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Biocontrol of Late Blight and Plant Growth Promotion in Tomato Using Rhizobacterial Isolates

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Seven bacterial isolates (*viz.*, AB05, AB10, AB11, AB12, AB14, AB15, and AB17) were derived from the rhizosphere and evaluated in terms of plant growth-promoting activities and the inhibition of *Phytophthora infestans* affecting tomatoes in Korea. According to 16S rDNA sequencing, a majority of the isolates are members of *Bacillus*, and a single isolate belongs to *Paenibacillus*. All seven isolates inhibited *P. infestans* by more than 60% *in vitro*. However, AB15 was the most effective, inhibiting mycelial growth of the pathogen by more than 80% *in vitro* and suppressing disease by 74% compared with control plants under greenhouse conditions. In a PGPR assay, all of the bacterial isolates were capable of enhancing different growth parameters (shoot/root length, fresh biomass, dry matter, and chlorophyll content) in comparison with non-inoculated control plants. AB17-treated plants in particular showed the highest enhancement in fresh biomass with 18% and 26% increments in the root and shoot biomass, respectively. However, isolate AB10 showed the highest shoot and root growth with 18% and 26% increments, respectively. Moreover, the total chlorophyll content was 14%~19% higher in treated plants.

Keywords: Biological control, PGPR, *Phytophthora infestans*, *Solanum lycopersicon*, inhibition effect

Introduction

Late blight disease, caused by *Phytophthora infestans* (Mont.) de Bary, is one of the most serious threats to tomato production worldwide [24]. It is well known for its explosive development when environmental conditions are suitable and host plants are susceptible to infection [14]. Control of plant disease is largely based on genetic resistance in host plants, cultural practices, and synthetic pesticides [20, 22, 25]. However, chemical control measures create imbalances in the microbial community, which may be unfavorable to the activity of beneficial organisms and may also lead to the development of resistant strains of pathogens [19]. Therefore, biocontrol using plant growth-promoting rhizobacteria (PGPR) represents a potentially attractive alternative disease management approach, since they are known to promote growth and reduce disease in crops [8].

For the biological control of late blight, several antagonistic microorganisms have been tested for their ability to inhibit *P. infestans*, including nonpathogenic *Phytophthora cryptogea* [23] and endophytic microorganisms such as *Cellulomonas flavigena*, *Candida* sp., and *Cryptococcus* sp. [12]. Although some fungal antagonists showed effective inhibition, bacterial antagonists have shown by far the most promising results. Bacteria with antagonistic activity toward *P. infestans* are found mainly in the genera *Pseudomonas* and *Bacillus* [6, 10, 18, 30].

The objectives of this study were to isolate rhizobacteria and to examine their *in vitro* and *in vivo* antagonistic activities toward *P. infestans* affecting tomatoes under greenhouse conditions. The successful *in vitro* and *in vivo* suppressions of *P. infestans* provide useful information on the potential use of these isolates as biocontrol agents against late blight disease. In addition, the results of the present study suggest that the rhizobacterial isolate

Paenibacillus polymyxa AB15 and other *Bacillus* spp. have a great ability to increase the yield, growth, and nutrition of tomato plants under greenhouse conditions. Therefore, they may be utilized as biofertilizer and biological control agents for fruit and vegetable production in sustainable and ecological agricultural systems.

Materials and Methods

Soil Sample Collection

Different soil samples were randomly collected from various parts of Gangwon Province, Korea. Several diverse habitats in different areas were selected for the collection of soil samples, which included the rhizosphere of plants, agricultural soil, preserved areas, and forest soils. Soil samples differed in cropping and tillage history, fertility, and texture. The samples were taken up to a depth of 20 cm after removing approximately 3 cm of the soil surface. Samples were placed in sterilized polythene bags and then taken to the laboratory. They were dried for 4 days. Large roots and stones were removed, and the remainder was passed through an autoclave-sterilized brass sieve with a 2 mm aperture size and then stored at 4°C until further examination.

