Broad-Spectrum Protection Against Several Pathogens by PGPR Mixtures Under Field Conditions in Thailand

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ABSTRACT

Jetiyanon, K., Fowler, W. D., and Kloepper, J. W. 2003. Broad-spectrum protection against several pathogens by PGPR mixtures under field conditions in Thailand. Plant Dis. 87:1390-1394.

Prior greenhouse experiments showed that four mixtures of plant growth-promoting rhizobacteria (PGPR) strains (all *Bacillus* spp.) elicited induced systemic resistance in several plants against different plant pathogens. Based on these findings, we sought to determine if systemic resistance induced by these PGPR would lead to broad-spectrum protection against several pathogens under field conditions in Thailand. Experiments were conducted during the rainy season (July to October 2001) and winter season (November 2001 to February 2002) on the campus of Naresuan University, Phitsanulok, Thailand. The specific diseases and hosts tested were southern blight of tomato (*Lycopersicon esculentum*) caused by *Sclerotium rolfsii*, anthracnose of long cayenne pepper (*Capsicum annuum* var. *acuminatum*) caused by *Colletotrichum gloeosporioides*, and mosaic disease of cucumber (*Cucumis sativus*) caused by *Cucumber mosaic virus* (CMV). Results showed that some PGPR mixtures suppressed disease more consistently than the individual PGPR strain IN937a. One PGPR mixture, *Bacillus amyloliquefaciens* strain IN937a + *B. pumilus* strain IN937b, significantly protected (P = 0.05) plants against all tested diseases in both seasons. Further, cumulative marketable yields were positively correlated with some treatments.

Additional keywords: biological control, plant growth promotion, systemic protection

Vegetable crops represent an important export commodity for Thailand. In 2001, Thailand's exports of edible vegetables and certain roots and tubers were valued at US$32 million. Among the most commonly grown vegetables are tomato (*Lycopersicon esculentum* Mill.), long cayenne pepper (*Capsicum annuum* L. var. *acuminatum* Fingerh), and cucumber (*Cucumis sativus* L.). Unfortunately, diseases of vegetables are often more prevalent and severe in the subtropics and the tropics, including Thailand, due to climates being conducive to disease dissemination and development (3,10,11,30).

Vegetable production in Thailand usually involves maximum inputs of various pesticides. Thai growers spend approximately 15% of total costs for chemicals to control plant diseases (23). At the same time, there is growing interest in Thailand in producing high-quality vegetables that have little or no pesticide residue. These factors have prompted the consideration of biological disease control strategies (9). In Thailand, the main mechanism for achieving biological control in fruit and vegetable crops has been antagonism, especially the use of fungal biocontrol agents (6,7,12,13,29). Induced systemic resistance (ISR) elicited by plant growth-promoting rhizobacteria (PGPR) has not yet been reported for biological control of diseases in Thai vegetable field crops but has attracted interest from scientists because it has led to disease reduction and promotion of plant growth and yield (22,33). Several greenhouse and field studies have demonstrated that treating cucumber seeds with either a single PGPR strain or a mixture of strains results in ISR activity against several pathogens (20,21,26–28,33). We previously reported (15) that compatible PGPR mixtures can elicit ISR in various plants including: cucumber (cv. Thong) against mosaic disease caused by *Cucumber mosaic virus* (CMV); green kuang futsoi (*Brassica chinensis* Jusl var. *parachinensis* (Bailey) Tsen & Lee cv. 610 Show-Jean) against damping-off disease caused by *Rhizoctonia solani* Kühn; long cayenne pepper (cv. 111 CHANYA) against anthracnose disease caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.; and tomato (cv. Srida) against wilt disease caused by *Ralstonia solanacearum* (Smith) Yabuuchi et al. (15).

