J. Microbiol. Biotechnol. (2007), **17**(1), 96–103

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

© The Korean Society for Microbiology and Biotechnology

Two Bacterial Entophytes Eliciting Both Plant Growth Promotion and Plant Defense on Pepper (*Capsicum annuum* L.)

KANG, SEUNG HOON[1,2], HYUN-SOO CHO[1], HOON CHEONG[1], CHOONG-MIN RYU[1], JIHYUN F. KIM[1], AND SEUNG-HWAN PARK[1,2]*

[1]*Laboratory of Microbial Genomics, Systems Microbiology Research Center, KRIBB, Daejeon 305-806, Korea*
[2]*Functional Genomics, School of Science, University of Science and Technology, Daejeon 305-333, Korea*

Received: August 18, 2006
Accepted: September 25, 2006

Abstract Plant growth-promoting rhizobacteria (PGPR) have the potential to be used as microbial inoculants to reduce disease incidence and severity and to increase crop yield. Some of the PGPR have been reported to be able to enter plant tissues and establish endophytic populations. Here, we demonstrated an approach to screen bacterial endophytes that have the capacity to promote the growth of pepper seedlings and protect pepper plants against a bacterial pathogen. Initially, out of 150 bacterial isolates collected from healthy stems of peppers cultivated in the Chungcheong and Gyeongsang provinces of Korea, 23 putative endophytic isolates that were considered to be predominating and representative of each pepper sample were selected. By phenotypic characterization and partial 16S rDNA sequence analysis, the isolates were identified as species of *Ochrobacterium*, *Pantoea*, *Pseudomonas*, *Sphingomonas*, *Janthinobacterium*, *Ralstonia*, *Arthrobacter*, *Clavibacter*, *Sporosarcina*, *Acidovorax*, and *Brevundimonas*. Among them, two isolates, PS4 and PS27, were selected because they showed consistent colonizing capacity in pepper stems at the levels of 10^6–10^7 CFU/g tissue, and were found to be most closely related to *Pseudomonas rhodesiae* and *Pantoea ananatis*, respectively, by additional analyses of their entire 16S rDNA sequences. Drenching application of the two strains on the pepper seedlings promoted significant growth of peppers, enhancing their root fresh weight by 73.9% and 41.5%, respectively. The two strains also elicited induced systemic resistance of plants against *Xanthomonas axonopodis* pv. *vesicatoria*.

Key words: *Pseudomonas rhodesiae*, *Pantoea ananatis*, bacterial endophyte, plant growth-promoting rhizobacteria, induced systemic resistance, pepper

*Corresponding author
Phone: 82-42-860-4410; Fax: 82-42-879-8595;
E-mail: shpark@kribb.re.kr

Plant growth-promoting rhizobacteria (PGPR) are free-living or root-associated bacteria that can increase plant growth and productivity [23]. PGPR are also used as biological control agents owing to their capacity to reduce the development of plant diseases caused by plant pathogenic fungi, bacteria, viruses, and nematodes [6, 27, 39–41]. Various mechanisms by which PGPR promote plant growth and elicit plant defense have been proposed, and some of these have been described in detail. Production of phytohormones such as auxins, gibberellins, cytokinins, and bacterial volatiles (*e.g.*, 2,3-butanediol and acetoin) as a mechanism of plant growth promotion by PGPR has been demonstrated [1, 3, 12, 13, 36, 44]. Direct suppression of the growth of plant pathogenic fungi by PGPR through the synthesis of antimicrobial compounds such as antibiotics, siderophores, and hydrolytic enzymes has been studied extensively [6, 10, 16, 46]. PGPR could also elicit plant defense mechanisms against foliar or soilborne pathogens; this is referred to as induced systemic resistance (ISR) [25, 47]. ISR by PGPR could be a factor in the suppression of plant diseases in the greenhouse and field against a broad range of plant pathogens, including viruses, fungi, bacteria, and nematodes [6, 24, 26, 45].

It is also known that some of the PGPR strains can colonize inside plant tissues, and bacterial strains that naturally exist in healthy plant tissues are referred to as "endophytes." Halmann *et al.* [14] defined endophytic bacteria as "bacteria that can be isolated from surface-disinfested plant tissue or extracted from within the plant, and that do not visibly harm the plant." Most of the endophytes reported previously were isolated by maceration of surface-sterile plant tissues [2, 11, 20, 35, 43]. Various endophytes have been isolated from agronomic crops and prairie plants [7, 14, 27, 49], and many of them have been

utilized as microbial inoculants to control plant pathogens and promote plant growth [2, 8, 9, 15, 28, 29, 30].