Isolation of Bacteria

Soil dilutions were prepared with each gram of soil sample suspended in 99 ml of sterile distilled water (SDW) and shaken vigorously for 2 min. The soil suspensions were serially diluted in SDW, and then a 1 ml aliquot of each 10^{-5} and 10^{-6} dilution was pipetted out, poured into a petri dish, and plated on sterile tryptic soy agar (TSA) medium [3]. The plates were incubated at 28°C for 12–14 days. Then, colonies with different morphologies were selected from countable plates and re-streaked on a new plate of the same medium to obtain pure colonies. For long-term storage, bacterial strains were maintained at −80°C in tryptic soy broth (TSB; Difco Laboratories, USA) supplemented with 20% glycerol.

Fungi and Cultural Conditions

The *P. infestans* used in this study was obtained from the Korean Agricultural Culture Collection (KACC), Suwon, Korea (Table 1), and had been isolated from tomato. It was grown on V8 juice agar (200 ml V8 juice, 800 ml distilled water, 3 g $CaCO_3$, and 15 g agar) medium and incubated in 17 ± 2°C for 7 days. Actively growing mycelia were then selected from plates, subcultured in freshly prepared agar medium, and incubated at their optimal growth temperature for further use.

In Vitro Inhibition Assay

A dual culture inhibition assay was conducted on V8-PDA (150 ml V8 juice, 10 g PDA, 3 g $CaCO_3$, 10 g agar, and 850 ml dH_2O) in plastic petri plates (8.5 cm diameter). In order to test the antagonistic activity of selected bacteria toward the pathogen, agar plugs containing fungi from the margins of young colonies and 20 µl of bacteria were placed on the dual medium plates at about 4.5 cm apart and assessed for about 15 days at 28°C to observe whether any inhibition zone appeared. Suppression of fungal growth was measured as the size of the clear zone, defined as the distance between the leading edge of fungal growth and the closest edge of the bacterial colony, and the strains were ranked accordingly. Inhibition was expressed relative to a control strain spotted on the same plate. Five replicates were done for the experiment for each pairing. The isolates showing the greatest inhibition were selected as potential antagonistic bacteria. The radii of the fungal colony toward and away from the bacterial colony were noted. The percentage growth inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = [(R - r)/R \times 100]$$

where r is the radius of the fungal colony opposite the bacterial colony, and R is the maximum radius of the fungal colony away from the bacterial colony.

Identification of Bacterial Isolates Based on 16S rDNA Homology

For the extraction of DNA, bacterial cells were harvested from 10 ml of overnight cultures, and pellets were lysed in 1 ml of lysis buffer (25% sucrose, 20 mM EDTA, 50 mM Tris-HCl, and 5 mg/ml of lysozyme). Chromosomal DNA was extracted according to the standard procedure of Weisburg *et al*. [27]. 16S rDNA was amplified using PCR with the universal primers 27F and 1492R [17]. PCR was performed in a thermocycler using 35 amplification cycles at 94°C (45 sec), 55°C (60 sec), and 72°C (60 sec), with a final

Table 1. Identification of rhizobacterial isolates to the species level by means of 16S rDNA sequencing.

Bacterial isolate	GenBank Accession No.	Closest GenBank library strain	Gram reaction	Similarity (%)
AB05	AB301002.1	*Bacillus amyloliquefaciens*	+	99
AB10	HM101166.1	*Bacillus subtilis*	+	99
AB11	HM032893.1	*Bacillus* sp.	+	99
AB12	HM101166.1	*Bacillus subtilis*	+	99
AB14	AJ880761.1	*Bacillus subtilis*	+	98
AB15	CP000154.1	*Paenibacillus polymyxa*	+	99
AB17	GQ375229.1	*Bacillus subtilis* subsp. *subtilis*	+	98

extension for 7 min at 72°C. The PCR products were purified using a Montage PCR Clean Up Kit (Millipore, USA). Purified PCR products of approximately 1,400 bp were sequenced using the universal primers 518F and 800R (Macrogen, Korea) and a Big Dye Terminator Cycle Sequencing Kit v.3.1 (Applied BioSystems, USA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA) at Macrogen, Inc., Seoul, Korea. The sequences were compared using the NCBI BLAST program (http://www.ncbi.nlm.nih.gov/Blast) for identification of the isolates.

