani* Using *B. thuringiensis* GS1

In this study, the growth of cucumber plants was determined based on the shoot height of the plant, the fresh weight, and the number of dead seedlings (Table 1). The germination of seedlings in RS-treated plants was 19%. The shoot height in the control and GS1-treated plants was higher than in GS1+RS- and RS-treated plants. The fresh weight in GS1-treated plants was higher than that of other treated plants.

The chitinase, β-1,3-glucanase, and GPOD activities in cucumber plants were assayed spectrophotometrically using swollen chitin, laminarin, and guaiacol as the substrate, respectively (Fig. 4). The activities of PR proteins were measured at 1, 2, 3, 4, 5, and 14 days after inoculation of *R. solani* KACC 4011 and/or *B. thuringiensis* GS1 into the soil of cucumber plants.

The chitinase activity was highest in RS-treated plants at 4 days after treatment and then rapidly decreased until 5 days after treatment (Fig. 4A). The chitinase activity decreased significantly by 65% and 33% in RS- and GS1+RS-treated plants, respectively, from 4 days to 5 days of treatment. At 5 days after treatment, the chitinase activity was 1.17 and 1.55 units/g FW in the RS- and GS+RS-treated groups, respectively.

The β-1,3-glucanase activity showed the same pattern as the chitinase activity (Fig. 4B). At 5 days, the β-1,3-glucanase activity was significantly decreased by 60% and 38% in RS- and GS1+RS-treated plants, respectively. At

### Table 1. Effects of *B. thuringiensis* GS1 on the growth of cucumber and protection from damping-off caused by *R. solani* KACC 4011 at 14 days after seeding.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination (%)</th>
<th>Shoot height (cm/plant)</th>
<th>Fresh weight (mg/pot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>75.0 ± 0.1a</td>
<td>6.4 ± 0.8a</td>
<td>952 ± 102ab</td>
</tr>
<tr>
<td>GS1</td>
<td>66.0 ± 0.1ab</td>
<td>6.0 ± 0.8a</td>
<td>1,014 ± 145a</td>
</tr>
<tr>
<td>RS</td>
<td>19.0 ± 0.1c</td>
<td>3.9 ± 0.7b</td>
<td>736 ± 125c</td>
</tr>
<tr>
<td>GS1+RS</td>
<td>50.0 ± 0.1b</td>
<td>4.1 ± 0.5b</td>
<td>806 ± 205bc</td>
</tr>
</tbody>
</table>

Values shown in each column are the means ± standard deviation based on three replicates. Data followed by the same letter within columns are not significantly different (p≤0.05) as determined by Tukey’s Studentized Range (HSD) test.

GS1: *B. thuringiensis* GS1; RS: *R. solani*; GS1+RS: *B. thuringiensis* + *R. solani*.

![Fig. 4 Activities of PR proteins of cucumber treated with *B. thuringiensis* GS1 (GS1) and/or *R. solani* (RS). Chitinase (A), β-1,3-glucanase (B), and GPOD (C) activities. Control (■), GS1 (●), RS (◆), and GS1+RS (○).](image)

![Fig. 5 Activities of APX and PPO of cucumber treated with *B. thuringiensis* GS1 (GS1) and/or *R. solani* (RS). APX (A) and PPO (B). Control (■), GS1 (●), RS (◆), and GS1+RS (○).](image)
5 days after treatment, the \( \beta-1,3 \)-glucanase activity was 11 and 13 units/g FW in RS- and GS+RS-treated plants, respectively.

The GPOD activity increased continuously until day 4 in all treated plants. There were no significant differences between the control and GS1-treated plants at day 4 or 5. The GPOD activity in RS- and GS1+RS-treated plants was increased 3- and 4-fold, respectively, at 4 days after treatment (Fig. 4C). At 4 days after treatment, the GPOD activities were 21.2 and 12.8 units/g FW in RS- and GS+RS-treated plants, respectively.