Pepper (*Capsicum annuum* L.) is an important agronomic crop of high economic value and is frequently used as a condiment worldwide [19, 34]. Pepper is one of the most important crops in Korea, because pepper powder has long been used as one of the main spices of *kimchi*, a traditional Korean food. Major yield losses during pepper cultivation in the field are generally the result of plant diseases [19, 34]. To protect pepper plants from disease, many disease control strategies, including application of chemical fungicides, adoption of disease-resistant varieties, and biological or cultural control, have been studied [5, 31, 34, 48]. However, few results have been published regarding microbe-mediated measures such as PGPR- or endophyte-mediated biological control for pepper plant diseases [21]. An analysis of the bacterial community in the rhizosphere of the pepper plant was conducted [22]. As a result of phylogenetic analysis of partial 16S rDNA clone sequences, the study found that 32 clones (41%) out of the total 78 clones belonged to the gamma-*Proteobacteria*, which include *Pseudomonas* and *Pantoea* spp. Lucas Garcia *et al.* [32] reported that *Pseudomonas fluorescens* strain Aur 6, which was isolated from *Lupinus hispanicus* and characterized as a plant growth-promoting bacterium, was an effective and persistent colonizer of pepper roots, but it did not behave as an endophyte. Besides Gram-negative endophytes, in Thailand, greenhouse screening of previously reported endophytic *Bacillus* spp. was conducted to demonstrate that ISR was elicited in other crops, including a local variety of pepper [21]. The long cayenne pepper (*C. annum* var. *acuminatum*) and *Colletotrichum gloeosporioides* pathosystem were used in the study. Mixed or single treatments of endophytic spore-forming bacteria elicited ISR in this system. However, no result indicated that a single PGPR strain elicits both plant growth promotion and ISR on pepper.

In this study, we intended to isolate endophytic PGPR strains that could be utilized as microbial inoculants for eliciting both plant growth promotion and ISR on pepper. Out of 23 preselected putative endophytes identified by 16S rDNA sequence analysis, we selected two strains, PS4 and PS27, which showed high, consistent colonizing capacity in pepper stems. The two endophytic strains showed consistent abilities to increase plant growth and plant defense against bacterial spot caused by *Xanthomonas axonopodis* pv. *vesicatoria*. This is the first report on bacterial endophytes that elicit both ISR and plant growth promotion on pepper plants.

MATERIALS AND METHODS

Isolation of Endophytic Bacteria

Samples of healthy pepper plants were collected at 25 locations in the Chungcheong and Gyeongsang provinces of Korea from June to September 2003. Whole pepper plants collected from the field and wrapped in plastic bags were brought to the laboratory within 6 h and kept at 4°C until further processing. Putative endophytic bacterial strains were collected from the pith of stems. In the laboratory, the stem of the whole pepper plant was wiped with 70% ethanol and flame-sterilized. A portion of the stem about 3 cm in length was severed aseptically at 10 cm above the crown, and the bark of the stem was peeled off to exclude potential contamination of epiphytic microorganisms. The pith samples were ground with a sterile mortar and pestle. Tissue extracts were then serially diluted in 12.5 mM potassium phosphate buffer (pH 7.4) and plated on 1/10 strength tryptic soy agar medium (TSA, BD Co, Sparks, U.S.A.). All colonies that displayed differentiable colony morphologies from each pepper sample were picked for further study after incubation at 30°C for two or three days. Bacterial isolates were stored at −70°C in tryptic soy broth with 15% glycerol.

Bacterial Growth Conditions and Phenotypic Characterization

The endophytic bacterial isolates were grown on 1/10 strength TSA at 30°C in a growth chamber. A plant-pathogenic bacterium, *X. anoxopodis* pv. *vesicatoria* (*Xav*), was grown on LB agar medium, and *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) and *P. syringae* pv. *tabaci* (*Ps tabaci*) were grown on King's B medium. Colony morphologies of each bacterial isolate, including color, shape, size, motility, and division mode, were observed with the naked eye or with a microscope (Nikon ECLIPSE E600, Nikon, Tokyo, Japan) at 1,000× magnification.

Amplification, Sequencing, and Phylogenetic Analysis of 16S rDNA

Bacterial isolates were identified by the analysis of 16S rDNA sequences and phylogenetic relatedness. Chromosomal DNA was isolated by following standard procedures [42]. For amplification of the partial 16S rRNA gene of approximately 1.3 kb, a pair of PCR primers, GF1 (5'-TAACACATGCAAGTCGAACG-3') and GR1 (5'-GTGT-GACGGGCGGTGTGTACAAG-3'), was used. To amplify approximately 1.5 kb, another set of 16S rRNA gene primers, BSF8 (5'-AGAGTTTGATCCTGGCTCAG-3') and BSR1541 (5'-AAGGAGGTGATCCAGCCGCA-3'), was used. The PCR mixtures consisted of 20 pmol of each primer, AccuPower PCR PreMix, *Taq* DNA polymerase, dNTP, Tris-HCl, KCl, $MgCl_2$, a stabilizer, and tracking dye (BIONEER Co., Daejeon, Korea) at a 20 µl volume. The amplification reaction, DNA sequencing, and phylogenetic analysis were performed as described by Cheong *et al.* [4].

Inoculation of Pepper Plants with Bacterial Isolates and Confirmation of the Reisolated Bacterial Strains

To test whether bacterial isolates were capable of colonizing inside the pepper stem, the putative endophytic isolates

were introduced into pepper seedlings grown aseptically under *in vitro* conditions. Chili pepper seeds (*C. annuum* L. cv. Bukang) were surface-sterilized using 5% sodium hypochlorite (NaOCl) for 30 min, 70% ethanol for 30 s, and rinsed ten times with sterile distilled water. The seeds were then placed on 3S medium (0.44% MS salt including vitamins, 3% sucrose, and 0.8% plant agar, pH 5.8) in a transparent sterile container. The pepper seedlings were grown in a growth chamber at 25°C under a 12-h/12-h of light/dark cycle. Four-week-old pepper seedlings were inoculated with 10 µl of bacterial suspension (10^6 CFU/ml) using a 26-gauge needle attached to a 1-ml syringe as described by Zinniel *et al.* [49]. The inoculated pepper seedlings were grown for two weeks, the surfaces of the stems were sterilized using 70% ethanol, and then the introduced bacterial strains were reisolated from the sterilized stems of pepper seedlings. Phosphate buffer was used as a control for the inoculation and reisolation studies. The experiment was repeated twice with 10 replications (one plant per replication).