Inhibition Assay in Greenhouse

Preparation of plant pathogen inocula. *P. infestans* (KACC 43070) was obtained from the Korean Agricultural Culture Collection (KACC) and grown on V8-agar medium at 17 ± 2°C for 14 days. To prepare inoculum for infection, 10 ml of distilled water was poured into each culture plate and kept in a refrigerator at 4°C for 2 h to release zoospores from sporangia. The zoospore suspension was filtered through two layers of miracloth, and the concentration was adjusted to 1.0×10^7 zoospores/ml for plant inoculation.

Seed sowing. Tomato (*Solanum lycopersicon* cv. 'Cool') seeds were surface sterilized for 20 min in 20% sodium hypochlorite, followed by a brief rinse with 50% methanol before planting in sterile soil. Seeds were sown in a plastic pot (50 holes, 4 cm diameter each) filled with commercial soil (Baroker, Seoul Bio Co., Ltd., Korea) containing 10% perlite (Parat Sam Son, Korea). Tomato seedlings were grown in a greenhouse, maintaining 28°C during the day and 25°C at night. Plants were watered with about 30 ml per plant once a day. After two weeks, individual tomato seedlings were planted in a plastic pot (15 cm in diameter) filled with the same commercial soil with 10% perlite as described above. The plants were grown in a greenhouse maintained at 60% RH, 28 ± 2°C during the day and 25°C at night, and watered daily. They were then used for the bioassay of inhibition activity toward late blight disease.

Preparation and application of bacterial inocula. A loop-ful of a bacterial isolate was inoculated into tryptic soy broth (TSB) and incubated in a rotary shaker at 150 rpm for 72 h at room temperature (28 ± 2°C). After incubation, the bacterial suspension was diluted in SDW to give a final concentration of 10^8–10^9 CFU/ml (OD = 0.1 – 0.5) at 550 nm, using a hemocytometer and optical density measurement. For greenhouse evaluation, targeted plants were left to dry for 2 days, and then 100 ml of bacterial spore solution (10^7 spores/ml) was added to each pot 7 days before infection so that only soil, but no above-ground parts, received any bacterial spores.

Application of fungal culture and disease assessment. Suspensions of zoospores and microbial cells of target pathogens in 0.04% Tween 80 were sprayed until runoff onto six seedlings of host plants per treatment (5 ml/seedling). Plants were kept in the greenhouse 12 h before and 12 h after inoculation to maintain high humidity and facilitate infection. Plants were scored as (1) uninfected; (2) 1/3 of the leaves infected; (3) 2/3 of the leaves infected; or (4) dead. Data for water-treated plants with or without pathogen inoculation were collected at the same intervals. Eight weeks later, plants were removed from the soil and the roots were washed with SDW. Roots were excised from the plant and data collected for analysis. Data included root and crown rot severity assessed on a scale of 0–4 [4], where, 0 = no infection, 1 = 1–25% infection, 2 = 26–50% infection, 3 = 51–75% infection, and 4 = 76–100% infection. Based on the disease severity index, the percentage suppression of root and crown rot was calculated as follows [26]:

$$\% \text{ Suppression} = [(A - B)/A] \times 100$$

where A is the disease severity exhibited in the leaves and stem region due to pathogen alone, and B is the disease severity exhibited on the leaf and stem region after inoculation with both the pathogen and bacterial antagonists.

Shoots and roots from six plants per treatment were excised, the fresh and dry weights were determined, and disease severity was assessed and the disease incidence rate was also calculated.

PGPR assay in greenhouse. A loop-ful of bacterial isolate was transferred to a 500 ml flask containing TSB and grown aerobically in a rotating shaker at 160 rpm for 48 h at 28 ± 2°C (Wisecube, Daihan Scientific Co. Ltd., Korea). The bacterial suspension was then diluted in SDW to a final concentration of 10^9 CFU/ml, using a hemocytomoter and optical density measurement (OD = 0.1 – 0.5 at 550 nm). The resulting suspensions were used to treat targeted plants under greenhouse conditions.

