ACADEMIC PRESS

Biological Control 24 (2002) 285–291

Biological Control

www.academicpress.com

Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases

Kanchalee Jetiyanon[a,*] and Joseph W. Kloepper[b]

[a] *Department of Agricultural Sciences, Faculty of Agriculture, Natural Resources, and Environmental Sciences, Naresuan University, Phitsanulok 65000, Thailand*

[b] *Department of Entomology and Plant Pathology, College of Agriculture, Auburn University, Auburn, AL 36849-5409, USA*

Received 7 September 2001; accepted 7 January 2002

Abstract

Studies of induced systemic resistance using strains of plant growth-promoting rhizobacteria (PGPR) have concentrated on the use of individual PGPR as inducers against multiple diseases of a single crop. To date, few reports have examined the potential of PGPR strain mixtures to induce systemic resistance against diseases of several different plant hosts. The objective of this study was to select mixtures of compatible PGPR strains with the capacity to elicit induced systemic resistance in four hosts. The specific diseases and hosts tested in this study included: bacterial wilt of tomato (*Lycopersicon esculentum*) caused by *Ralstonia solanacearum*, anthracnose of long cayenne pepper (*Capsicum annuum* var. *acuminatum*) caused by *Colletotrichum gloeosporioides*, damping off of green kuang futsoi (*Brassica chinensis* var. *parachinensis*) caused by *Rhizoctonia solani*, and cucumber mosaic virus (CMV) on cucumber (*Cucumis sativus*). To examine compatibility, seven selected PGPR strains were individually tested for in vitro antibiosis against all other PGPR strains and against three of the tested pathogens (*R. solanacearum*, *C. gloeosporioides*, and *R. solani*). No in vitro antibiosis was observed among PGPR strains or against pathogens. Twenty-one combinations of PGPR and seven individual PGPR were tested in the greenhouse for induced resistance activity. Results indicated that four mixtures of PGPR and one individual strain treatment significantly reduced the severity of all four diseases compared to the nonbacterized control: 11 mixtures reduced CMV of cucumber, 16 mixtures reduced bacterial wilt of tomato, 18 mixtures reduced anthracnose of long cayenne pepper, and 7 mixtures reduced damping off of green kuang futsoi. Most mixtures of PGPR provided a greater disease suppression than individual PGPR strains. These results suggest that mixtures of PGPR can elicit induced systemic resistance to fungal, bacterial, and viral diseases in the four hosts tested.

Keywords: PGPR; *Ralstonia solanacearum*; *Colletotrichum gloeosporioides*; *Rhizoctonia solani*; Cucumber mosaic virus; Induced resistance

1. Introduction

Biological control using introduced microorganisms with the capacity to elicit induced systemic resistance (ISR) against plant diseases has been extensively studied under greenhouse and field conditions. Several studies have shown that individual strains of plant growth-promoting rhizobacteria (PGPR) could elicit ISR against multiple diseases on one plant host (Hoffland et al., 1996; Liu et al., 1995a–c; Raupach et al., 1996; Wei et al., 1996). However, little research has been conducted to determine whether PGPR strains can elicit ISR in a range of plant hosts.

Compared to the use of individual PGPR strains, mixtures of several strains may result in a more stable rhizosphere community, provide several mechanisms of biological control, and may suppress a broader range of pathogens (Pierson and Weller, 1994). Compatible mixtures of certain biocontrol strains with antagonism as the main mechanism of action have provided a greater disease suppression than that used individually (Datnoff et al., 1995; Duffy and Weller, 1995; Duffy et al., 1996; Janisiewicz, 1988; Janisiewicz and Bors, 1995; Raupach and Kloepper, 1998). One study by Raupach and Kloepper (1998), which used PGPR that elicit ISR, indicated that mixtures of PGPR provided

* Corresponding author. Fax: +66-55-261040.
E-mail address: kanchaleej@nu.ac.th (K. Jetiyanon).