Growers in Thailand generally use multi- or inter-cropping systems for growing vegetables. Several different diseases often occur in different crops in the same field during a single growing season. Thus, the objective of this study was to evaluate the effectiveness of compatible PGPR mixtures that elicit ISR against multiple diseases in different hosts during the rainy and winter seasons in Thailand. We studied three major diseases that occur on vegetable crops in Thailand: anthracnose disease caused by *C. gloeosporioides* on long cayenne pepper, southern blight disease caused by *Sclerotium rolfsii* on tomato, and mosaic disease caused by CMV on cucumber.

MATERIALS AND METHODS

Bacterial cultures. The PGPR used in this study were *Bacillus amyloliquefaciens* strain IN937a and *Bacillus pumilus* strains IN937b, SE34, SE49, T4, and INR7. All strains were obtained from the culture collection of the phytobacteriology laboratory of Auburn University. In previous greenhouse tests, individual PGPR strain IN937a and compatible PGPR mixtures (IN937a + IN937b, IN937b + SE34, IN937b + SE49, and T4 + INR7) induced systemic protection in various plants (15).

The bacteria were maintained in tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI) supplemented with 20% glycerol at –80°C for long-term storage. For experimental use, bacteria were transferred into TSB for 24 h. The concentration was then adjusted in sterile distilled water to 10^{11} CFU/ml for seed treatment or 10^{8} CFU/ml for root treatment.

Pathogens, hosts, media, and culture conditions. A culture of *C. gloeosporioides* was provided by the Plant Pathology and Microbiology Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. Conidial suspensions of *C. gloeosporioides* were maintained in cryovials containing TSB supplemented with 20% glycerol and kept at –80°C for long-term storage. For experimental use, conidia were transferred to plates of potato dextrose agar (PDA; Difco) and incubated at 30°C for 7 days. Dried leaf tissue of tobacco (*Nicotiana tabacum* L. cv. White Burley) infected with CMV was provided by Orawan Chatchawankanphanich of Kasetsart University, Nakorn Pathom, Thailand, who identified the pathogen by both enzyme-linked immunosorbent assay (ELISA) and reverse transcriptase technique. One gram

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Accepted for publication 26 June 2003.

Publication no. D-2003-0908-03R

of leaf tissue was ground in a mortar with 10 ml of 0.01 M sodium phosphate buffer (SPB; pH 7.0). The supernatant containing CMV inoculum was swabbed onto the cotyledons of 10-day-old cucumber (cv. Thong) using sterile cotton. When symptomatic leaves appeared on inoculated cucumber plants, the young infected leaves were harvested and maintained at –80°C for further experimental use.

Field experiments. Field experiments were conducted to determine whether selected PGPR treatments could elicit ISR activity against target pathogens. Field trials were conducted in the rainy season (July to October 2001) and the winter season (November 2001 to February 2002) at the Field Research Experimental Unit of the Faculty of Agriculture, Natural Resources, and Environmental Sciences, Naresuan University, Phitsanulok, Thailand. During each season, there were three experiments (one each for cucumber, tomato, and long cayenne pepper). The experimental design was a randomized complete block (RCB) with six treatments and three replications. Plots were 1 m wide by 3 m long. Each plot was separated by 1 m, and six plants within were spaced at 50 cm. Plots were covered with white polyethylene plastic film. Weeds were periodically eradicated either mechanically or by hand. Treatments consisted of a nonbacterized control, individual PGPR strain IN937a, and four mixtures of PGPR (IN937a + IN937b, IN937b + SE34, IN937b + SE49, and T4 + INR7).

Seeds of cucumber, tomato, and long cayenne pepper were soaked in bacterial suspensions (10^{11} cells per ml) maintained in 100-ml flasks and were then incubated in an orbital incubator shaker (100 rpm, Gyromax 707, Amerex Instrument, Lafayette, CA) at 30°C for 60 min. They were planted in a plastic seedling tray containing sterile soilless peat (Florafleur, NEVEMA B. V., Zwolle, Holland). The nonbacterized control treatment was soaked in TSB. All plants were grown in the greenhouse. Each cucumber plant was transferred into a plastic pot (10 cm diameter) 4 days after seeding. Tomato and pepper plants were transferred into plastic pots 7 days after seeding. At 5 days after planting, each cucumber pot was drenched with 50 ml of bacterial suspension (10^8 cells per ml). At 1 month after planting, each pot of tomato and pepper was drenched with the same amount and the same bacterial concentration. Control pots were drenched with 50 ml of diluted TSB in sterile distilled water (1.0 ml of TSB in 1.0 liter of sterile distilled water). Plants were transferred to the field site 9 days after soil drench. Liquid fertilizer (15-15-15) was sprayed onto plant leaves until runoff 1 day before transplanting to the field.

