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Biological Control of Phytopathogenic Fungi by *Bacillus amyloliquefaciens* 7079; Suppression Rates are Better Than Popular Chemical Fungicides

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Abstract Rhizobacteria are actively sought for the substitution of chemical fertilizers and pathogen control agents in environment-friendly sustainable agriculture. To be successfully commercialized in the current Korean market as agriculture biomaterials, microbial agents should exhibit both properties of plant growth promotion and pathogen control. That is, the organism must be a phytostimulator as well as a biocontrol agent. These criteria and the survival rate of a rhizobacterium, *Bacillus amyloliquefaciens* 7079, in the soil system were investigated to evaluate the suitability for future commercialization. *B. amyloliquefaciens* 7079-treated seedlings showed 22.8% maximum increase in leaf-length growth, compared with water-treated controls, showing the phytostimulating property. The disease suppression rates of *Phytophthora*-blight of peppers and *Fusarium*-wilt of tomatoes by *B. amyloliquefaciens* 7079 were 1.5 and 2.2 times better, respectively, than by three popular chemical fungicides used in actual agricultural practices to control the respective pathogens. Survival of *B. amyloliquefaciens* 7079 on the rhizoplane and in the rhizosphere was favorable up to 50 days in the soil system employed. These positive properties show that *B. amyloliquefaciens* 7079 is likely to be a suitable candidate for commercialization to market as agricultural biomaterials.

Key words: Rhizobacterium, *Bacillus*, biocontrol, phytostimulator, colonization, *Phytophthora*-blight, *Fusarium*-wilt, commercialization

Plants are either directly or indirectly influenced by rhizobacteria. Among the cultivable rhizosphere bacterial community, the majority of the strains were shown to be harmful to plant growth and less than 20% of them promoted the plant development [24]. These beneficial microorganisms are termed plant-growth-promoting rhizobacteria (PGPR). As an effort to reduce chemical fungicide usage in sustainable agriculture, rhizobacteria application is considered as an alternative method for environment-friendly pathogen control.

The biological mechanism behind pathogen suppression by PGPR is habitat occupation, induction of plant defense system, and antibiosis. Habitat occupation results when competition for nutrients [34] and space occur and the antagonistic strain successfully colonizes the area in question to exclude any proliferation of the pathogen. Specific PGPR also indirectly protects the plant against post-pathogen challenge by stimulating the defense system of the host plant to produce various substances involved in the resistant mechanism [42, 43, 52]. The so-called induced systemic resistance (ISR), predominantly studied with pseudomonads, identified bacterial determinants of lipopolysaccharides, siderophores, and salicylic acid [reviewed in 52]. Antibiosis is a direct method of biocontrol-PGPR, where the bacteria produce various antibiotic compounds to inhibit the growth of the pathogens. Such substances that are shown to be effective for the biological control of plant pathogenic fungi include pyoverdin [30], phenazine [49], pyrrolnitrin [17, 19], and pyoluteorin [20], all studied in *Pseudomonas fluorescens*, and altericidins [26] and pyrrolnitrin [21, 40], identified in *Burkholderia cepacia*. Several cases of mutants overexpressing these antifungal compounds have shown increased efficacy and potential in biological control [32, 46]. In real situations, however, PGPR is likely to execute one method after another or combination of different methods at a time to achieve the goal of preventing the disease.

Biocontrol by *Bacillus* spp. may also involve multiple mechanisms of actions, and several cases of ISR induction have been reported with *Bacillus* spp., both in pot experiments and field conditions [27, 39]. In other studies,

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root colonization of the young plant by inoculant *Bacillus* strains has been associated with resistance towards a variety of pathogens [3, 4, 16, 23]. Antibiosis, however, is the most studied method of pathogen control, and purified compounds alone have been shown to be effective in the biocontrol of plant pathogenic fungi in pot experiments [2]. Antibiotic substances researched in *Bacillus* spp. comprise three groups; the surfactin group [41], the plipastatin-fengycin group [50, 51], and the iturin group [2, 36]. Identification of more new antibiotics and derivatives of previously known antifungal substances in *Bacillus* spp. continues [22]. Extracellular hydrolytic enzymes of chitinases, extensively studied in both *Bacillus* spp. and *Serratia* spp. [13, 29], and proteases might also play a significant role in the antibiosis of biological control.

In planning a microbial agent for commercialization, several factors of the user's expectation should be met in order to successfully infiltrate the current chemical fungicide market; firstly, because the current Korean market circulates all microbial agents as plant growth enhancers, microbial agents must show positive effect on the growth promotion of plants. Secondly, if a microbial agent is to be accepted as a biomaterial for pathogen control, the efficacy must be comparable or higher than agrochemicals favorably used in real practices; the comparison fungicides should not necessarily be those recommended by the manufacturer, but those employed in real situations by the end-users. Moreover, the controlling effect must not be narrowly limited to one pathogen only, but should be effective against multiple pathogens. Thirdly, the introduced microorganism must compete with the endogenous microbial community and survive to some extent on the rhizoplane as well as in the rhizosphere, to successfully colonize and protect the root system.