1049-9644/02/$ - see front matter
PII: S1049-9644(02)00022-1

synergistic activity against a broader range of pathogens on one host.

Agriculture in many parts of the world involves intercropping or mixtures of different crops in a confined geographical area, in contrast to large-acreage monoculture which typifies agriculture in North America. Under conditions of intercropping or small fields with multiple crops, induced resistance should be operable against several diseases of different crops. We hypothesize that mixtures of PGPR, which elicit ISR, will be useful in Thai agriculture for protecting against diseases on several different crops. The objective of this project was to select mixtures of compatible PGPR strains with the capacity to elicit induced systemic resistance in several hosts.

2. Materials and methods

2.1. PGPR strains, media, and culture conditions

Seven selected PGPR strains were used: *Bacillus amyloliquefaciens* strain IN937a, *Bacillus sphaericus* strain SE56, and *Bacillus pumilus* strains IN937b, SE34, SE49, T4, and INR7. Strains SE34, SE49, SE56, T4, and INR7 elicit ISR in cucumber against anthracnose caused by the fungus *Colletotrichum orbiculare* (Berk. and Mont.) von Arx (Jetiyanon, 1997; Wei et al., 1996). Strains IN937a and IN937b elicit ISR in tomato against cucumber mosaic virus (Zehnder et al., 2000). For long-term storage, these bacteria were maintained in tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI, USA), supplemented with 20% glycerol at −80 °C.

For experimental use, bacteria were transferred onto tryptic soy agar (TSA) (Difco) and were incubated at 28–30 °C for 24 h. The bacterial cells were harvested and suspended in 10 ml of 0.1 M sodium phosphate buffer (SPB; pH 7.0). The concentration was adjusted to 10^9 CFU/ml for seed treatment or to 10^8 CFU/ml for root treatment.

2.2. Pathogens, hosts, media, and culture conditions

Colletotrichum gloeosporioides (Penz.) Penz. and Sacc. (the causal agent of anthracnose disease), *Rhizoctonia solani* Kühn (the causal agent of damping-off disease), and *Ralstonia solanacearum* (Smith) Yabuuchi et al. (the causal agent of wilt disease) were provided by the Plant Pathology and Microbiology Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. Spore suspensions of *C. gloeosporioides* and bacterial suspensions of *R. solanacearum* were maintained in cryovials containing TSB supplemented with 20% glycerol and were kept at −80 °C for a long-term storage. For experimental use, the frozen fungus was revived by growing on potato dextrose agar (PDA; Difco) at 30 °C for 7 days. The frozen bacterium was grown on TSB at 30 °C for 24 h before use. The fungus *R. solani* was lyophilized for long-term storage and was transferred onto PDA for further experimental use. Dried tobacco (*Nicotiana tabacum* L. cv. White Berley) leaf tissues infected with cucumber mosaic virus (CMV) were provided by Dr. Orawan Chatchawankanphanich, Plant Genetic Engineering Unit, Kasetsart University, Nakorn Pathom, Thailand. Identification of the pathogen was confirmed by the institution using both enzyme-linked immunosorbent assay (ELISA) and reverse transcriptase technique. One gram of the 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 inoculated onto 10-day-old cucumber (*Cucumis sativus* L. cultivar Thong) plants using sterile cotton to swab-inoculate cotyledons. When symptomatic leaves appeared on inoculated cucumber plants, the young infected leaves were harvested and were maintained at −80 °C for further experimental use.