The APX activity was highest in RS-treated cucumber plants at 4 days after treatment, after which it rapidly decreased throughout the remaining experimental period (Fig. 5A). At 5 days after treatment, the APX activities were 8.7 and 12.6 units/g FW in RS- and GS1+RS-treated plants, respectively.

The PPO activity in cucumber plants followed the same trend as the APX activity (Fig. 5B). The PPO activity in RS-treated plants increased sharply for 3 days after treatment, after which it rapidly decreased. At 5 days after treatment with RS and GS1+RS, the PPO activity had decreased to 41.4 and 67.9 units/g FW, respectively.

**Activity Staining of Pathogenesis-Related (PR) Proteins**

Three bands with chitinase activity, designated Ch1, Ch2, and Ch3, were observed on the SDS-PAGE gel (Fig. 6A). One major chitinase from cucumber plants, Ch1, was visible as a strong band in response to treatment with GS1, whereas the same chitinase produced a band in GS1+RS-treated plants. At 5 days after treatment, all three active chitinase bands were observed in cucumber plants treated with GS1+RS, whereas plants treated with RS alone showed only one band (Ch2).

**Activity Staining of Antioxidant Enzymes of Cucumber on Native-PAGE (10%)**

Fifty \( \mu \)g of enzyme was loaded onto native-PAGE gels. APX (A) and PPO (B). M: Standard protein marker; SE: seed extract; Lane 1: Control; 2: *B. thuringiensis* GS1; 3: *R. solani*; and 4: *B. thuringiensis* GS1 + *R. solani*.
SDS-PAGE revealed only one active β-1,3-glucanase band (B1) for the control, and GS1- and GS1+RS-treated cucumber plants at 2 days after treatment, whereas this band was no longer present in RS-treated plants (Fig. 6B).

Five major isozymes of GPOD, designated Gp1, Gp2, Gp3, Gp4, and Gp5, were observed upon native-PAGE in response to all treatments at 14 days after treatment (Fig. 6C). GPOD activity staining revealed that the strongest activity occurred in RS-treated plants. In addition, one isozyme of GPOD, Gp3, was observed in response to treatment with RS and GS1+RS at 4 days after treatment.

Three bands with APX activity, designated Ap1, Ap2, and Ap3, were observed on the native-PAGE gel (Fig. 7A). All three bands were observed for the control, and GS1- and GS1+RS-treated cucumber plants on the native-PAGE gel at 1 day after treatment, whereas Ap2 in RS-treated plants disappeared.

Three bands with PPO activity were observed on native-PAGE and designated Po1, Po2, and Po3 (Fig. 7B). Po1 in RS- and GS1+RS-treated cucumber plants showed stronger activity than that in the control and GS1-treated plants upon native-PAGE at 5 days after treatment. At least three dark bands (Po1, Po2, and Po3) were observed in response to all treatments after 14 days.

**Discussion**

Chitinases have been reported to induce plant resistance to fungal pathogens owing to their antifungal activities in vitro [50]. In this study, B. thuringiensis GS1 was isolated from bracken (Pteridium aquilinum Kuhn) (Fig. 2). The highest level of chitinase was observed after 5 days of culture, after which it gradually declined (Fig. 1). Driss et al. [13] reported that the chitinase activity of B. thuringiensis subsp. kurstaki increased during vegetative growth to reach a maximum of 28.31 mU/ml after 70 h of incubation. At 6 days after culture, the enzyme activity decreased, and this was likely due to the presence of proteolytic enzyme [44, 58], an increase in pH, and/or the accumulation of sugars [34, 57]. As shown in Fig. 5B, crude enzyme from B. thuringiensis GS1 has three chitinase isozymes (50, 40, and 36 kDa). Using the same approaches, at least four isozymes (66, 60, 47, and 32 kDa) in B. thuringiensis subsp. pakistani [51] and five isozymes (62, 54-52, 43, 38, and 21 kDa) in Serratia marcescens [14] were detected.