To confirm whether the reisolated bacteria were identical to the inoculated strains, randomly amplified polymorphic DNA (RAPD) analysis was performed with a RAPD primer, OPY-7 (CTGGACGTCA, Operon Biotechnologies, Inc., Huntsville, U.S.A.). The thermocycling conditions consisted of one cycle of pre-PCR at 94°C for 5 min, 36°C for 2 min, and 72°C for 2 min, followed by 35 amplification cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min, and a final polymerization step of 72°C for 10 min with a GeneAmp PCR System 9700 (Perkin-Elmer, Foster City, U.S.A.).

Hypersensitive Response and Pathogenicity Test

To test whether the isolates were pathogenic to plants, we assessed plant responses after infiltration of bacterial suspensions at 10^6–10^7 CFU/ml into the leaves of pepper (*C. annuum* L. cv. Bukang) and tobacco (*Nicotiana benthamiana*) plants, as described previously [33]. To assay non-host hypersensitivity responses, *Pst* DC3000 and 12.5 mM potassium phosphate buffer (pH 7.4) were used as a positive and negative control, respectively. For the pathogenicity assay, *Xav* was used as a control pathogen. The presence of the main symptom (bacterial spot) was assessed seven days after infiltration.

Evaluation of Plant Growth Promotion and Induced Resistance Against Bacterial Leaf Spot Disease

To test the effect of the bacterial isolates on growth promotion in pepper seedlings, 10 ml bacterial suspensions adjusted to an optical density of 1.0 at 600 nm were used to drench three-week-old pepper seedlings. The same volume of 0.05 M $MgSO_4$ solution was used as a control treatment. Seedlings were grown further under a 12-h/12-h of light/dark cycle in a greenhouse at 25°C. Two weeks after drenching with the bacterial isolates, growth parameters such as height, total fresh weight, and root fresh weight were measured. Additionally, to assess the effect of the selected isolates on induced resistance against the bacterial spot pathogen, 10 ml of bacterial suspensions diluted to an optical density of 1.0 at 600 nm were also used to drench three-week-old pepper seedlings. The same volumes of 0.05 M $MgSO_4$ and a chemical inducer, 1 mM benzothiadiazole (BTH), were used as a negative and positive control, respectively. Four days after application, bacterial suspensions of *Xav* (OD_{600}=0.01) were forced to penetrate pepper leaves using the needleless syringe method [33]. Seven days after the pathogen challenge, disease severity was assessed as described by Ryu *et al.* [37]. The severity of symptoms on the leaf was scored from 0 to 5; 0=no symptoms, 1=mild chlorosis, 2=chlorosis only, 3=chlorosis and mild necrosis, 4=necrosis, and 5=severe necrosis of the inoculated area. The experiment was repeated three times with 10 replications (one plant per replication).

Statistical Analysis

Analysis of variance for the experimental datasets was performed using JMP software version 5.0 (SAS Institute Inc., Cary, U.S.A.). Significance of the effect of each treatment was determined by the magnitude of the *F*-value (P=0.05). When a significant *F*-value was obtained for treatments, separation of means was accomplished by Fisher's protected least significant difference (LSD) at P=0.05.

Results and Discussion

Isolation and Characterization of Endophytic Bacteria from Pepper Stems

Whole pepper plants were collected at 25 locations in the Chungcheong and Gyeongsang provinces of Korea from June to September 2003, and endophytic bacterial strains were isolated from the plant samples. To avoid contamination during isolation of endophytic bacteria from inner plant tissues, the pepper stems were peeled after surface-sterilization. By observing colony morphologies, a total of 150 bacterial isolates were collected from the pith of pepper stems. Among them, 23 putative endophytic isolates that predominated and were representative of each pepper sample were chosen for further study. All 23 bacterial isolates were identified by partial 16S rDNA sequence analyses as described in Materials and Methods. The lengths of the 16S rDNA sequences of the isolates determined in this study ranged from 431 to 679 nucleotides, and the analysis of the sequences revealed that the closest species of these isolates include those of genera *Ochrobacterium*, *Pantoea*, *Pseudomonas*, *Sphingomonas*, *Janthinobacterium*, *Ralstonia*, *Arthrobacter*, *Clavibacter*, *Sporosarcina*, *Acidovorax*, and *Brevundimonas* (Table 1). The 16S rDNA similarity

Table 1. Identification and characterization of bacterial strains isolated from the pith tissue of pepper stems.