Cucumber mosaic disease. Cucumber plants were artificially challenged with CMV by rub-inoculation with infected sap at the first true leaves 7 days after transplanting. Inoculated leaves were immediately rinsed with water to remove the sap extract. The length of the main runner of each plant was recorded 1 month after transplanting. Mosaic symptoms were observed daily until 22 days after challenge in rainy season and 28 days after challenge in winter season. Disease incidence was calculated by the percentage of plants showing mosaic symptoms on younger leaves. Liquid fertilizer was applied again when plants began flower set. Marketable cucumber fruits (3.5 to 4 cm in diameter, 10 to 14 cm in length) were harvested every 3 days until the end of the season, and the fruit weight from each treatment was totaled for statistical analysis.

Tomato southern blight disease. Liquid fertilizer was applied again when plants began flower set. Inoculum of the southern blight pathogen, *S. rolfsii*, was already present in the tomato field site. The height of plants was recorded 1 month after planting. All plants were rated for disease severity approximately 2 months after transplanting. The number of symptomatic leaves (yellowing and wilting leaves) per plant was recorded. Disease severity was calculated as the percentage of symptomatic leaves per plant. Tomato fruits with marketable size (2.5 to 3.0 cm diameter) were harvested three times from each treatment, and the fruit weight from each treatment was totaled for statistical analysis.

Pepper anthracnose disease. Liquid fertilizer was applied again when plants began flower set. Approximately 55 days after soil drench, pepper fruits were challenged with spore suspensions of *C. gloeosporioides* (10^5 spores per ml) supplemented with 0.05% of Triton X100 (Fluka, Switzerland). The suspensions were sprayed onto pepper fruits in the evening (6:30 P.M.) until runoff. At 14 days after challenge, fully developed pepper fruits were collected from each plant and rated for disease severity. Disease severity was calculated as the percentage of fruit area covered with symptoms.

Statistical analyses. All data were analyzed by analysis of variance (ANOVA). The percent values for disease incidence on cucumber and disease severity for tomato and long cayenne pepper were transformed by the arcsine procedure prior to analysis. Least significant difference (LSD) tests were performed to compare treatment means at the 5 and 1% level. All analyses were performed with the GLM procedure of SAS version 8, 1990 (SAS Institute, Cary, NC).

RESULTS

Cucumber mosaic disease. In the rainy season, all plants in the nonbacterized con-

Table 1. Protection by plant growth-promoting rhizobacteria (PGPR) against *Cucumber mosaic virus* on cucumber in field trials[v]

Treatment[w]	Mean[x] (rainy)	Mean[y] (winter)
Control	6.0 a[z]	6.0 a
IN937a alone	2.3 b	3.0 b
IN937a+IN937b	2.3 b	3.3 b
IN937b+SE34	1.3 b	3.3 b
IN937b+SE49	1.3 b	3.0 b
T4+INR7	2.3 b	2.7 b
$LSD_{0.01}$	2.8	1.1

[v] Field experiments were conducted in the rainy season (July–October 2001) and winter season (November 2001–February 2002). The experimental design was a randomized complete block of six treatments with three replications, each with six plants.
[w] PGPR strain identifications: Strain IN937a = *Bacillus amyloliquefaciens*; Strains IN937b, SE34, SE49, T4, and INR7 = *B. pumilus*.
[x] Mean of mosaic disease incidence (number of plants showing symptoms) on cucumber plants was obtained 22 days after challenge.
[y] Mean of mosaic disease incidence on cucumber plants was obtained 28 days after challenge.
[z] Numbers with different letters show significant differences at $P = 0.01$ according to the least significant difference (LSD) test.