A soil bacterium, *Bacillus amyloliquefaciens* 7079, that exhibited growth inhibition toward a wide spectrum of plant pathogens, was isolated in a previous study [13]. Due to the effective antibiosis, the strain subsequently became a candidate to be commercialized as an agricultural biomaterial. In this study, the plant growth promoting ability and the biocontrol activity of *B. amyloliquefaciens* 7079 to suppress the two main diseases in South Korea, *Phytophthora*-blight of peppers and *Fusarium*-wilt of tomatoes, were investigated. To obtain an objective idea on how effective the biological control could be, suppression rates were compared with those of chemical fungicides favorably used in actual practices: Three fungicides were selected for each disease by interviewing farmers and sellers of agrochemicals in Gyeongbook and Gyeongnam provinces. The survival of *B. amyloliquefaciens* 7079 on the rhizoplane and in the rhizosphere was also monitored to evaluate the effective colonization of the root area.

MATERIALS AND METHODS

Strains, Media, and Growth Condition

Bacillus amyloliquefaciens 7079 was isolated from the soil of the suburban area of Daegu in South Korea and cultured in Luria Burtani medium [45] at 30°C [13]. A comparison soil strain of a *Serratia* sp. was grown in the same medium at 25°C. *Phytophthora capsici* Leonian and *Fusarium oxysporum* f. sp. *lycopersici* were stock pathogens of the laboratory and were propagated on PDA (Potato Dextrose Agar, Merck) at 28–30°C. Virulence was maintained by isolating fresh strains from the diseased pepper and tomato plants.

Materials and Conditions for Cultivation of Pepper Plants

Artificial soil employed was TKS 2, purchased from Korea Agriculture Materials Ltd. (Seoul, Korea). Asian pepper seeds of *Capsicum annum* Leonian were used in germination and survival-rate-determination experiments. For the biocontrol assays, 7–9 leaf-stage pepper seedlings (*Capsicum annum* Leonian) and 18–20 leaf-stage tomato seedlings (*Lycopersican esculetum* Mill) were purchased from Green Plug Seedlings Ltd. (Milyang, Gyeongnam, Korea). Chemical control agents that were used in comparison experiments of disease suppression were Ethaboxamtriflumizole, Oxadixylcopper hydroxide, and Propamocarbhydrochloride for *Phytophthora*-blight control, and Metalaxyl, Fluquinconazole, and Copper hydroxide for *Fusarium*-wilt control. These agrochemicals were chosen not because the manufacturer suggested these agents for controlling respective pathogens, but because farmers in Gyeongbook and Gyeongnam provinces of South Korea recommended and frequently used these fungicides in actual practice to control *Phytophthora*-blight and *Fusarium*-wilt. Young plants were cultivated in growth chamber at 28°C and 70% humidity. Fluorescent light was left on 24 h.

Production of Zoospores of *Phytophthora capsici*

For the production of zoospores, a 5-mm agar plug from a 6-day-old PDA was inoculated onto V8 juice agar medium (20% V8 juice, 0.4% $CaCO_3$, 1.5% agar, pH 5.0), and the pathogen was propagated for 6 days at 30°C. After flattening the mycelium with a bacterial glass spreader, zoospore induction was carried out under fluorescent light for 2 days. To obtain the zoospores, sterile water was poured directly to the plates and the zoospores were resuspended into the liquid by brushing the mycelium as indicated by Lee and Kim [29]. Final concentration of the zoospores was determined by hemacytometer counts. Typical concentration was 10^6 zoospores/ml with 5 ml of sterile water in a 90-mm plate.

Production of Spores of *Fusarium oxysporum*

For the production of spores, a 5-mm agar plug from a 6-day-old PDA was inoculated into 200 ml of fresh Potato

Dextrose Broth in a 500-ml flask, and the pathogen was propagated for 6 days at 30°C with constant shaking at 160 rpm. By following the method of Hahn *et al.* [13], the liquid culture was then passed though a cheese cloth to remove the mycelium. The spores in the liquid phase were centrifuged at 1,000 ×*g* for 10 min and washed once with water. The spores were resuspended in 200 ml of distilled water. The concentration of spores was determined by hemacytometer counts. Typical concentration was 10^6 spores/ml.

Biocontrol Assay

***Phytophthora*-Blight in Peppers.** In a 10 cm×10 cm (diameter×depth) pot holding 200 ml of artificial soil, 7–9 leaf-stage pepper seedlings were transplanted, and 1,000 zoospores in 5 ml of sterile water were poured directly into the pot in order to artificially induce the disease. After 2 days, 5 ml of chemical or biological control agents were applied to suppress the disease; bacterial cultures were at 10^8 cfu/ml, and the amount of chemical control agents that was recommended by the manufacturer for the pot area of 78.5 cm^2 was resuspended in 5 ml of water (recommended dosage was based on the area of the farmland). Survivals were counted 1 week after the introduction of the control agents. To calculate the disease suppression rates, survivals out of 5 plants were counted with 8 repetitions. Analysis of variance (ANOVA) was performed, and differences between means of treatments were determined by Tukey's test using SAS ver. 8.2.