Strains	Identification[a]	HR (24 h)[b]		Symptom (day 5)[c]	
		Pepper	Tobacco	Pepper	Tobacco
PS1	*Ochrobactrum* sp.	–	–	–	–
PS2	*Pantoea* sp.	–	–	–	–
PS3	*Pseudomonas* sp.	+/–	–	–	–
PS4	*Pseudomonas* sp.	–	–	–	–
PS5	*Sphingomonas* sp.	–	–	–	–
PS6	*Sphingomonas* sp.	–	–	–	–
PS7	*Janthinobacterium* sp.	–	–	–	–
PS8	*Pseudomonas* sp.	–	–	+	–
PS9	*Ralstonia* sp.	+/–	+/–	–	–
PS11	*Pseudoxanthomonas* sp.	–	–	–	–
PS12	*Arthrobacter* sp.	–	–	–	–
PS13	*Pseudomonas* sp.	+	+	–	–
PS14	*Janthinobacterium* sp.	+	–	–	+
PS15	*Janthinobacterium* sp.	+/–	–	–	–
PS17	*Janthinobacterium* sp.	–	–	–	–
PS19	*Janthinobacterium* sp.	–	–	–	–
PS21	*Sporosarcina* sp.	–	–	–	–
PS24	*Acidovorax* sp.	–	–	–	–
PS25	*Brevundimonas* sp.	–	–	–	–
PS26	*Janthinobacterium* sp.	–	–	–	–
PS27	*Pantoea* sp.	–	–	–	–
PS28	*Clavibacter* sp.	+/–	+/–	–	–
PS29	*Pseudomonas* sp.	+	+	–	–
Controls	*X. axonopodis* pv. *vesicatoria*	–	+	+	–
	P. syringae pv. *tabaci*	+	–	–	+
	P. syringae pv. *tomato* DC3000	+	+	–	–
	Potassium phosphate buffer	–	–	–	–

[a]Identification of isolates was conducted by partial 16S rDNA sequence analysis.
[b]–: no HR (visual cell death on bacterial inoculated area); +: HR within 24 hrs; +/–: late HR after 48 h.
[c]–: no symptoms; +: symptoms seven days after infiltration.
Plant responses were detected 24 h and five days after infiltration of bacterial suspensions of each isolate at 10^6–10^7 CFU/ml into the leaves of tobacco (*N. benthamiana*) for hypersensitivity response (HR) and pepper (*C. annuum* L. cv. Bukang) for disease assay, respectively.

values of each isolate to its closest strain were found to be between 95.7 and 100%.

To examine whether the endophytic isolates were capable of causing plant diseases, we conducted a hypersensitive response (HR) assay. As shown in Table 1, seven isolates produced an HR on pepper leaves after infiltration of a 10^7–10^8 CFU/ml bacterial suspension. Four isolates out of these also elicited an HR on tobacco leaves. These results indicated that the seven strains have the potential to act as pathogens in certain plants. We also measured lesion development (symptoms) at 5–7 days after inoculation with 10^5–10^6 CFU/ml bacterial suspension. Inoculation of strain PS8 (*Pseudomonas* sp.) and strain PS14 (*Janthinobacterium* sp.) resulted in development of symptom-like lesions on pepper and tobacco, respectively. For the plant pathogenic bacteria, two pathovars (tobaci and tomato) of *P. syringae* induced an HR on the non-host pepper plant, and infiltration of *Xav* and *Pst* DC3000 showed an HR on tobacco. In contrast, each host plant that was challenged with *Xav* on pepper or *Ps tabaci* on tobacco showed severe symptoms at the area of inoculation. Of 23 selected isolates, these seven isolates were eliminated from the list of candidates for further study because they could be potential pathogens in some plants. Potential plant pathogens cannot be utilized as microbial inoculants for enhancing plant growth and reducing the development of plant disease. After eliminating the potential pathogens, the remaining 15 isolates were assessed for their capacity to colonize plant tissues.

Confirmation and Assessment of the Capacity of Endophytic Colonization

To confirm the endophytic growth and to assess the colonization capacity of the selected bacterial isolates in pepper tissue, we performed an inoculation and reisolation experiment twice using four-week-old pepper seedlings

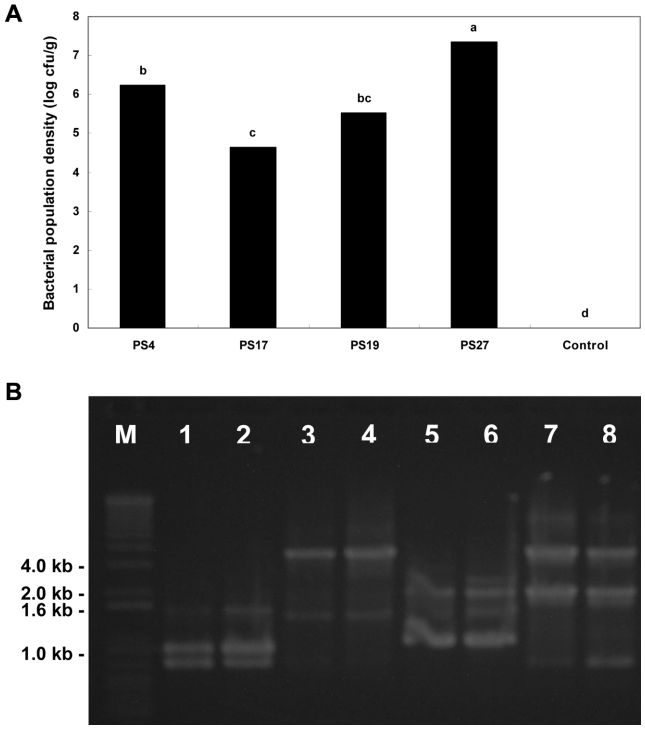

Fig. 1. Colonization of four endophytic isolates in pepper stems and confirmation of the reisolates.