Chitinase-producing bacteria were co-cultured with phytopathogens in medium containing chitin/PDA [1:1 (v/v)] (Fig. 3). The results revealed that the mycelial growth inhibition of R. solani KACC 40111 was likely primarily due to the chitinolytic enzymes produced by B. thuringiensis GS1. Reyes-Ramirez et al. [43] reported that chitinase produced by B. thuringiensis ITV20 inhibited the growth of some phytopathogenic fungi such as Fusarium sp., Sclerotium rolfsii, Curvularia sp., and Rhizopus sp.

Results from the dual inoculation of phytopathogen and bacteria for the early growth stage of cucumber plants indicated that chitinase produced by B. thuringiensis GS1 induced strong systemic resistance mechanisms in plants against R. solani KACC 40111. Inoculation with chitinase-producing B. thuringiensis GS1 inoculum in vivo significantly suppressed the incidence of damping-off by R. solani in cucumber seedlings (Table 1). In this case, the suppressive effect of B. thuringiensis is believed to be associated with the action of hydrolytic enzymes, causing hyphal degradation of the fungi. Chitinase produced by B. thuringiensis ITV20 has been shown to be involved in the biological control of phytopathogenic fungi in soybean seeds [43]. In addition, Jung et al. [21] reported that chitinase produced by P. illinoisensis KJA-424 plays an important role in the suppression of damping-off in cucumber, and antibiosis and hydrolytic enzyme activities are the most recognized mechanisms involved in these effects [18].

The change in chitinase activity in plants in response to antagonist and/or phytopathogens has been well demonstrated in cases such as the control of R. solani by P. aureofaciens in soybean [23] and sheath blight caused by R. solani in rice cultivars [48]. In the present study, the highest chitinase activity was observed in RS-treated cucumber plants at 4 days after treatment (Fig. 4A). In addition, the chitinase activity of RS- and GS1+RS-treated cucumber plants was increased 3.2- and 2.3-fold, respectively, compared with the control. Analysis of SDS-PAGE gels revealed two chitinases (Ch1 and Ch2) in GS1+RS-treated plants at 4 days after treatment (Fig. 6). Ch1 was induced only in GS1-treated plants at 4 days after treatment. Accordingly, we suggest that the two isoenzymes, Ch1 and Ch2, were induced by inoculation with the phytopathogen (R. solani) and the antagonistic microorganism (B. thuringiensis GS1), respectively. Yedidia et al. [56] reported that the expression of two chitinase isozymes (102 and 73 kDa) in cucumber roots at 3 days after treatment were induced by Trichoderma harzianum T-203 through activation of the plant defense response. In this study, one chitinase isozyme (Ch3) in cucumber was induced in all treatments at 1 day after treatment (Fig. 6A). Furthermore, it was reported that Class III acidic chitinase was present in tamarind seeds [42] and a 30 kDa thermostable chitinase was found in peronias seeds [45]. The presence of chitinase in seeds, fruits, and vegetative tissue also suggests that they may be storage proteins that act as a source of amino acids for the synthesis of other proteins during the development of plants [41].

The highest β-1,3-glucanase activity was observed in GS1-treated cucumber plants at 5 days after treatment (Fig. 4B). In addition, the β-1,3-glucanase activity of GS1-treated cucumber plants was increased 1.7-fold compared
with the control. β-1,3-Glucanase activity in plants has been demonstrated in the control of Botrytis cinerea by selected grapevine-associated bacteria [33] and the control of R. solani by P. aeruginosa in soybean plants [23]. Furthermore, recent studies have provided evidence that β-1,3-glucanase plays a role in several developmental processes [49, 55]. One β-1,3-glucanase isozyme (B1) was induced in the control and GS1-treated cucumber plants at 5 days after treatment (Fig. 6B). Jung et al. [23] reported that five β-1,3-glucanase isozymes (80, 70, 50, 46, and 19 kDa) were present in soybean plants. The accumulation of the 33 kDa β-1,3-glucanase isoform observed in the present study was correlated with an increase in its level of translatable mRNA that was observed in response to infection with the fungus Fusarium moniliforme [11].