Table 2. Effect of plant growth-promoting rhizobacteria (PGPR) on cucumber fruit fresh weight and runner length in field trials[v]

	Mean total fruit weight (kg/plot)[x]		Mean main runner length (cm)[y]	
Treatment[w]	Rainy season	Winter season	Rainy season	Winter season
Control	3.3 b[z]	5.5 b	104.3 de	111.7 b
IN937a alone	4.5 ab	8.4 ab	119.8 bc	137.8 a
IN937a+IN937b	5.0 a	6.0 ab	129.1 ab	139.3 a
IN937b+SE34	3.7 ab	7.5 ab	114.4 cd	116.3 b
IN937b+SE49	3.5 ab	7.9 ab	99.7 e	129.2 ab
T4+INR7	4.7 ab	9.4 a	135.3 a	142.6 a
$LSD_{0.05}$	1.5 ab	3.6	13.0	18.2

[v] Field experiments were conducted in the rainy season (July–October 2001) and winter season (November 2001–February 2002). The experimental design was a randomized complete block of six treatments with three replications, each with six plants.
[w] PGPR strain identifications: Strain IN937a = *Bacillus amyloliquefaciens*; Strains IN937b, SE34, SE49, T4, and INR7 = *B. pumilus.*
[x] Mean total fruit weight from six plants per plot from cumulative harvests until the end of the season.
[y] Mean length of main runner was measured 1 month after transplanting.
[z] Numbers with different letters show significant differences at $P = 0.05$ according to least significant difference (LSD) test.

trol showed mosaic symptoms 13 days after challenge, especially in the younger leaves. In contrast, symptoms were noted on only some plants in the bacterized treatments (data not shown). Even though more plants in the bacterized treatments exhibited disease symptoms at 22 days after challenge than at 13 days, the disease incidence in all bacterized treatments was significantly lower than in the control (Table 1). Some infected plants in the control had severe systemic disease and were stunted. PGPR strain mixtures IN937b + SE34 and IN937b + SE49 provided approximately 80% disease suppression, and the remaining PGPR treatments provided approximately 60% disease suppression compared with the control. In the middle of the season, cucurbit leaf beetles (*Aulacophora similis* Oliver) infested cucumber plants and increased in population quickly throughout the season. These beetles severely damaged cucumber leaves, stems, and flowers. Total cucumber fruit weight in most PGPR treatments was greater than that for the control (Table 2). However, only mixture IN937a + IN937b produced significantly higher yield ($P = 0.05$).

In the winter season field trial, the appearance of mosaic symptoms on cucumber plants was delayed both in nonbacterized and PGPR treatments. All plants in the control showed mosaic symptoms on young leaves 28 days after challenge. Even though the level of disease suppression (45 to 55%) in PGPR treatments was lower than in the rainy season, disease incidence in all PGPR treatments was significantly less than in the control (Table 1). The damage caused by the beetles was negligible in this season. Only PGPR mixture T4 + INR7 gave significantly ($P = 0.05$) higher yields than plants in the control (Table 2).

For plant growth promotion, individual PGPR strain IN937a and two PGPR strain mixtures, IN937a + IN937b and T4 + INR7, significantly enhanced the length of the main runner compared with the nonbacterized control in both the rainy and winter season trials (Table 2). Further, PGPR strain mixtures (IN937a + IN937b and T4 + INR7) resulted in greater growth promotion than did individual strain IN937a (Table 2).