To test for in vitro antibiotic activity, tests were first conducted among selected PGPR strains, followed by testing each PGPR strain against all the target pathogens except CMV (an obligate parasite). Tests for antibiosis between PGPR strains and against the bacterial pathogen *R. solanacearum* were conducted in TSA plates. PDA plates were used to test for antibiosis against fungal pathogens *C. gloeosporioides* and *R. solani*. Each tested combination of microorganisms was replicated at least three times. For testing antibiosis between PGPR strains, 100 μl of a 10^5 CFU/ml suspension of one PGPR strain was spread over the agar surface and dried. A sterile assay disk (6 mm in diameter; Schleicher & Schuell, Einbeck, Germany) was placed on the agar and a suspension of each challenged PGPR (10^{11} CFU/ml, 30 μl/disk) was dropped onto each disk. Tetracycline disks (30 μg/disk; Becton Dickinson, Sparks, MD, USA) and vancomycin disks (30 μg/disk; HiMedia Laboratories Limited, Mumbai, India) were used as positive controls and each plate contained two antibiotic disks and two challenged bacteria. Plates were incubated at 28–30 °C for 24 h and examined for signs of clear zones indicating growth inhibition. The tests were done twice.

Tested fungi (*C. gloeosporioides* and *R. solani*) were applied as mycelial plugs from the edge of a pregrown colony and were placed in the middle of each PDA plate. Four sterile disks (6 mm in diameter) were placed onto each PDA plate and 30 μl of a 10^{11} CFU/ml PGPR suspension was applied to each disk. Plates were incubated at 28–30 °C for 7 days for *R. solani* and 10 days for *C. gloeosporioides*. The inhibition zone was observed and was recorded. There were three replications per treatment. The experiments were repeated at least twice.

2.3. Greenhouse experiments

There were four experimental groups based on the type of the challenged pathogen and each experimental group was conducted separately. The experiment had a randomized complete block design consisting of 29 treatments. Treatments for all experimental groups consisted of an SPB disease control, compatible mixtures of PGPR strains, and each PGPR strain individually. The mixtures of PGPR strains were prepared by combining equal proportions of each strain prior to application to the seed. Within each experimental group, there were three plants (pots) used as replicates. The whole experiment with each group was repeated at least 4 times. The plants were grown in the greenhouse at 32/25 °C day/night.

Seeds of cucumber (*Cucumis sativus* L. cv. Thong), green kuang futsoi (*Brassica chinensis* Jusl. var. *parachinensis* [Bailey] Tsen and Lee cv. 610 Show-Jean), long cayenne pepper (*Capsicum annuum* L. var. *acuminatum* Fingerh cv. 111 CHANYA), and tomato (*Lycopersicon esculentum* Mill. cv. Srida) were soaked in a suspension of 10^9 CFU/ml of either PGPR mixtures or individual PGPR strains for 1 h before planting seeds into a plastic seedling tray containing sterile soilless peat-based medium (Florafleur, NEVEMA, Zwolle, Holland). A control treatment was soaked in 0.1 M SPB.

2.4. Cucumber mosaic virus

Four days after seeding, cucumber seedlings were transplanted into 10-cm-diam pots containing a soilless peat-based medium. Each pot contained one plant. The following day, pots were drenched with 100 ml of a bacterial suspension (10^8 CFU/ml) of either PGPR mixtures or individual PGPR strains. A control treatment was drenched with 0.1 M SPB (pH 7.0). After 5 days, plants were transferred from the greenhouse and were held in an indoor building for 3–4 h before challenge. For challenge, 1.0 g of frozen, CMV-infected cucumber leaf tissue was ground in 0.01 M SPB (pH 7.0) and the plants were inoculated by rubbing the cotyledons with a sterile cotton swab dipped in the CMV containing leaf extract. Inoculated cotyledons were immediately rinsed with water to remove the sap extract. Incidence of disease was recorded as the number of symptomatic plants 14 days after challenge. The experiment was conducted five times.