Phytopathogen resistance has often been correlated with elevated GPOD activity in diseased cucumber tissues. In this study, we observed a constant increase in GPOD activity for 5 days in GS1+RS-treated cucumber plants (Fig. 4C). In addition, the GPOD activity of GS1+RS-treated plants was increased 4.7-fold compared with the control. GPOD activity increased in diseased cucumber tissues. In this study, we observed a constant increase in GPOD activity for 5 days in GS1+RS-treated cucumber plants (Fig. 4C). In addition, the GPOD activity of GS1+RS-treated plants was increased 4.7-fold compared with the control. GPOD activity has also been demonstrated in wheat heads infected with Fusarium graminearum [33], in pepper roots challenged with Phytophthora capsici [22], and in R. solani-inoculated soybean plants infected with P. aeruginosa [23]. We also found that GPOD from cucumber plants was present as three isoenzymes (Gp1, Gp2, and Gp3) (Fig. 6C). These isozymes were observed in RS- and GS+RS-treated plants at 5 days after treatment. Activity staining on native-PAGE gels revealed several isozymes in F. graminearum-infected wheat heads [33]. Moreover, two isozymes (59 and 27 kDa) were induced in R. solani-infected soybean plants by exposure to P. aeruginosa [23]. GPOD is also involved in lignin synthesis and degradation of cytotoxic levels of hydrogen peroxide generated in plant tissues as a result of pathogen attack [10, 27].

APX activity increased in RS-treated cucumber plants at 4 days after treatment (Fig. 5A) and was increased 1.5-fold compared with the control. APX activity has also been observed in pepper infected by P. capsici [17], in rice in response to infection with R. solani [38], and in the relationship between R. solani and P. aeruginosa in rice [12]. We also found that APX from cucumber plants was present as three isoenzymes (Ap1, Ap2, and Ap3) (Fig. 7A). Ap2 was no longer observed in RS-treated plants at 1 day after treatment. Activity staining of a native-PAGE gel revealed an APX of 28 kDa in Japanese radish [36]. APX is one of the most important antioxidant enzymes produced by plants for detoxification of H$_2$O$_2$, and ascorbate is used in this reaction. Most importantly, APX reduces H$_2$O$_2$ to water using ascorbate as the specific electron donor [47].

PPO activity in RS-treated plants increased sharply for 3 days after treatment, after which it rapidly decreased (Fig. 5B). In addition, the PPO activity of RS-treated plants was increased 1.5-fold compared with the control. PPO activity was 2-fold greater in resistant cucumber plants than in the control. The PPO from cucumber plants was present as three isoenzymes (Po1, Po2, and Po3) (Fig. 7B). Two PPO isozymes (Po1 and Po2) were strongly induced in RS- and GS1+RS-treated plants at 4 days after treatment. Activity staining on native-PAGE gels revealed the presence of one basic and six acidic isozymes in wheat heads [33]. This may suggest that induced PPO-specific activity in resistant cucumber plants could be a defensive response against R. solani infection and suggests that this phenomenon is related to disease resistance.

In conclusion, the endophytic bacterium B. thuringiensis GS1 is a rich source of chitinase. B. thuringiensis GS1 was found to be associated with strong antifungal activity against the phytopathogen R. solani KACC 40111 in vitro. The induction of PR proteins by B. thuringiensis GS1 may have suppressed the damping-off caused by R. solani in cucumber. Based on these findings, B. thuringiensis GS1 is a feasible biocontrol agent for the suppression of phytopathogens and for the development of environmentally friendly agriculture.

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References