Southern blight disease of tomato. In the rainy season, when symptoms occurred, they were first apparent on lower leaves 1 month after transplanting. The leaves began to turn yellow, wilt, or die from the tips downward in the nonbacterized control and in some plants of the bacterized treatments. *S. rolfsii* grew upward in the plant and covered the tomato stem with a cotton-like, white mass of mycelium. Invaded stem tissues were pale brown. The fungus produced numerous small sclerotia of uniform size that were white when immature and then became dark brown to black when they matured. IN937a alone and the mixture IN937a + IN937b showed significant ($P = 0.05$) reductions in disease severity of 75 and 65%, respectively, compared with the control (Table 3). Mixture IN937a + IN937b resulted in a significant ($P = 0.05$) yield increase of total marketable fruit that was 1.5 times that of the control treatment (Table 4).

In the winter season, the lower leaves on most tomato plants began to turn yellow, wilt, or die from the tips downward at 1 month after transplanting. Disease development progressed more quickly and with greater severity than in the rainy season. Two months after transplanting, the mixtures IN937a + IN937b, T4 + INR7, and IN937b + SE34 provided significant ($P = 0.05$) disease suppression of 47, 43, and 35%, respectively, compared with the nonbacterized control (Table 3). There were no significant effects of PGPR treatments on tomato yield, although all PGPR treatments resulted in numerically higher yields than the control (Table 4).

For plant growth promotion on tomato, strain mixtures IN937b + SE49 and T4 + INR7 significantly increased the length of the main runner of tomatoes ($P < 0.05$) compared with that of the control in both experiments (Table 4). Cotton bollworms (*Helicoverpa armigera* (Hübner)) were present in some tomato plants in both seasons. They were eradicated by hand during frequent field inspections and did not cause significant damage.

Anthracnose disease on pepper. In the rainy season, several large symptoms developed on pepper fruits in the nonbacterized control at the infection site 7 days after challenge. The symptoms first appeared as light to dark brown sunken lesions. The lesions then merged and caused the collapse of tissues, which resulted in a large patch of sunken lesions 14 days after challenge. This finally distorted the shape of pepper fruits. In bacterized treatments, fewer and smaller lesions developed on pepper fruits. All bacterized treatments provided significant ($P = 0.05$) disease suppression ranging from 55 to 68% against anthracnose disease when compared with the control 14 days after challenge (Table 5). Lesion symptoms on pepper fruits appeared later in the winter season than in the rainy season. PGPR mixtures T4 + INR7, IN937a + IN937b, and IN937b + SE49 elicited significant ($P = 0.05$) disease suppression of 58, 54, and 28%, respectively, against anthracnose (Fig. 1 and Table 5).

PGPR mixtures IN937a + IN937b, IN937b + SE34, IN937b + SE49, and T4 + INR7 resulted in significantly ($P = 0.05$) greater yields than the control (Table 6) in the rainy season experiment. PGPR combinations IN937b + SE49 and T4 + INR7

Table 3. Protection by plant growth-promoting rhizobacteria (PGPR) of tomato against southern wilt disease caused by *Sclerotium rolfsii* in field trials[w]

Treatment[x]	Mean[y] (%) (rainy)	Mean[y] (%) (winter)
Control	70.8 a[z]	72.7 a
IN937a alone	16.7 b	48.1 ab
IN937a+IN937b	23.9 b	38.7 b
IN937b+SE34	58.0 ab	46.9 b
IN937b+SE49	34.7 ab	53.6 ab
T4+INR7	57.5 ab	41.4 b
$LSD_{0.05}$	44.4	25.2

[w] Field experiments were conducted in the rainy season (July–October 2001) and winter season (November 2001–February 2002). The experimental design was a randomized complete block of six treatments with three replications, each with six plants.

[x] PGPR strain identifications: Strain IN937a = *Bacillus amyloliquefaciens*; Strains IN937b, SE34, SE49, T4, and INR7 = *B. pumilus*.

[y] Mean disease severity. Disease was rated 2 months after transplanting by determining percentage of symptomatic leaves (yellowing and wilting) per plant.

[z] Numbers with different letters show significant differences at $P = 0.05$ according to least significant difference (LSD) test.