A. The colonizing capacity of four selected endophytic isolates, PS4, PS17, PS19, and PS27, in stems of pepper seedlings was analyzed by inoculation and reisolation experiments. The population densities of the bacteria reisolated from pepper stems were measured at two weeks after inoculation. No indigenous bacteria were found in the pepper seedlings. The experiment was repeated twice with 10 replications (one plant per replication). **B**. The identities of the reisolated bacteria and the inoculated strains were confirmed by RAPD analysis with OPY-7 primer (CTGGACGTCA, Operon Biotechnologies, Inc., Huntsville, U.S.A.). In each case, the DNA band patterns between the two strains were shown to be the same. Lane M, DNA size marker (1-kb ladder); lane 1, PS4 (inoculated strain); lane 2, PS4 (reisolated strain); lane 3, PS17 (inoculated strain); lane 4, PS17 (reisolated strain); lane 5, PS19 (inoculated strain); lane 6, PS19 (reisolated strain); lane 7, PS27 (inoculated strain); lane 8, PS27 (reisolated strain).

grown aseptically *in vitro*, as described in Materials and Methods. Population densities of the bacteria reisolated from pepper stems and grown on the MS medium ranged from 7.0×10^3 to 3.1×10^7 CFU/g tissue (fresh weight) at two weeks after inoculation, depending on the strain. No indigenous bacteria were isolated from the control plants in which only phosphate buffer was used for inoculation (Fig. 1A). We could observe only one type of colony when we plated macerated pepper stems inoculated with each strain. For further confirmation of reisolated strains compared with the introduced strains, RAPD analysis was performed for the two strains, and the two strains did, indeed, show identical band patterns (Fig. 1B). In this assessment, four isolates, PS4 (*Pseudomonas* sp.), PS17 (*Janthinobacterium* sp.), PS19 (*Janthinobacterium* sp.), and PS27 (*Pantoea* sp.), out of 15 isolates showed consistently high colonizing capacity in pepper stems at levels ranging from 10^5 to 10^7 CFU/g at 14 days after inoculation (Fig. 1A).

Pepper Endophytes Elicit Plant Growth Promotion and Induce Systemic Resistance on Pepper

Two endophytic strains, PS4 and PS27, showing the highest levels of colonizing capacity [1.9×10^6 and 3.1×10^7 CFU/g tissue (fresh weight), respectively] in pepper stems were chosen in order to test whether they are capable of promoting plant growth and inducing systemic resistance against *Xav* on pepper. Drenching application of the two strains on the crown part of the pepper seedlings significantly promoted the growth of pepper at two weeks after treatment (Table 2). The two strains, PS4 and PS27, enhanced shoot height as much as 16.6 and 17.2%, total fresh weight as much as 27.7 and 15.3%, and root fresh weight as much as 73.9 and 41.5%, respectively. From these results, it was found that the two endophytic strains were effective, especially in promoting root growth. Considering that limited root growth is one of the major problems in production of plug seedlings, the increase of root biomass induced by the two strains is an interesting result in terms of application. In many cases, pepper growers have disregarded this factor because root growth is not readily recognizable when transplanting the seedlings to the field. In fact, when seedlings are transplanted to the field or greenhouse, root growth limitations often result in unhealthy seedlings that are more susceptible to environmental stresses such as heat or drought. Further identification of the two strains by additional analyses of 16S rDNA sequences showed that strain PS4 was closest to *Pseudomonas rhodesiae* strain CIP 104664 (perfectly matched in 1,522 bp of 16S rDNA sequences, GeneBank Accession No. AF064459), and

Table 2. Effect of endophytic bacterial strains PS4 (*Pseudomonas* sp.) and PS27 (*Pantoea* sp.) on the growth of pepper seedlings.

Treatments	Height (cm)	Total fresh weight (g)	Root fresh weight (g)
PS4	21.35±0.40	16.85±0.52	5.53±0.29
PS27	21.45±0.57	15.21±0.61	4.50±0.33
Control	18.30±0.88	13.19±0.88	3.18±0.24

The number indicates the mean and standard deviation of ten replicated experiments.

Ten ml of bacterial suspension diluted to OD_{600}=1 was used to drench three-week-old pepper seedlings. The same volume of 0.05 M $MgSO_4$ was used as a control. Growth parameters were measured two weeks after bacterial inoculation.

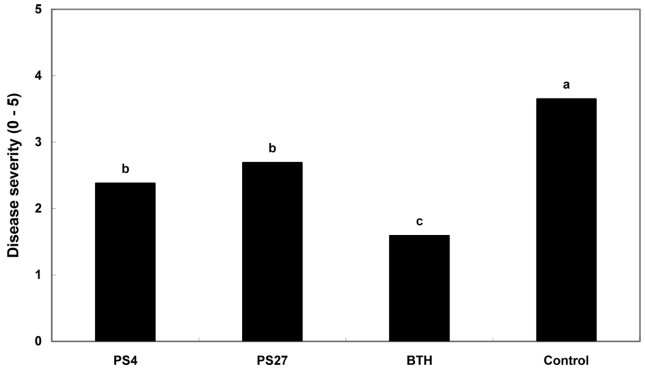

Fig. 2. Two endophytic bacterial strains, PS4 (*Pseudomonas* sp.) and PS27 (*Pantoea* sp.), elicited induced systemic resistance against *X. axonopodis* pv. *vesicatoria*.