Tomato seeds were surface sterilized for 20 min in 20% sodium hypochlorite, followed by a brief rinse with 70% methanol before planting in sterile soil. Seeds were sown in a plastic pot (50 holes, 4 cm diameter each) filled with commercial soil (Baroker, Seoul Bio Co., Ltd., Korea) containing 10% perlite (Parat, Sam Son, Korea). Seedlings were grown in a greenhouse, maintaining 28°C during the day and 25°C at night. Plants were watered at about 30 ml per plant once a day. After 2 weeks, individual tomato seedlings were planted in a plastic pot (15 cm in diameter) filled with the same commercial soil with 10% perlite as described above. For each treatment, one replicate and three replications were used in a randomized block design. The bacterial inoculum (10^8–10^9 CFU/ml) was applied in the form of soil drenching twice, one week apart. The temperature of the greenhouse was maintained at 28 ± 2°C and watering was done twice daily. The plants were harvested 3 weeks after the last inoculation. Shoot and root length as well as fresh and dry weight measurements were compared with the non-inoculated control. Chlorophyll content was also measured with a chlorophyll meter (SPAD-502; Konica Minolta Sensing, Inc., Japan).

Statistical analysis. Data from the *in vitro* and greenhouse experiments under production conditions were analyzed using analysis of variance in a completely randomized design. Duncan's multiple range test was used to compare the means of the treatments in each experiment. All statistical analyses were conducted using SAS software (Ver. 9.2.; SAS Institute Inc., USA).

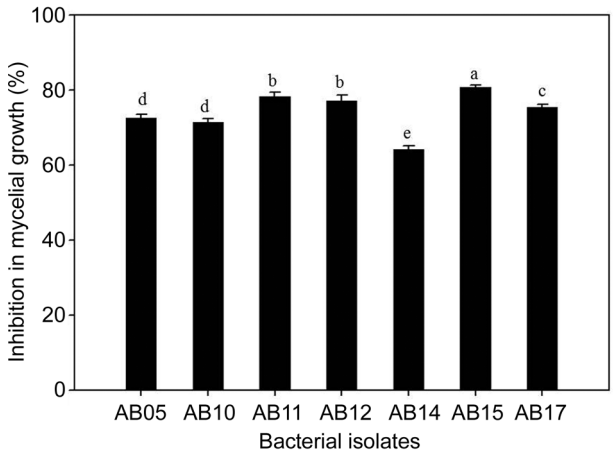

Fig. 1. Inhibition effect of rhizobacterial isolates on the mycelial growth of *P. infestans* in *in vitro* dual culture assay.
Error bars represent the mean standard deviations of three replicates. Bars headed by different letters are significantly different ($p \leq 0.05$) according to Duncan's multiple range tests.

Table 2. Antagonistic activity of rhizobacterial isolates toward *P. infestans* in dual culture assay.

Bacterial isolate	Inhibition zone	Antagonistic activity[a]
AB05	18.3 ± 1.5[b]	++++
AB10	13.0 ± 2.0	+++
AB11	19.6 ± 1.5	++++
AB12	18.3 ± 1.1	++++
AB14	12.0 ± 2.6	+++
AB15	23.0 ± 1.0	++++
AB17	17.6 ± 2.0	++++
Control (SDW)	-	-

[a]Zone of inhibition in dual culture assay: +, 0–5 mm; ++, 5–10 mm; +++, 10–15 mm; ++++, >15 mm; -, absent.

[b]Values are the mean ± SD of three independent observations. The experiments were repeated twice with five replications per assay.

Results

Screening and Identification of Bacterial Isolates

Out of 125 bacterial strains isolated from rhizospheric soil, seven were capable of inhibiting the mycelial growth of *P. infestans*, on *in vitro* dual culture screening, in various degrees. Among them, six were identified as species of *Bacillus* and one was identified as *Paenibacillus polymyxa*, according to 16S rDNA sequencing (Table 1). AB10, AB12, and AB14 were identified as *B. subtilis* and AB05 was characterized as *B. amyloliquefaciens*. However, the other two isolates, AB15 and AB11, were identified as *P. polymyxa* and *Bacillus* sp., respectively. The GenBank accession number of the isolate most similar to the selected bacteria used in this study is presented in Table 1. All of the species were identified as Gram-positive bacteria through a Gram staining procedure (Table 1).