Table 4. Effect of plant growth-promoting rhizobacteria (PGPR) on tomato growth and yield in field trials[v]

	Mean total fruit weight (kg/plot)[x]		Mean plant height (cm)[y]	
Treatment[w]	Rainy season	Winter season	Rainy season	Winter season
Control	1.4 b[z]	2.5 a	97.1 c	83.8 bc
IN937a alone	2.0 ab	2.9 a	111.5 a	74.4 c
IN937a+IN937b	2.4 a	3.7 a	112.0 a	87.8 ab
IN937b+SE34	1.6 ab	3.4 a	101.4 abc	79.0 bc
IN937b+SE49	1.7 ab	2.8 a	109.3 ab	97.6 a
T4+INR7	1.6 ab	3.4 a	98.9 bc	100.6 a
$LSD_{0.05}$	0.8	1.9	11.7	13.1

[v] Field experiments were conducted in the rainy season (July–October 2001) and winter season (November 2001–February 2002). The experimental design was a randomized complete block of six treatments with three replications, each with six plants.

[w] PGPR strain identifications: Strain IN937a = *Bacillus amyloliquefaciens*; Strains IN937b, SE34, SE49, T4, and INR7 = *B. pumilus*.

[x] Mean total fruit weight from six plants per plot from two or three harvests for rainy and winter seasons.

[y] Mean height was obtained 1 month after transplanting.

[z] Numbers with different letters show significant differences at $P = 0.05$ according to least significant difference (LSD) test.

resulted in significant (P = 0.05) yield increases in the winter (Table 6).

In the rainy season, broad mites (*Polyphagotarsonemus latus* Banks) infested some pepper plants and caused damage on the top parts of the plants. Red thrips (*Scirtothrips dorsalis* Hoods) were also found in this season. They caused the edges of lower leaves to curl downward and the upper plant to develop a reddish-brown color. A few leaf-eating caterpillars (*Spodoptera litura* (Fabr.)) and cotton bollworms were seen in some plants in both seasons, especially among fully developed pepper fruits. These insect pests did not cause significant damage, as the infested plant parts were immediately trimmed and destroyed by burning.

DISCUSSION

Our results demonstrate that PGPR strain IN937a and mixtures can elicit systemic protection against multiple diseases in different hosts under two field conditions, rainy and winter seasons, in Thailand. The systemic protection was associated with increased plant growth in most cases and sometimes with enhanced total yield. However, treatments that best reduced disease incidence or severity were not always the same as those that best enhanced plant growth or yield. Some tested PGPR strain mixtures more consistently suppressed either disease severity or disease incidence in both seasons than the individual PGPR strain IN937a. Mixture IN937a + IN937b elicited systemic protection activity against all diseases (mosaic disease, wilt disease, and anthracnose disease) in both seasons. The individual strain IN937a elicited disease suppression against all diseases only in the rainy season for unknown reasons. PGPR strain mixtures IN937b + SE49 and T4 + INR7 suppressed mosaic and anthracnose diseases in both seasons. All PGPR treatments significantly reduced mosaic disease in both seasons when compared with the nonbacterized control.

Several host defenses have been reported to be involved with ISR elicited by

Fig. 1. Anthracnose symptoms on pepper fruits grown 3 months under field conditions during the winter season. Top = nonbacterized control. Bottom = treatment with plant growth-promoting rhizobacteria (PGPR) strain mixture IN937a + IN937b.

Table 5. Protection by plant growth-promoting rhizobacteria (PGPR) of long cayenne pepper against anthracnose disease caused by *Colletotrichum gloeosporioides* in field trials[w]

Treatment[x]	Mean[y] (%) (rainy)	Mean[y] (%) (winter)
Control	51.4 a[z]	39.5 a
IN937a alone	20.0 b	33.0 ab
IN937a+IN937b	16.5 b	18.4 c
IN937b+SE34	20.1 b	34.1 ab
IN937b+SE49	18.0 b	28.3 b
T4+INR7	22.9 b	16.5 c
$LSD_{0.05}$	8.1	8.7

[w] Field experiments were conducted in the rainy season (July–October 2001) and winter season (November 2001–February 2002). The experimental design was a randomized complete block of six treatments with three replications, each with six plants.
[x] PGPR strain identifications: Strain IN937a = *Bacillus amyloliquefaciens*; Strains IN937b, SE34, SE49, T4, and INR7 = *B. pumilus*.
[y] Mean disease severity. Disease was rated 14 days after challenge by determining percentage of fruit area covered with lesions.
[z] Numbers with different letters show significant differences at P = 0.05 according to least significant difference (LSD) test.