2.5. Postemergence damping-off disease

Ten days after seeding, green kuang futsoi seedlings were transplanted into 10-cm-diam pots containing a soilless peat-based medium. Each pot contained one plant. Fifteen days after planting, pots were drenched with 100 ml of a bacterial suspension (10^8 CFU/ml) of either PGPR mixtures or individual PGPR strains. A control treatment was drenched with 0.1 M SPB (pH 7.0). A fertilizer (16–16–16; N–P–K) was applied as soil drench when plants were 1 month old. Each pot was drenched with 100 ml of the fertilizer at a concentration of 340 μg/ml. For challenge-inoculation, a potato–dextrose agar culture of *R. solani* (9-cm diam), completely covering the plate, was cut into pieces and was mixed with 50 g of sterile soilless peat. The mixture was incubated for 2 days at 25–27 °C, before use as the challenge inoculum. All plants were challenged by placing 0.5 g of the peat containing the pathogen around the stem base, 14 days after soil drench. Disease was rated 7 days after challenge and any plant showing dark brown lesion at the infection site was scored as diseased. The experiment was conducted four times.

2.6. Wilt disease

Four days after seeding, tomato seedlings were transplanted into 10-cm-diam pots containing a soilless peat-based medium. Each pot contained one plant. A fertilizer (16–16–16; N–P–K) with the same concentration and the same amount as described above was applied 20 days after planting. When plants were 30 days old, the pots were drenched with 100 ml of a bacterial suspension (10^8 CFU/ml) of either PGPR mixtures or individual PGPR strains. After 14 days, plants were challenged with *R. solanacearum* by cutting their roots with a sterile scissors and then drenching the cut roots with the pathogen suspension (10^8 CFU/ml; 100 ml/pot) or 0.1 M SPB (control). Plants were kept in a moist chamber for 24 h and were transferred to the greenhouse. Diseased plants were recorded 11 days after challenge and were observed for another 14 days. Based on the observations of cross-sections of the tap root, plants showing either external (wilting) or internal symptoms (browning of the vascular tissue) were scored as diseased. The experiment was conducted four times.

2.7. Anthracnose disease

Seven days after seeding, pepper seedlings were transplanted into 10-cm-diam pots containing a soilless peat-based medium. Each pot contained one plant. A fertilizer (16–16–16; N–P–K) was applied 30 days after planting. PGPR treatments were applied by soil drenching when plants were 45 days old. A fertilizer (13–13–21; N–P–K) was applied after fruit setting, which was 6 days after the soil drench. The plants were challenged 60 days after planting with *C. gloeosporiodes* (10^6 spores/ml) by drenching the pepper fruit with a fungal spore suspension or 0.1 M SPB (control). At this stage, the fruits were fully developed, having at least four fruits per plant. The plants were then maintained in a moist chamber for 24 h and were transferred to a greenhouse.

Disease was rated 9 days after challenge. Two fruit samples were randomly collected from each plant for disease rating. (0 = all fruits were healthy, 1 = 10% of fruit area was destroyed, 2 = 25% of fruit area was destroyed, 3 = 50% of fruit area was destroyed, 4 = 75% of fruit area was destroyed, and 5 = 100% of fruit area was destroyed). The experiment was conducted four times.

All data were analyzed by analysis of variance (ANOVA) and the treatment means were separated by using Fisher's protected least significant difference (LSD) test at $P = 0.05$ and $P = 0.01$ using SAS software (SAS Institute, Gary, NC, USA).

3. Results

3.1. In vitro antibiosis

All tested bacterial strains were inhibited by tetracycline and vancomycin. However, no antibiosis was exhibited by any combination of PGPR strains. None of the PGPR strains exhibited antibiosis against the tested pathogens. Both *R. solani* and *C. gloeosporiodes* grew toward PGPR strains and eventually overgrew the PGPR strains. From these results, all PGPR strains tested were selected for further investigation to determine which compatible mixtures of PGPR could provide a broad spectrum of disease suppression in greenhouse experiments.