In Vitro Inhibition of Late Blight

Of the seven isolates evaluated *in vitro*, five isolates (*viz.*, AB05, AB10, AB11, AB12, and AB17) reduced the mycelial growth of *P. infestans* by more than 70% on dual culture plates (Figs. 1 and 2). However, maximal inhibition was achieved with the isolate AB15 (80.75%). Control plates without bacterial isolates were completely covered by the

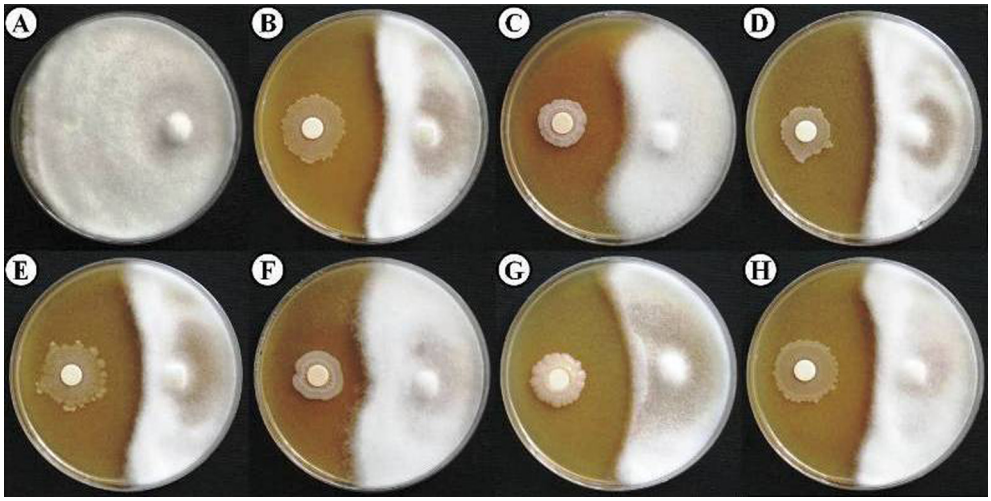

Fig. 2. Dual culture assay for *in vitro* inhibition of mycelial growth of *P. infestans* by selected rhizobacterial isolates.
(**A**) Control, (**B**) AB05, (**C**) AB10, (**D**) AB11, (**E**) AB12, (**F**) AB14, (**G**) AB 15, and (**H**) AB17.

phytopathogen, showing no inhibition zone (Fig. 2A). Mycelial growth inhibition of the target pathogen revealed that the suppression rate was highly reciprocal, with a wider inhibition zone. Isolate AB14 showed the least inhibition, with a 64.17% reduction in mycelial growth of *P. infestans*. The antagonistic activity was higher in isolates AB05, AB11, AB12, AB15, and AB17, with a more than 15 mm inhibition zone observed on dual culture plates (Table 2).