Table 6. Effect of plant growth-promoting rhizobacteria (PGPR) on yield of long cayenne pepper in field trials[w]

Treatment[x]	Mean (kg/plot)[y] (rainy)	Mean (kg/plot)[y] (winter)
Control	1.8 d[z]	1.4 c
IN937a alone	1.9 cd	1.7 bc
IN937a+IN937b	2.0 bc	1.8 abc
IN937b+SE34	2.1 b	1.9 abc
IN937b+SE49	2.4 a	2.0 ab
T4+INR7	2.5 a	2.3 a
$LSD_{0.05}$	0.1	0.5

[w] Field experiments were conducted in rainy (July–October 2001) and winter seasons (November 2001–February 2002). The experimental design was a randomized complete block of six treatments with three replications, six plants each.
[x] PGPR strain identifications: Strain IN937a = *Bacillus amyloliquefaciens*; Strains IN937b, SE34, SE49, T4, and INR7 = *B. pumilus*.
[y] Mean total fruit weight from six plants per plot from one harvest (14 days after challenge).
[z] Numbers with different letters show significant differences at P = 0.05 according to least significant difference (LSD) test.

PGPR. These defenses include lignification, peroxidase, and superoxide dismutase (SOD) production in cucumber (16), production of peroxidase (1) and phenolic compounds in cell wall appositions in pea (4), phytoalexin production in carnation (31), and lignification in bean (2). van Wees et al. (32) suggested that a single bacterium may induce systemic resistance through more than a single mechanism.

Pierson and Weller (25) reported that some combinations of fluorescent pseudomonad strains increased wheat yield compared with the same strains used individually. They also found that the best combinations did not always produce the same results in different crops. We found that strain mixture IN937a + IN937b generally improved yield of all plants compared with that of plants treated with individual strain IN937a, suggesting that the mixture could be useful on tomato, long cayenne pepper, and cucumber. Part of the reason for the difference in our results and those of Pierson and Weller may be that we used spore-forming strains of *Bacillus* spp. and different crop species and conducted our work under tropical environmental conditions.

Many mechanisms have been related to plant growth elicited by PGPR, including fixing atmospheric nitrogen and supplying it to plants, chelating iron from the soil and providing it to the plant cell, synthesizing several different phytohormones to enhance various stages of plant growth, solubilizing phosphorus for plant growth, and synthesizing some uncharacterized, low-molecular-mass compounds or enzymes that modulate plant growth and development (5,8,17–19,24). All PGPR strains in our study were *Bacillus* spp., which have been reported to produce a monocatecholate siderophore, 2,3-dihydroxybenzol glycine (14). Further research is needed to discern the mechanisms elicited by the PGPR mixtures that promoted plant growth in our study.

Our results indicate the potential of using PGPR strain mixtures for broad-spectrum protection against multiple diseases in Thai vegetable inter-cropping systems. Compared with the current use of pesticides to achieve disease protection, the use of PGPR mixtures presents fewer environmental risks. Another benefit of using PGPR strain mixtures is the enhancement of plant growth and yield. However, studies in different geographical areas in Thailand and of modified methods of PGPR application are needed to assess the consistency and effectiveness of PGPR strains as a component of an integrated program for management of vegetable diseases.

ACKNOWLEDGMENTS

This work was funded from the Thailand Research Fund (TRF) (grant no. PDF4380048). We thank Natthapong Hoysung and Chusak Raksanau for technical assistance in harvesting and processing samples.

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