3.2. Greenhouse experiments

3.2.1. Cucumber mosaic virus

Initial symptoms in the SPB control treatment and some PGPR-treated plants appeared 10 days after the challenge. The symptom was seen mainly in the third and fourth leaves of cucumber plants. Subsequently, disease symptoms appeared systemically in the second, fifth, and sixth leaves 4 days later but not in virus-inoculated cotyledons. Older leaves of PGPR-untreated plants developed mottling and discoloration 14 days after challenge. Infected developing leaves became mottled, distorted, and wrinkled, while induced resistant plants were still green and had a normal shape. Eleven mixtures of PGPR and two individual PGPR treatments provided significant disease suppression ($P \leqslant 0.05$) throughout all five repeated experiments against CMV, when compared to the SPB control (Table 1). Additionally, all effective mixtures of PGPR treatments gave better protection than the individual strains.

Table 1
PGPR-mediated reductions in cucumber mosaic virus (CMV) symptoms in cotyledons of cucumber plants challenged with CMV

Treatment [b]	No. of symptomatic plants[a]					
	Trial					
	1	2	3	4	5	Mean[c]
Buffer control	3	3	3	3	3	3.0
IN937a alone	0	2	2	2	2	1.6**
IN937a + IN937b	0	2	1	1	2	1.2**
IN937a + SE34	1	3	3	3	3	2.6
IN937a + SE49	2	1	0	0	1	0.8**
IN937a + SE56	3	3	3	3	3	3.0
IN937a + T4	2	3	3	2	3	2.6
IN937a + INR7	2	2	1	2	0	1.4**
IN937b alone	1	2	3	2	2	2.0*
IN937b + SE34	0	1	1	1	2	1.0**
IN937b + SE49	0	1	1	1	1	0.8**
IN937b + SE56	0	2	2	2	2	1.6**
IN937b + T4	0	1	1	3	3	1.6**
IN937b + INR7	0	2	2	2	3	1.8*
SE34 alone	2	3	3	3	3	2.8
SE34 + SE49	1	3	3	3	3	2.6
SE34 + SE56	0	3	3	3	3	2.4
SE34 + T4	3	2	1	2	2	2.0*
SE34 + INR7	3	3	3	3	3	3.0
SE49 alone	0	3	3	3	3	2.4
SE49 + SE56	1	1	1	1	1	1.0**
SE49 + T4	2	2	3	3	3	2.6
SE49 + INR7	3	3	2	2	3	2.6
SE56 alone	1	3	3	3	3	2.6
SE56 + T4	2	1	3	3	3	2.4
SE56 + INR7	3	2	3	3	3	2.8
T4 alone	1	2	3	3	3	2.4
T4 + INR7	1	1	2	1	2	1.4**
INR7 alone	3	3	3	3	3	3.0
$LSD_{0.05}$						0.8
$LSD_{0.01}$						1.3

[a] The experimental design was a randomized complete block with three replications per treatment. Symptomatic plants were recorded 14 days after inoculation with CMV.

[b] Strain IN937a = *Bacillus amyloliqefaciens*, IN937b, SE34, SE49, T4, and INR7 = *B. pumilus*, and SE56 = *B. sphaericus*.

[c] * and ** indicate significant treatment effects compared to the control at $P = 0.05$ and $P = 0.01$, respectively.

3.2.2. Damping-off disease

A typical disease symptom consisting of dark brown lesions developed at the infection site (stem-base) of PGPR-untreated plants, 7 days after challenge. The PGPR-treated plants showed only a small brown spot at the infection site. The PGPR-untreated plants were also thinner and less vigorous than PGPR-treated plants. Seven mixtures of PGPR and three individual PGPR treatments provided significant disease suppression ($P \leqslant 0.05$) against *R. solani* when compared to the SPB control treatment (Table 2).