In Vivo Inhibition of Late Blight

The results demonstrated that isolates AB05, AB11, AB15, and AB17 significantly inhibited *P. infestans* (Table 3). Treated plants looked healthy and showed a lower incidence of late blight. Treatment with isolate AB15 resulted in a more than 70% suppression of late blight, whereas treatment with AB05, AB11, AB12, and AB17 resulted in a disease reduction of more than 60% (Table 3). Control plants without bacterial treatment but inoculated with *P. infestans* alone exhibited an up to 98–100% disease incidence, with the majority of plants being completely stunted or dead. Plants inoculated with neither the pathogen nor bacterial isolates also survived, but showed some infection, presumably from a low level of cross-contamination (Table 3). All infected shoots were characterized by dark blight discoloration and rotting. The leaves of infected seedlings were pale green and plants were stunted. Infection with the fungus resulted in a pronounced decrease in the fresh and dry weight of shoots compared with the non-inoculated control and some of the treatments with the bacterial isolates. The reduction in fresh weight of shoots in the control treatment inoculated with *P. infestans* alone amounted to 53%, whereas a 12% reduction in fresh shoot weight was recorded for treatments involving inoculation with both the pathogen and isolate AB15. Isolates AB11, AB12, and AB17 also showed a minimal reduction in shoot fresh weight. The shoot dry weight of the control treatment inoculated with only *P. infestans* was decreased by 64% in comparison with the non-inoculated control. Among the selected biological control agents in this study, isolate AB17 and isolates AB15 and AB12 reduced the shoot and root dry weights by 28% and 26%, respectively. The result presented in Table 3 shows that AB15 suppressed disease by more than 70%, and plants treated with the same isolate also showed less leaf necrosis compared with the others. In addition, treatment with isolates AB12, AB11, and AB05 resulted in a lower percentage of leaf necrosis in tomato plants (Table 3).

Efficacy of PGPR on Tomato

Of the seven isolates analyzed in the greenhouse test on tomato, all resulted in a significant increase ($p \leq 0.05$) in at least two growth parameters. Treatment with isolates AB10, AB11, AB15, and AB17 showed a significant increase in at least five growth parameters (Table 4). Among the treatments, isolate AB17 displayed the highest growth-promoting activity in all of the parameters evaluated, except shoot/root length and root dry weight. However, isolate AB10 showed the highest increase in shoot and root length at 18% and 26%, respectively. AB17-treated plants showed a 27% and 32% increase in shoot and root biomass, respectively (Table 4, Figs. 3 and 4), and the chlorophyll

Table 3. Effects of rhizobacterial isolates on suppression of late blight caused by *Phytophthora infestans* under greenhouse conditions.

Bacterial isolate	Shoot fresh weight (g)	Shoot dry weight (g)	% Leaf necrosis	% Disease suppression
AB05	67.20f*	15.96cd	36.33e	62.00d
AB10	72.96e	16.90cd	46.33c	55.57e
AB11	84.80d	18.80cb	35.33fe	63.26dc
AB12	94.03c	20.60b	33.00f	65.24c
AB14	63.06g	15.36d	76.66b	24.41f
AB15	97.83b	20.63b	26.66g	74.01b
AB17	82.00d	20.06b	42.00d	61.47d
Control a	51.90h	10.00e	98.16a	2.88g
Control b	111.33a	27.86a	7.00h	98.07a

Percent disease suppression was determined as [A-B/A] x 100, where A is the disease severity index in the root/shoot of control plants inoculated with only *P. infestans* without bacterial antagonists, B is the disease severity index in the root/shoot of plants treated with both the pathogen and bacterial antagonists.

Control a = pathogen only inoculated (disease control); Control b = non-inoculated (healthy control).

*Means followed by different letter(s) in each column are significantly different at $p \leq 0.05$ according to the Duncan's multiple range test. Experiments were repeated twice, and each replicate consisted of a single pot with one plant per pot.

Table 4. Effect of PGPR strains on different growth parameters in tomato in greenhouse pot trials.

Bacterial isolate	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Chlorophyll (spad units)
AB05	59.26dc*	27.30cb	46.50ba	9.83b	8.16ba	2.16bac	36.83a
AB10	69.46a	33.70a	49.83ba	11.0b	8.50ba	1.66b	35.93a
AB11	68.73a	31.50a	53.33ba	16.0ba	8.33ba	3.0a	36.93a
AB12	65.23bac	28.16b	53.33ba	15.0ba	7.16ba	2.83a	35.60a
AB14	67.0ba	27.76b	52.0ba	14.50ba	8.33ba	2.0bac	35.0a
AB15	67.16ba	28.23b	53.0ba	13.66ba	8.66ba	2.50a	36.20a
AB17	61.13bdc	27.90b	55.16a	20.16a	9.33a	2.50a	37.50a
Control	57.10d	24.93c	40.50b	10.83b	6.33b	1.33c	30.56b

Means were obtained from an individual trial, with six replicates per treatment. The experiment was repeated twice, and each replicate consisted of a single pot with one plant per pot.