Ten ml of bacterial suspension diluted to an optical density of 1.0 at 600 nm was used to drench three-week-old pepper seedlings. The same volumes of 0.05 M $MgSO_4$ and a chemical inducer, 1 mM benzothiadiazole (BTH), were used as a negative and positive control, respectively. Four days after application, a bacterial suspension of *X. axonopodis* pv. *vesicatoria* at OD_{600}=0.01 was used to infiltrate pepper leaves using the needleless syringe method. Seven days after pathogen challenge, the disease severity was assessed. The experiment was repeated three times with 10 replications (one plant per replication).

strain PS27 was closest to *Pantoea ananatis* strain LMG 20103 (99.1% similarity in 1,393 bp of 16S rDNA sequences, GeneBank Accession No. AF364847).

To examine the capacity for ISR on pepper by the two strains PS4 and PS27, bacterial suspensions were applied to pepper seedlings by drenching, and the pepper leaves were then challenged by the plant pathogenic bacterium, *Xav*, as described in Materials and Methods. Severity of disease caused by *Xav* was visually measured using the ratings of 0 to 5 (no symptoms to severe necrosis) at seven days after the pathogen challenge. Disease severity by the two strains was decreased to 34.7% and 26.3%, respectively, compared with the control treatment. Pepper leaves treated with strains PS4 and PS27 showed only chlorosis (disease severity=2.38) or chlorosis and mild necrosis (disease severity=2.69), respectively, whereas control treatment showed severe necrosis (3.65) (Fig. 2). Drenching with a chemical inducer, BTH, which is well-known to elicit strong ISR, showed only mild yellowish color (1.59). To exclude direct contact between the two bacterial strains and the pathogen, we confirmed that no introduced endophytes were detected on the pepper leaves at the area in which they were challenged with *Xav*. Intriguingly, pepper plants treated with 0.1 M BTH showed dwarfism one week after application. In previous studies, application of chemical inducers such as salicylic acid and BTH often resulted in reduced plant growth [17, 18]. In particular, BTH significantly decreased wheat growth under limited nitrogen conditions in the greenhouse and field [18]. A reduction of plant growth after triggering of induced resistance might be explained as a trade-off in terms of the cost in conditions in which nutrient or mineral resources for growth and plant defense against pathogen attack are limited [17]. However, our study indicated that bacterial endophytes induced both ISR and plant growth promotion.

In conclusion, from the pepper stems, we isolated bacterial endophytes that were evaluated as microbial inoculants for the management of plant pathogens and also for enhancement of plant productivity. Two strains, PS4 and PS27, were finally selected for useful characteristics such as high levels of colonization capacity and lack of plant pathogenicity, and were identified to be most closely related to *P. rhodesiae* and *P. ananatis*, respectively. The two strains showed significant pepper growth-promoting effects, especially on root growth, and the strains also elicited ISR against a bacterial pathogen, *Xav*. The exact mechanisms and reasons for this phenomenon are not currently understood. However, recent results on plant responses to endophytic bacteria demonstrated that specific volatile organic compounds produced by the endophyte *B. amyloliquefaciens* strain IN937a elicit both plant growth promotion and ISR [36, 38]. Efforts to find a specific determinant among bacterial VOCs produced by IN937a revealed that 2,3-butanediol and acetoin elicited plant growth promotion and ISR against *Erwinia carotovora* subsp. *carotovora*. To our knowledge, this is the first report of bacterial endophytes isolated from pepper (*C. annuum* L.) that elicited both ISR and plant growth promotion.

Acknowledgments

This research was supported by grants from the BioGreen 21 Program, funded by the Rural Development Administration, and from the Crop Functional Genomics Center of the 21st Century Frontier Research Program, funded by the Ministry of Science and Technology, Republic of Korea.

REFERENCES

1. Bottini, R., F. Cassan, and P. Piccoli. 2004. Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. *Appl. Microbiol. Biotechnol.* **65:** 497–503.
2. Cao, L., Z. Qiu, J. You, H. Tan, and S. Zhou. 2004. Isolation and characterization of endophytic *Streptomyces* strains from surface-sterilized tomato (*Lycopersicon esculentum*) roots. *Lett. Appl. Microbiol.* **39:** 425–430.
3. Chanway, C. P. 1997. Introduction of tree roots with plant growth promoting soil bacteria: An emerging technology for reforestation. *Forest Sci.* **43:** 99–112.
4. Cheong, H., S.-Y. Park, C.-M. Ryu, J. F. Kim, S.-H. Park, and C. S. Park. 2005. Diversity of root-associated *Paenibacillus* spp. in winter crops from the southern part of Korea. *J. Microbiol. Biotechnol.* **15:** 1286–1298.