3.2.3. Wilt disease

Initial symptoms occurred in susceptible plants 3 days after challenge, appearing as one-sided wilting during the daytime and recovering at night. Afterwards, the wilt gradually developed throughout the whole plant, until permanent wilting occurred. Sixteen mixtures of PGPR and six individual PGPR strains provided sig-

Table 2
PGPR-mediated reductions in postemergent damping-off symptoms in green kuang futsoi plants challenged with *Rhizoctonia solani*

Treatment[b]	No. of symptomatic plants[a]				
	Trial				
	1	2	3	4	Mean[c]
Buffer control	3	3	3	3	3.0
IN937a alone	2	1	2	1	1.5**
IN937a + IN937b	1	1	1	0	0.7**
IN937a + SE34	1	1	1	2	1.2**
IN937a + SE49	2	2	3	2	2.2
IN937a + SE56	3	3	3	3	3.0
IN937a + T4	3	3	3	3	3.0
IN937a + INR7	3	1	2	2	2.0
IN937b alone	3	2	1	2	2.0
IN937b + SE34	2	3	1	1	1.7*
IN937b + SE49	3	1	1	1	1.5**
IN937b + SE56	3	1	1	2	1.7*
IN937b + T4	3	3	2	2	2.5
IN937b + INR7	3	1	2	2	2.0
SE34 alone	2	1	1	1	1.2**
SE34 + SE49	3	1	3	1	2.0
SE34 + SE56	3	1	1	1	1.5**
SE34 + T4	3	2	1	2	2.0
SE34 + INR7	3	3	3	3	3.0
SE49 alone	3	3	2	1	2.2
SE49 + SE56	2	3	3	3	2.7
SE49 + T4	2	3	3	3	2.7
SE49 + INR7	3	3	1	1	2.0
SE56 alone	2	2	1	2	1.7*
SE56 + T4	3	2	2	2	2.2
SE56 + INR7	3	1	2	3	2.2
T4 alone	3	2	2	1	2.0
T4 + INR7	3	2	1	1	1.7*
INR7 alone	3	2	2	1	2.0
$LSD_{0.05}$					1.1
$LSD_{0.01}$					1.4

[a] The experimental design was a randomized complete block with three replications of each treatment. Symptomatic plants were recorded 7 days after inoculation with *R. solani*.

[b] Strain IN937a = *Bacillus amyloliquefaciens*, IN937b, SE34, SE49, T4, and INR7 = *B. pumilus*, and SE56 = *B. sphaericus*.

[c] * and ** indicate significant treatment effects compared to the control at $P = 0.05$ and $P = 0.01$, respectively.

Table 3
PGPR-mediated reductions in wilt disease in tomato plants challenged with *Ralstonia solanacearum*

Treatment[b]	No. of symptomatic plants[a]				
	Trial				
	1	2	3	4	Mean[c]
Buffer control	3	3	3	3	3.0
IN937a alone	1	2	1	0	1.0**
IN937a + IN937b	1	1	0	2	1.0**
IN937a + SE34	2	3	1	1	1.8
IN937a + SE49	1	1	2	0	1.0**
IN937a + SE56	1	0	3	1	1 2**
IN937a + T4	0	3	1	1	1.2**
IN937a + INR7	1	2	3	3	2.2
IN937b alone	2	2	1	0	1.2**
IN937b + SE34	2	2	2	0	1.5*
IN937b + SE49	0	2	1	1	1.0**
IN937b + SE56	1	3	3	2	2.2
IN937b + T4	0	0	1	1	0.5**
IN937b + INR7	0	1	2	2	1.2**
SE34 alone	0	1	1	2	1.0**
SE34 + SE49	2	0	2	2	1.5*
SE34 + SE56	2	2	0	1	1.2**
SE34 + T4	2	1	2	2	1.8
SE34 + INR7	2	1	2	1	1.5*
SE49 alone	0	1	1	0	0.5**
SE49 + SE56	2	2	1	1	1.5*
SE49 + T4	3	0	0	0	0.8**
SE49 + INR7	1	1	1	0	0.8**
SE56 alone	0	0	2	2	1.0**
SE56 + T4	0	1	1	3	1.2**
SE56 + INR7	2	1	3	1	1.8
T4 alone	3	2	1	1	1.8
T4 + INR7	2	1	1	1	1.2**
INR7 alone	0	1	2	3	1.5*
$LSD_{0.05}$					1.3
$LSD_{0.01}$					1.7

[a] The experimental design was a randomized complete block with three replications of each treatment. Symptomatic plants were recorded 11 days after inoculation with *R. solanacearum*.