*Means followed by the same letter(s) in each column are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

content was also the highest, with a significant difference from the non-inoculated control. There was no significant variation in the chlorophyll content of tomato plants among the bacterial treatments (Table 4). However, foliar observation revealed that non-inoculated (control) plants had less chlorophyll, with a yellowish leaf color (Fig. 3A).

Fig. 3. Promotion of tomato plant growth in the greenhouse by different rhizobacterial isolates.

(**A**) Control (non-inoculated), (**B**) AB05, (**C**) AB10, (**D**) AB11, (**E**) AB12, (**F**) AB14, (**G**) AB14, and (**H**) AB17. Note that the non-inoculated control resulted in retarded shoot growth compared with the treatments with bacterial isolates.

The total chlorophyll content was increased in treatments at over a range of 14% to 19% (Table 4).

Discussion

The application of PGPR is a potentially attractive approach to disease management and improved crop productivity in sustainable agriculture [16]. The present study was carried out to evaluate the efficacy of PGPR for the reduction of late blight disease caused by *P. infestans* and promoting growth in tomato under greenhouse conditions. The results show that treatment with most of the bacterial isolates significantly reduced the severity of disease in host plants caused by artificial inoculation with *P. infestans*. The bacterial isolates not only inhibited pathogen symptoms but also enhanced growth. In the experiment, some bacterial isolates were found to be highly inhibitory to fungal growth, whereas others showed only minor activity. This result suggests that the mode of action exerted and/or the type of antifungal metabolite produced may vary among the isolates and that they are taxonomically different from one another [28]. The isolates that most effectively inhibited fungal growth in the dual culture experiment generated such a large zone of inhibition that there was no physical contact with the pathogen, indicating that the rhizobacteria produce certain antifungal metabolites [15]. Among them, AB15 (*Paenibacillus polymyxa*) was the most effective in terms of disease reduction *in vitro* and under greenhouse conditions.

Several antagonistic bacteria act upon plants by different mechanisms. Mainly, rhizobacteria that produce antibiotics [7] or fungal cell-wall-degrading enzymes such as chitinase [21] have sometimes been sprayed on foliar parts of plants

Fig. 4. Growth enhancement in tomato roots treated with selected rhizobacterial isolates.
Note that the non-inoculated control resulted in retarded root growth compared with the treatments with bacterial isolates.

to reduce disease through direct suppression of the development and infection of fungal pathogens [2]. However, rhizobacteria are usually applied to soil, seeds, or seedlings, wherein they reduce disease through induction of local or systemic resistance in plants [5, 11, 16]. This experiment was also based on soil treatment with antagonistic bacteria for the control of *P. infestans* affecting tomatoes under greenhouse conditions.

In the greenhouse, AB10, AB11, AB15, and AB17 showed significant growth promotion in tomato. However, all the bacterial isolates were capable of enhancing different growth parameters (shoot/root length, fresh biomass, dry matter, and chlorophyll content) in comparison with non-inoculated control plants. Studies regarding the potential of PGPR for biofertilization have been widely reported [1]. Beneficial effects reported for PGPR include increases in a number of parameters such as germination rate, root and shoot growth (length and/or weight), yield, leaf area, chlorophyll content, nutrients, and protein, as well as induction of delayed leaf senescence and tolerance to drought and other stress factors [13, 29]. Another study showed that drenching with PGPR isolates increased the total weight of tubers per potato plant, in addition to effectively controlling late blight disease caused by *P. infestans* [9].

The main conclusions from the current study are that rhizobacteria isolated from the rhizosphere in Gangwon Province, South Korea, have the ability to control late blight disease in tomato *in vitro* and under greenhouse conditions. It has also been concluded that these rhizobacteria possess growth-promoting activities when applied to the soil. The present study also provides information on the application of rhizobacteria as inoculants in agricultural practice. However, in order to develop the best performing PGPR strains for commercial applications, further selection through molecular study and screening in field trials will be necessary.

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