***Fusarium*-Wilt in Tomatoes.** In a 10 cm×10 cm (diameter×depth) pot holding 200 ml of artificial soil, 18–20 leaf-stage tomato seedlings were transplanted, and 5×10^6 spores in 5 ml of sterile water were poured directly into the pot in order to artificially induce the disease. After 2 days, 5 ml of chemical or biological control agents were applied to suppress the disease; bacterial cultures were at 10^8 cfu/ml, and the amount of chemical control agents that was recommended by the manufacturer for the pot area of 78.5 cm^2 was resuspended in 5 ml of water. Survivals were counted 1 week after the introduction of the control agents. To calculate the disease suppression rates, survivals out of 5 plants were counted with 8 repetitions. Analysis of variance (ANOVA) was performed, and differences between means of treatments were determined by Tukey's test using SAS ver. 8.2.

Seed Germination Assay

To coat the seeds with *Bacillus amyloliquefaciens* 7079 or *Serratia* sp., seeds were dipped in bacterial cultures that had been washed and resuspended in water. Each seed had 10^7 cfu (*Bacillus* sp. 7079) or 10^8 cfu (*Serratia* sp.) of bacterial cells, and the inoculated seeds were immediately planted in 4 cm×4 cm (diameter×depth) pots. Controls were dipped in water. After 2 weeks, germinated seedlings were counted. One set of experiment included 50 seeds, and 5 repetitions were carried out to determine the germination rate.

Determination of Plant Growth Promotion

About 14 days after planting the pepper seeds in 4 cm× 4 cm (diameter×depth) pots, the whole rhizosphere of the 2-leaf-stage pepper seedlings was dipped in either water (control) or bacterial suspensions of 10^8 cfu/ml. Pepper seedlings were then transplanted in 20 cm×15 cm (diameter× depth) pots holding 800 ml soil. The leaf-lengths were measured every 2 days until the 9th leaf, just before flowering.

Microbial Root Colonization Assay

Pepper seeds were coated with 10^7 cfu of *Bacillus amyloliquefaciens* 7079 or 10^8 cfu of *Serratia* sp., as indicated in seed germination assay. Coated seeds were immediately planted in 20 cm×15 cm (diameter×depth) pots holding 800 ml of soil. The artificial soil with endogenous microbial community was used as it was, and not autoclaved. Pots were watered with regular tap water every 2 days. To see the degree of root colonization, bacterial cells were counted for rhizoplane, rhizosphere, and soil, every 10 days until day 50. Rhizosphere was defined as soil tightly held by the root, and the volume of the rhizosphere increased from 20 g to 30 g, 70 g, and to 230 g on day 50, as a root developed. Cell counts were expressed for the total weight of the rhizosphere soil for each measurement. Rhizoplane was determined as the root surface after brushing off the rhizosphere soil as much as possible. The cell counts were measured for the total root weight which increased from 0.04 g on day 10 to 3.2 g on day 50. Soil was defined as the soil left inside the pot after removing the plant and the rhizosphere. A survival curve of the live cells was generated by employing means of two independent experiments.

RESULTS

Determination of Germination Rate and Plant Growth Promoting Activity by *Bacillus amyloliquefaciens* 7079

To find out if the bacteria stimulate the germination of pepper seeds, 50 seeds were coated with *Bacillus amyloliquefaciens* 7079 at 10^7 cells per seed and planted in 4 cm×4 cm (diameter×depth) pots containing 5 ml of artificial soil. Total of 5 repetitions, however, showed only a small effect of the bacteria on germination, and revealed no statistically significant difference between the bacteria-treated samples and water-treated controls (at $P\leq0.05$, results not shown). However, when the leaf-length growth was investigated, a substantial promoting effect was detected with the treated seedlings; the growth promotion was detectable from day 13 with the 5th leaves and maximum stimulation was observed in the 8th leaves, which were

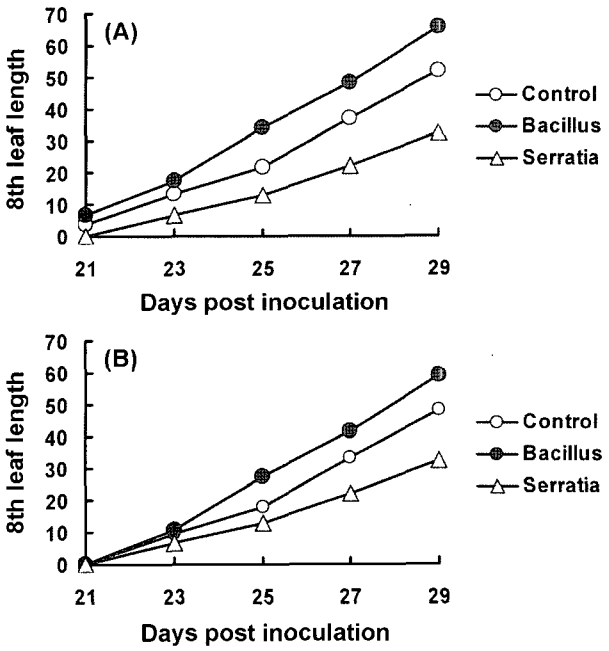

Fig. 1. Leaf growth promotion of pepper plants (*Capsicum annum* L.) by *Bacillus amyloliquefaciens* 7079.
(A) Growth stimulation curve of the 8th leaves. (B) Normalized at day 21 to show the growth comparison of the 8th leaves from day 21 to day 29. Values are means of 4 independent experiments. Abbreviations: Control, treated with water; Bacillus, treated with *Bacillus* sp. 7079. Note that the growth was stimulated in *Bacillus* sp. 7079-treated sample, but was inhibited in *Serratia* sp.-treated sample. Error bars are omitted for clarity.