5. Chung, E., C.-M. Ryu, S.-K. Oh, R. N. Kim, J. M. Park, H. S. Cho, S. Lee, J. S. Moon, S.-H. Park, and D. Choi. 2006. Suppression of pepper SGT1 and SKP1 causes severe retardation of plant growth and compromises basal resistance. *Physiol. Plant.* **126:** 605–617.
6. Compant, S., B. J. Nowak, C. Clément, and E. Ait Barka. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. *Appl. Environ. Microbiol.* **71:** 4951–4959.
7. Compant, S., B. B. Reiter, A. Sessitsch, J. Nowak, C. Clement, and E. Ait Barka. 2005. Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. *Appl. Environ. Microbiol.* **71:** 1685–1693.
8. Dickie, G. A. and C. R. Bell. 1995. A full factorial analysis of nine factors influencing *in vitro* antagonistic screens for potential biocontrol agents. *Can. J. Microbiol.* **41:** 284–293.
9. Downing, K. J., G. Leslie, and J. A. Thomson. 2000. Biocontrol of the sugarcane borer *Eldana saccharina* by expression of the *Bacillus thuringiensis cry1Ac7* and *Serratia marcescens chiA* genes in sugarcane-associated bacteria. *Appl. Environ. Microbiol.* **66:** 2804–2810.
10. Emmert, E. A. and J. Handelsman. 1999. Biocontrol of plant disease: A (Gram-) positive perspective. *FEMS Microbiol. Lett.* **171:** 1–9.
11. Garbeva, P., L. S. Overbeek, J. W. van Vuurde, and J. D. van Elsas. 2001. Analysis of endophytic bacterial communities of potato by plating and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments. *Microb. Ecol.* **41:** 369–383.
12. Glick, B. R. 1995. The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol.* **41:** 109–117.
13. Glick, B. R. 1999. Overview of plant growth-promoting bacteria, pp. 1–13. *In* B. R. Glick, C. L. Patten, G. Holguin, and D. M. Penrose (eds.), *Biochemical and Genetic Mechanisms Used by Plant Growth Promoting Bacteria*. Imperial College Press, London.
14. Halmann, J., A. Quadt-Hallmann, and J. W. Kloepper. 1997. Bacterial endophytes in agricultural crops. *Can. J. Microbiol.* **43:** 895–914.
15. Hallmann, J., A. Quadt-Hallmann, W. G. Miller, R. A. Sikora, and S. E. Lindow. 2001. Endophytic colonization of plants by the biocontrol agent *Rhizobium etli* G12 in relation to *Meloidogyne incognita* infection. *Phytopathology* **91:** 415–422.
16. Handelsman, J. and K. Stabb. 1996. Biocontrol of soil-borne plant pathogens. *Plant Cell* **8:** 1855–1869.
17. Heil, M. and I. Baldwin. 2002. Costs of induced resistance: Emerging experimental support for a slippery concept. *Trend. Plant Sci.* **7:** 61–67.
18. Heil, M., A. Hilpert, W. Kaiser, and K. E. Linsenmair. 2000. Reduced growth and seed set following chemical induction of pathogen defence: Does systemic acquired resistance (SAR) incur allocation costs? *J. Ecol.* **88:** 645–654.
19. Hwang, B. K. and C. H. Kim. 1995. Phytophthora blight of pepper and its control in Korea. *Plant Dis.* **79:** 221–227.
20. James E. K. and F. L. Olivares. 1998. Infection and colonization of sugar cane and other graminaceous plants by endophytic diazotrophs. *Crit. Rev. Plant Sci.* **17:** 77–119.
21. Jetiyanon, K. and J. W. Kloepper. 2002. Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. *Biol. Contr.* **24:** 285–291.
22. Kim, J. S., S. W. Kwon, F. Jordan, and J. C. Ryu. 2003. Analysis of bacterial community structure in bulk soil, rhizosphere soil, and root samples of hot pepper plants using FAME and 16S rDNA clone libraries. *J. Microbiol. Biotechnol.* **13:** 236–242.
23. Kloepper, J. W. and M. N. Schroth. 1978. Plant growth-promoting rhizobacteria on radishes, pp. 879–882. *In* Station de pathologie vegetale et phyto-bacteriologie (ed.), *Proceedings of the 4th International Conference on Plant Pathogenic Bacteria, vol. II.* Gilbert-Clarey, Tours.
24. Kloepper, J. W. 1992. Plant growth-promoting rhizobacteria as biological control agents, pp. 255–274. *In* F. B. Metting Jr. (ed.), *Soil Microbial Ecology: Applications in Agricultural and Environmental Management*. Marcel Dekker Inc., N.Y.
25. Kloepper, J. W., S. Tuzun, and J. Kuc. 1992. Proposed definitions related to induced disease resistance. *Biocontrol Sci. Technol.* **2:** 349–351.
26. Kloepper, J. W., R. Rodriguez-Kabana, G. W. Zehnder, J. Murphy, E. Sikora, and C. Fernandez. 1999. Plant root-bacterial interactions in biological control of soilborne diseases and potential extension to systemic and foliar diseases. *Austral. J. Plant Pathol.* **28:** 27–33.
27. Kloepper, J. W., C.-M. Ryu, and. S. Zhang. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* **94:** 1259–1266.
28. Kloepper, J. W. and C.-M. Ryu. 2006. Bacterial endophytes as elicitors of induced systemic resistance, pp. 33–52. *In* B. Schulz, C. Boyle, and T. Sieber (eds.), *Microbial Root Endophytes*. Kluwer Inc.
29. Krechel, A., A. Faupel, J. Hallmann, A. Ulrich, and G. Berg. 2002. Potato-associated bacteria and their antagonistic potential towards plant-pathogenic fungi and the plant-parasitic nematode *Meloidogyne incognita* (Kofoid & White) Chitwood. *Can. J. Microbiol.* **48:** 772–786.
30. Kuklinsky-Sobral, J., W. L. Araujo, R. Mendes, O. I. Geraldi, A. A. Pizzirani-Kleiner, and J. L. Azevedo. 2004. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. *Environ. Microbiol.* **6:** 1244–1251.
31. Lee, H. J., K. H. Park, J. H. Shim, R. D. Park, Y. W. Kim, J. Y. Cho, H. Hwangbo, Y. C. Kim, G. S. Cha, H. B. Krishnan, and K. Y. Kim. 2005. Quantitative changes of plant defense enzymes in biocontrol of pepper (*Capsicium annuum* L.) late blight by antagonistic *Bacillus subtilis* HJ927. *J. Microbiol. Biotechnol.* **15:** 1073–1079.
32. Lucas García, J. A., M. Schloter, T. Durkaya, A. Hartmann, and F. J. Gutiérrez Mañero. 2003. Colonization of pepper roots by a plant growth promoting *Pseudomonas fluorescens* strain. *Biol. Fertil. Soils* **6:** 381–385.