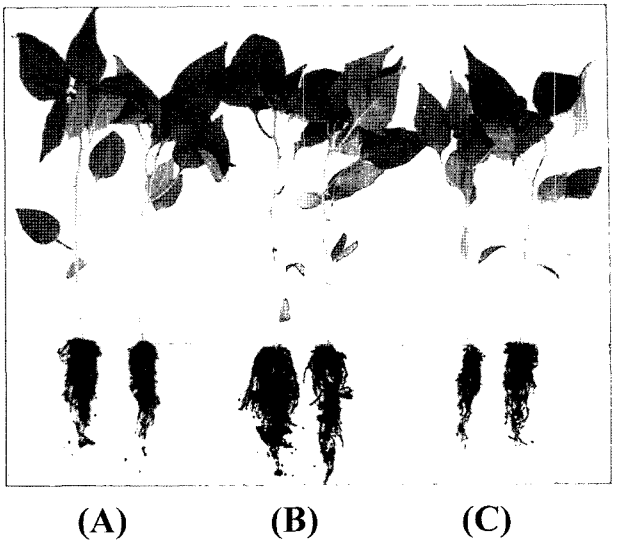

Fig. 2. Root systems of 50-day-old pepper plants.
(A) Water treated. (B) *Bacillus* sp. 7079 treated. (C) *Serratia* sp. treated. Note the extensive root hair in the *Bacillus*-treated sample.

sprouted 21 days after the bacterium inoculation (Fig. 1, closed circles). To demonstrate that not all bacteria can achieve this growth promotion, pepper seedlings were treated with another soil isolate of a *Serratia* sp., which resulted in inhibition of pepper growth (Fig. 1, open triangles). The stimulating effect of *B. amyloliquefaciens* 7079 was accelerated at a later stage of the leaf growth (Fig. 1A), and the 8th leaf length difference of the treated and control seedlings was most prominent on day 29, as shown on the normalized graph in Fig. 1B. During the 8 days investigated, the leaves of the treated samples grew 1.28 mm more each day than the controls, resulting in 22.8% longer leaves in the treated samples than those of the controls, on day 29 (Table 1). On the 50th day after the bacteria inoculation, the overall state of the leaves were extensively developed in the *B. amyloliquefaciens* 7079-treated sample, and the robust growth of root hair system was also noticed (Fig. 2). On the contrary, *Serratia* sp. negatively influenced the root development, and retarded the growth of the root hair system, compared with the controls (Fig. 2C). These results indicate that not all bacteria can promote plant growth, and that *B. amyloliquefaciens* 7079 can be one of the plant growth promoting bacteria that can be categorized as a plant stimulator.

Determination of *Phytophthora*-Blight Suppression Rate of Peppers by *Bacillus amyloliquefaciens* 7079

The efficacy of the biological control of *Phytophthora*-blight of peppers was determined by artificially inducing the disease with 1,000 zoospores of *Phytophthora capsici* in each pot containing 200 ml soil and one 10–12 leaf-stage pepper seedling. The seedlings planted in soils infested with pathogen showed symptoms of the disease 70% of the time in 3–4 days after the transplantation, and

Table 1. Leaf growth promotion of Asian peppers by *Bacillus amyloliquefaciens* 7079.

Treatment[1]	Mean growth rate[2]	Increased leaf length[3]	Growth increase[4]
Water	6.13 mm/day	48.25±2.02 mm	
Bacillus sp. 7079	7.41 mm/day	59.25±4.03 mm	+22.8%

[1]The rhizosphere of two-leaf-stage pepper seedlings (*Capsicum annum* L.) were dipped in water or bacterial cultures (10^8 cfu/ml) and transplanted into 20 cm×15 cm (diameter×depth) pots holding 800 ml soil. The overall lengths and the growth rates of the 8th leaves are compared for the 8 days indicated in Fig. 1.

[2]Values are means of 4 independent experiments for 8 days.

[3]Increased leaf length from day 21 to day 29. Standard errors were determined at $P \leq 0.05$ level.

[4]Percent increased leaf length compared with controls.

Table 2. Suppression rates of *Phytophthora*-blight disease in peppers by biocontrol strain *Bacillus amyloliquefaciens* 7079 and popular chemical fungicides.

Treatment[1]	Survivals[2]	Suppression rate	Effectiveness[3]
No pathogen	5.00 A		
Pathogen only (1,000 zoospores/pot)	1.50 D	30%	
Pathogen+*B. amyloliquefaciens* 7079	3.25 B	65%	1.5×
Pathogen+Ethaboxamtriflumizole[4]	2.50 C	50%	
Pathogen+Oxadixylcopper hydroxide[4]	2.00 C	40%	
Pathogen+Propamocarbhydrochloride[4]	2.00 C	40%	

Five ml of chemical or biological control agents were poured into pots (10 cm×10 cm, diameter×depth) containing 200 ml of soil and one 7–9 leaf-stage pepper plant (*Capsicum annum* L.), 2 days after the pathogen (*Phytophthora capsici*) inoculation. Bacterial cultures were at 10^8 cfu/ml in water. The amount of chemical control agents was employed as directed by the manufacturer. Survivals were counted 1 week after the introduction of the control agents.

[2]Number of survivals out of 5 plants. Values are means of 8 independent experiments. Treatments within an experimental trial followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Tukey's test.

[3]Effectiveness of the disease suppression by *B. amyloliquefaciens*, compared with that of the mean value of the three chemical control agents.

[4]Comparison fungicides are those used in actual situations by pepper growers in Gyeongbook and Gyeongnam provinces, and not necessarily those recommended by the manufacturer.

the ones with symptoms died within a week in every case investigated. In contrast to the pathogen-only control pots, 65% of the seedlings survived in bacteria-treated samples (Table 2). To examine if the biological control is as effective as chemical control of *Phytophthora*-blight of peppers, survival rates by *Bacillus amyloliquefaciens* 7079 treatments were compared with those of the 3 popular chemical control agent treatments (Table 2). The chemical fungicides used were Ethaboxamtriflumizole, Oxadixylcopper hydroxide, and Propamocarbhydrochloride, and they were actively used and recommended by pepper growers in Gyeongbook and Gyeongnam provinces in South Korea. In every case investigated, the survival rates of the biocontrol samples were greater than those of the fungicide-treated samples, being 1.5 times better, regardless of the different fungicides (Table 2). These results indicate that biological control can be as effective as or even superior to chemical control, when a suitable biocontrol agent is employed.

Determination of *Fusarium*-Wilt Suppression Rate of Tomatoes by *Bacillus amyloliquefaciens* 7079

The effectiveness of *Bacillus amyloliquefaciens* 7079 on *Fusarium*-wilt was determined in an artificial system also using pots holding 200 ml soil and one 18–20 leaf-stage tomato seedling in each pot. With the seedlings planted, pathogens were introduced at 5×10^6 spores per pots and incubated for 2 days before the introduction of control agents to sufficiently induce the disease. Within a week, seedlings in the pathogen-only control pots showed symptom of wilting 75% of the time, and the diseased seedlings died in every case investigated (Table 3; Fig. 3B, lanes 2–5). However, tomato seedlings survived 73% of the time, when the infested soil was treated with *B. amyloliquefaciens* 7079 (Table 3). The survived seedlings of the bacteria-treated samples showed robust growth despite of the pathogen (Fig. 3C, lanes 1-4). To ascertain how effective this control rate was, the same conditions

Table 3. Suppression rates of *Fusarium*-wilt disease in tomatoes by biocontrol strain *Bacillus amyloliquefaciens* 7079 and popular chemical fungicides.

Treatment[1]	Survivals[2]	Suppression rate	Effectiveness[3]
No pathogen	5.00 A		
Pathogen only (5×10^6 spores/pot)	1.25 C	25%	
Pathogen+*B. amyloliquefaciens* 7079	3.63 B	73%	2.2×
Pathogen+Metalaxyl[4]	1.75 C	35%	
Pathogen+Fluquinconazole[4]	1.63 C	33%	
Pathogen+Copper hydroxide[4]	1.63 C	33%	

[1]Five ml of chemical or biological control agents were poured into pots (10 cm×10 cm, diameter×depth) containing 200 ml of soil and one 18–20 leaf-stage tomato plant (*Lycopersican esculetum* Mill), 2 days after the pathogen (*Fusarium oxysporum*) inoculation. Bacterial cultures were at 10^8 cfu/ml in water. The amount of chemical control agents was employed as directed by the manufacturer. Survivals were counted 1 week after the introduction of the control agents.

[2]Number of survivals out of 5 plants. Values are means of 8 independent experiments. Treatments within an experimental trial followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Tukey's test.

[3]Effectiveness of the disease suppression by *B. amyloliquefaciens*, compared with that of the mean value of the three chemical control agents.

[4]Comparison fungicides are those used in actual situations by tomato growers in Gyeongbook and Gyeongnam provinces, and not necessarily those recommended by the manufacturer.