33. Oh, S.-K., S. Lee, E. Chung, J. M. Park, S. H. Yu, C.-M. Ryu, and D. Choi. 2006. Insight into Types I and II nonhost resistance using expression patterns of defense-related genes in tobacco. *Planta* **223:** 1101–1107.
34. Pernezny, K. L., P. D. Roberts, J. F. Murphy, and N. P. Goldberg. 2003. *Compendium of Pepper Diseases*. 1st Ed. APS Press, St. Paul, MN, U.S.A.
35. Reiter, B., U. Pfeifer, H. Schwab, and A. Sessitsch. 2002. Response of endophytic bacterial communities in potato plants to infection with *Erwinia carotovora* subsp. *atroseptica*. *Appl. Environ. Microbiol.* **68:** 2261–2268.
36. Ryu, C.-M., M. A. Farag, C.-H. Hu, M. S. Reddy, H. X. Wei, P. W. Paré, and J. W. Kloepper. 2003. Bacterial volatiles promote growth in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **100:** 4927–4932.
37. Ryu, C.-M., C.-H. Hu, M. S. Reddy, and J. W. Kloepper. 2003. Different signaling pathways of induced resistance by rhizobacteria in *Arabidopsis thaliana* against two pathovars of *Pseudomonas syringae*. *New Phytol*. **160:** 413–420.
38. Ryu, C.-M., M. Farag, C.-H. Hu, M. S. Reddy, P. Pare, and J. W. Kloepper. 2004. Bacterial volatiles induced systemic resistance in *Arabidopsis*. *Plant Physiol*. **134:** 1017–1026.
39. Ryu, C.-M., J. Kim, O. Choi, S.-Y. Park, S.-H. Park, and C. S. Park. 2005. Nature of a root-associated *Paenibacillus polymyxa* from field-grown winter barley in Korea. *J. Microbiol. Biotechnol.* **12:** 984–991.
40. Ryu, C.-M., J. Kim, O. Choi, S. H. Kim, and C. S. Park. 2006. Improvement of biological control capacity of *Paenibacillus polymyxa* E681 by seed pelleting on sesame. *Biol. Control* (in press).
41. Ryu, C.-M., J. F. Murphy, K. S. Mysore, and J. W. Kloepper. 2004. Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against cucumber mosaic virus by a salicylic acid and NPR1-independent and jasmonic acid-dependent signaling pathway. *Plant J*. **39:** 381–392.
42. Sambrook, J. and D. W. Russel. 2001. *Molecular Cloning: A Laboratory Manual*, 3rd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, U.S.A.
43. Sessitsch, A., B. Reiter, and G. Berg. 2004. Endophytic bacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities. *Can. J. Microbiol.* **50:** 239–249.
44. Timmusk, S., B. Nicander, U. Granhall, and E. Tillberg. 1999. Cytokinin production by *Paenibacillus polymyxa*. *Soil Biol. Biochem*. **31:** 1847–1852.
45. Van Loon, L. C., P. A. Bakker, and C. M. Pierterse. 1998. Systemic resistance induced by rhizosphere bacteria. *Annu. Rev. Phytopath*. **36:** 453–483.
46. Weller, D. M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annu. Rev. Phytopathol*. **26:** 379–407.
47. Wei, G., J. W. Kloepper, and S. Tuzun. 1991. Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. *Phytopathology* **81:** 1508–1512.
48. Zheng, H. Z., Y. W. Kim, H. J. Lee, R. D. Park, W. J. Jung, Y. C. Kim, S. H. Lee, T. H. Kim, and K. Y. Kim. 2004. Quantitative changes of PR proteins and antioxidative enzymes in response to *Glomus intraradices* and *Phytophthora capsici* in pepper (*Capsicum annuum* L.) plants. *J. Microbiol. Biotechnol*. **14:** 553–562.
49. Zinniel, D. K., P. Lambrecht, N. B. Harris, Z. Feng, D. Kuczmarski, P. Higley, C. A. Ishimaru, A. Arunakumari, R. G. Barletta, and A. K. Vidaver. 2002. Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. *Appl. Environ. Microbiol.* **68:** 2198–2208.