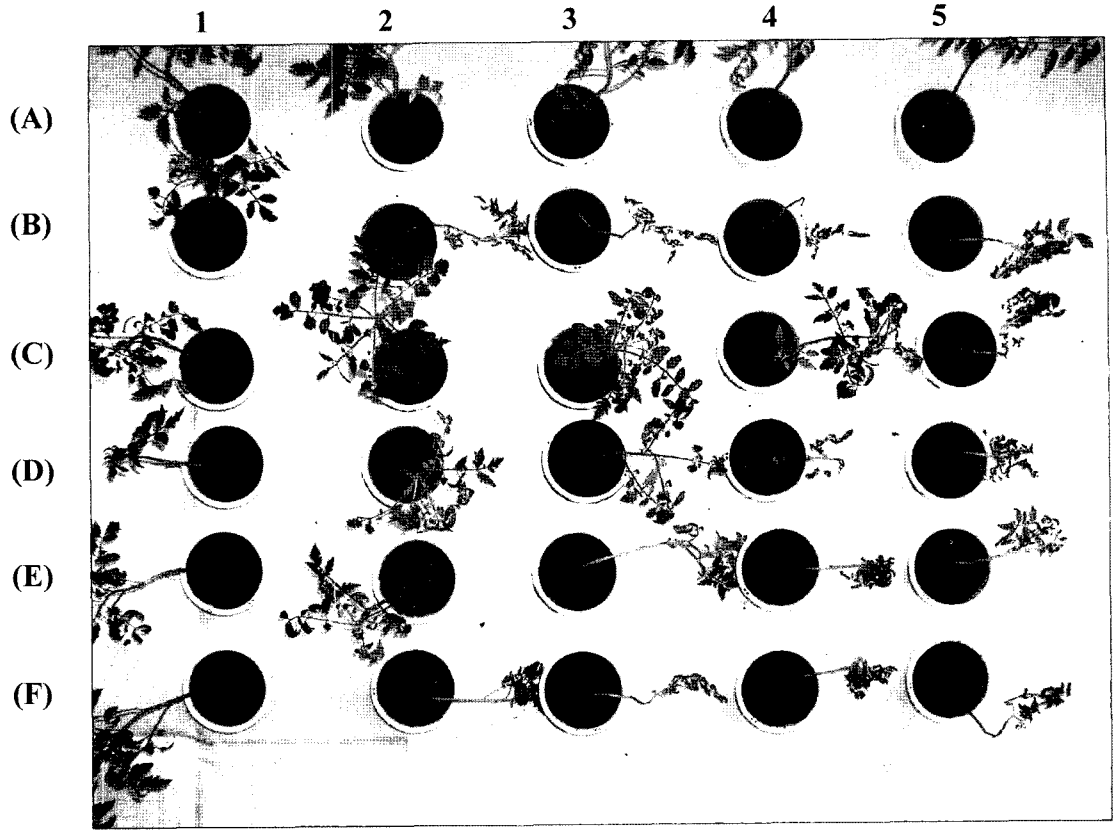

Fig. 3. A picture of *Fusarium*-wilt suppression in tomatoes (*Lycopersican esculetum* Mill) by *Bacillus amyloliquefaciens* 7079 and popular chemical fungicides.
(A) No pathogen (all survived). (B) Pathogen only (only the one in lane 1 survived). (C) Treated with *B. amyloliquefaciens* 7079 (the ones in lanes 1-4 survived). (D) Treated with Metalaxyl (the ones in lanes 1 and 2 survived). (E) Treated with Fluquinconazole (the ones in lanes 1 and 2 survived). (F) Treated with Copper hydroxide (only the one in lane 1 survived).

were employed in chemical control treatment experiments with three fungicides, Metalaxyl, Fluquinconazole, and Copper hydroxide (Figs. 3D, 3E, 3F). These agrochemicals were chosen not because the manufacturer suggested them, but because farmers recommended and frequently used them in actual practices to control *Fusarium*-wilt. As indicated in Table 3, only 30% of tomato seedlings in chemical fungicide-treated pots survived, regardless of the different fungicide treatments. This comparison of the survival rates shows that the control efficacy of *Fusarium*-wilt by *B. amyloliquefaciens* 7079 is over 2 times more effective than those by the popular chemical fungicides. These results, together with the data in Table 2, indicate that biological control by *B. amyloliquefaciens* 7079 is better than chemical fungicide treatment in the control of both *Phytophthora*-blight of peppers and *Fusarium*-wilt of tomatoes.

Determination of the Survival Rate of *Bacillus amyloliquefaciens* 7079 on the Rhizoplane and in the Rhizosphere

To determine how long the biocontrol strains survive in the soil system employed, 10^7 *Bacillus amyloliquefaciens* 7079 cells were coated on pepper seeds and planted in pots holding 800 ml of the artificial soil. Controls were coated with water. Total live cells on the rhizoplane and in the rhizosphere were counted every 10 days up to day 50. Dilution plates obtained with water-treated samples were used as controls for background bacterial community of the potting soil. While no cells of *B. amyloliquefaciens* 7079 were detected on control plates, *Bacillus* sp. 7079 in the treated samples could easily be distinguished from the background bacteria that were originally in the artificial soil; *B. amyloliquefaciens* 7079 colony morphology had a large and opaque appearance. Despite the variety of the background bacteria, the number of *B. amyloliquefaciens* 7079 cells was not significantly reduced but rather proliferated and maintained to 10^5–10^6 cells in both areas, during the period investigated (Fig. 4A). The fact that the live cells in the rest of the pot soil (Fig. 4A, open triangles) were not detected during the same period suggests that *B. amyloliquefaciens* 7079 had colonized primarily the root area. To clarify whether the survival shown was not due to some kind of plant induced action, the same experiment was conducted with cucumbers, and a similar number of

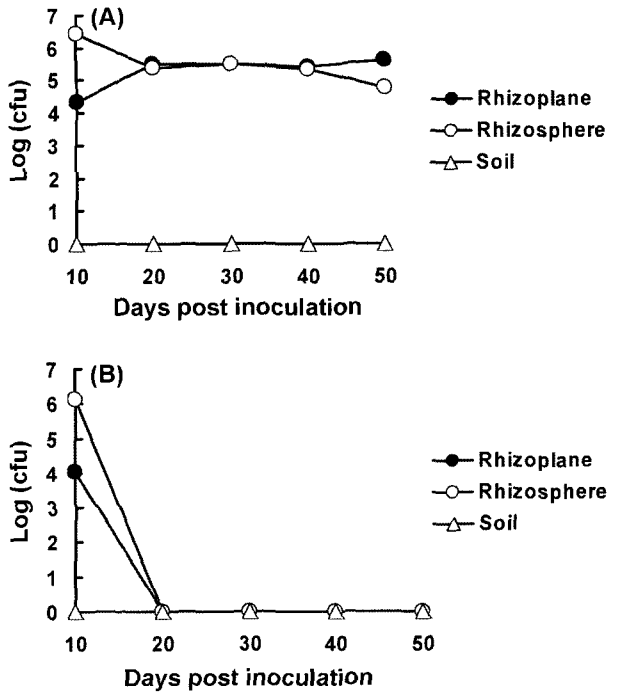

Fig. 4. Survival of *Bacillus amyloliquefaciens* 7079 in the rhizosphere, soil, and on the rhizoplane of peppers (*Capsicum annum* L.). Values are means of 2 independent experiments.
(A) Seeds inoculated with *B. amyloliquefaciens* 7079. (B) Seeds inoculated with a comparison soil strain, *Serratia* sp. Error bars are omitted for clarity.

survivals was detected (results not shown). This suggests that the survival power was not due to a specific plant induced phenomenon, but rather to a true nature of *B. amyloliquefaciens* 7079. In contrast, another soil bacterium of a *Serratia* sp. survived only until day 10 even on the rhizoplane and in the rhizosphere (Fig. 4B). These results indicate that *B. amyloliquefaciens* 7079 was stable up to at least 50 days in the soil system employed, and the bacteria inoculated on seeds had sufficiently proliferated to inoculate the rhizoplane and the rhizosphere.

DISCUSSION

The predominant effort of disease prevention for soilborne pathogens has been chemical fungicide treatment [34], usually by spray irrigation. Unlike airborne pathogens, however, because the disease source and the target sites of the pathogens are both inside the soil, the optimum amount of agrochemicals often cannot reach all the sites where needed. This frequently results in inconsistent and unsatisfying pathogen control efficacies. Selection of resistant strains is another problem encountered with continued use of chemical pesticides. As shown in this study, however, more consistent and superior control efficacies were seen with microorganism than with chemical fungicides, in the artificially infested pot environment (Tables 2 and 3). This seems to be possible because *B. amyloliquefaciens* 7079 could restrain the individual pathogens through direct antibiosis [32], as in the case with general fungicides, but an effective shield generated around the root area by the microorganism might have played a role in the effective control; as the antagonists lived, proliferated, and died, they were likely to form a mutual relationship with the plant and provided a continuous barrier against the pathogen. Therefore, with microbiological control, a multiple mode of preventive action can be expected in the course of producing the final outcome of the healthy plant. One condition, however, is that the survival of the microorganism must be provided to some extent (see below).

Just because a microorganism has good pathogen control efficacy, it does not necessarily mean that the commercialized strain could successfully infiltrate the current market. That is, in addition to the biocontrol ability, the microorganism must also enhance plant growth. Researchers, however, have not always been able to observe plant growth promotion among different cultivars of *Bacillus* sp. [5, 12, 18]. On the contrary, *B. amyloliquefaciens* 7079 exhibited effective growth promotion for both leaf-elongation (Fig. 1 and Table 1) and root development (Fig. 2), satisfying the first condition for an agriculture biomaterial. In the root, it has been reported that the deaminase produced by various rhizobacteria establishes a sink for 1-aminocyclopropane-1-carboxylate, thereby lowering the endogenous ethylene levels [9, 11, 14]. As a consequence, root elongation is enhanced in bacteria-treated seeds or seedlings, and extensive growth of the root hair seems to have resulted in the *B. amyloliquefaciens* 7079-treated sample (Fig. 2). This healthy root system could have contributed to the development of the leaves shown in Table 1. It is also possible that *B. amyloliquefaciens* 7079 contributed as nutrients for the plant, as the strain colonized, grew, and died on the rhizoplane and in the rhizosphere to give the robust growth of the leaves.

Another aspect to consider in commercialization is that if the biocontrol microbial agent is to be readily used by the end-users (farmers), the product must be as effective or superior to the conventional chemical fungicides that they currently employ in actual agricultural practices. Farmers often use chemicals that are not recommended by the manufacturer because of their previous experience of the efficacy of certain fungicides in controlling specific diseases. One example is metalaxyl (Table 3); despite the recommendation for *Phytophthora*-blight control, the fungicide was favorably used and circulated to control *Fusarium*-wilt in Gyeongbook and Gyeongnam provinces. One possible explanation could be that a metalaxyl-resistant strain of *Phytophthora capsici* had been selected, and the fungicide was no longer effective in this area. Another factor to consider is that, for convenience purposes, farmers

usually prefer products that are applicable to several pathogens and crop cultivars, just as they would expect for conventional agrochemicals (S. C., personal communication). To provide an idea as to how effective biological control can be, this study compared the efficacy directly with those of the chemical control agents favorably used by farmers. Because the goal of this study was to provide information for farmers and to present ideas for a successful infiltration of the biocontrol agents into the current market, the fungicides used for comparison were not necessarily those recommended by the manufacturer, but those employed in real situations by the end-users. Moreover, the efficacies were compared for two problematic diseases of peppers and tomatoes to widen the application spectrum. As indicated in Tables 2 and 3, *B. amyloliquefaciens* 7079 was not only effective in controlling the two major pathogens, but the control rates were also consistently 1.5–2.2 times better than the popular fungicides. These results seem to imply that biological control can be better than or, at least, as effective as popular chemical control when a proper antagonist is employed at the proper time point. Furthermore, since one strain can be applied for two different pathogens for two different plants, application of a biological control agent is shown not to be limited to a specific pathogen or plant.

For a biocontrol strain to exhibit a continuously positive effect on the plant and negative effect on the soilborne pathogens, it is required that the strain should survive to some extent on the rhizoplane and in the rhizosphere. This will assure the plant to receive a continuous shield from the invading pathogen. Survival of *B. amyloliquefaciens* 7079 in the soil system was superior to other soil strains investigated; relatively stable numbers of cells were detected on the rhizoplane and in the rhizosphere of pepper seedlings, until 50 days from the strain inoculation on seeds (Fig. 4A). Therefore, *B. amyloliquefaciens* 7079 seems to compete well with the endogenous microbial community in the soil, to successfully colonize the root area. This is, however, not the case for all soil bacteria, since the survival of a *Serratia* sp. was shown to be relatively poor (Fig. 4B). The degree of survival of the two strains was roughly the same for cucumber seedlings, suggesting that the efficient survival power of *B. amyloliquefaciens* 7079 was not due to some kind of plant dependent action, but a true nature of the bacteria. It cannot, however, exclude the possibility that this survival rate occurs only in the soil system employed and under the well-regulated artificial environment.

Environmental factors that have been reported to influence the survival of the microorganisms included crop cultivar [7], cropping pattern [8], and soil type [47]. Yet other studies indicate that soil type is a minor factor for the variation of *Bacillus* sp. [10, 38]. Invariant survival rate was also seen with *B. amyloliquefaciens* 7079 inoculated in different types of soil (results not shown). Moreover, colonization seems to be independent of crop cultivars of peppers and cucumbers for *B. amyloliquefaciens* 7079. Among the abiotic factors (i.e. nutrients, humidity, and temperature), however, temperature might play a key role in influencing both the colonization of rhizobacteria and expression of biocontrol mechanisms [28]. One environment often considered to be a good candidate for maintaining consistent survival of the introduced antagonist is greenhouse condition, where the abiotic and biotic conditions are constantly monitored throughout the plant development. To maximize the survival and obtain the full benefit of *B. amyloliquefaciens* 7079, the greenhouse soil can be inoculated with the bacterium before the transplant of the seedlings, or the strains can be used as root drench to colonize the rhizosphere of the young plants being transplanted to the greenhouse soil. Another useful application of *B. amyloliquefaciens* 7079 would be at the seedling production stage; seeds could be coated with the antagonist strain before sowing, as in the case for *Bacillus subtilis* GB03 used on cottons [4], but a more practical method is to mix the microorganisms into the potting soil and let the bacteria colonize the rhizosphere of the developing young plant. The bacterial coating of the root would likely provide a protection against plant pathogens that might be present in the transplantation soil. Whether the strain will show the same amount of survival rate in field environment, however, is another issue. In fact, several field cases of unsuccessful biocontrol results have been correlated to poor root colonization of the antagonists. Provided that a commercialized antagonist is well formulated to support the survival during the circulation period, the abundance of *Bacillus* strain externally introduced is likely to decrease in the soil with time [1, 15]. Therefore, a booster treatment with additional microorganisms during the plant growth season seems to be a wise decision [27].

Microbiological control researches have been focused on isolating strains showing antagonism towards specific phytopathogenic fungi, and mainly identified species in 3 genera, *Pseudomonas*, *Bacillus*, and *Trichoderma* [37]. Recent studies also revealed several species in *Serratia* [27, 44]. Efforts have been concentrated on the identification of the antagonistic substances that these biocontrol strains produce, and the majority of the contribution was made in the isolation and purification of antibiotics produced by *Pseudomonas* spp. This is largely because the gene introduction system and genetic manipulation techniques are well-established for *Pseudomonas* spp. The problem, however, is that these organisms are generally not commercially available for use by farmers, primarily because of difficulties in maintaining the survival of the strains during the circulation period. Commercialized microbial products are rather widely formulated with *Bacillus* spp. both in the

United States and in South Korea [26, 30]. If one important goal of agricultural research is to provide solid scientific information for farmers in selecting agricultural practices [27], biocontrol mechanisms in *Bacillus* spp. as well as isolating various antagonists of *Bacillus* strains [6] should require more interests in future research directions. Identification of antibiotic substances produced by these antagonists contributed to our knowledge in one method of biological control, but the biological mechanisms behind other methods, such as induced systemic resistance and root colonization in *Bacillus* spp., have not been studied with equal emphasis. It should be noted that the property of a biological material is different from homogenous composition of a single chemical compound and does not achieve its goal of preventing diseases by one specific mechanism, especially in real environment.

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