[b] Strain IN937a = *Bacillus amyloliquefaciens*, IN937b, SE34, SE49, T4, and INR7 = *B. pumilus*, and SE56 = *B. sphaericus*.

[c] * and ** indicate significant treatment effects compared to the control at $P = 0.05$ and $P = 0.01$, respectively.

nificant disease suppression ($P \leqslant 0.05$) against *R. solanacearum*, when compared to the SPB control treatment (Table 3). The plants treated with PGPR were taller and more vigorous than plants not treated with PGPR. Furthermore, the number of diseased plants did not increase over time after the initial disease rating.

3.2.4. Anthracnose disease

Sunken brown lesions developed at the infection site of susceptible pepper fruits 4 days after challenge. Later, the lesions expanded and coalesced quickly into a large sunken lesion, leading to the distortion of pepper fruits. However, the lesions on fruits treated with PGPR developed more slowly, resulting in smaller lesions than those observed on PGPR-untreated plants. Treatments with seven individual PGPR strains and 18 mixtures caused a significant disease suppression ($P \leqslant 0.01$) against *C. gloeosporioides*, compared to the SPB control treatment (Table 4). Treatments applied with mixtures of PGPR provided better disease suppression than those applied with PGPR alone.

4. Discussion

The results indicated that several mixtures of PGPR and several individual strains provided significant disease suppression against specific tested pathogens. The in vitro antibiosis study showed that the selected PGPR strains were not antibiotic against the tested fungal and

Table 4
PGPR-mediated reductions in anthracnose disease in long cayenne pepper plants challenged with *Colletotrichum gloeosporioides*

Treatment[b]	Disease severity of infected plants[a]				
	Trial				
	1	2	3	4	Mean[c]
Buffer control	83.3	87.5	87.5	91.7	87.5
IN937a alone	58.3	66.7	62.5	54.1	60.4**
IN937a + IN937b	50.0	45.8	45.8	41.7	45.8**
IN937a + SE34	54.2	66.7	62.5	54.2	59.4**
IN937a + SE49	41.7	58.3	58.3	62.5	55.2**
IN937a + SE56	70.8	87.5	79.1	87.5	81.2
IN937a + T4	45.8	54.2	58.3	54.2	53.1**
IN937a + INR7	45.8	58.3	50.0	58.3	53.1**
IN937b alone	54.2	62.5	70.8	58.3	61.4**
IN937b + SE34	29.2	37.5	45.8	33.3	36.4**
IN937b + SE49	41.7	33.3	41.7	41.7	39.5**
IN937b + SE56	20.0	37.5	41.7	29.2	32.0**
IN937b + T4	62.5	66.7	75.0	62.5	66.7**
IN937b + INR7	79.2	87.5	87.5	87.5	85.4
SE34 alone	66.7	66.7	75.0	70.8	69.8**
SE34 + SE49	58.3	66.7	62.5	58.3	61.4**
SE34 + SE56	62.5	70.8	66.7	66.7	66.7**
SE34 + T4	62.5	75.0	70.8	66.7	68.8**
SE34 + INR7	54.2	66.7	66.7	66.7	63.6**
SE49 alone	58.3	66.7	66.7	66.7	64.6**
SE49 + SE56	70.8	79.2	87.5	79.2	79.2
SE49 + T4	54.2	66.7	66.7	62.5	62.5**
SE49 + INR7	45.8	58.3	66.7	62.5	58.3**
SE56 alone	50.0	58.3	62.5	58.3	57.3**
SE56 + T4	41.7	58.3	58.3	58.3	54.2**
SE56 + INR7	33.3	62.5	54.2	50.0	50.0**
T4 alone	50.0	58.3	50.0	58.3	54.2**
T4 + INR7	20.0	33.3	37.5	33.3	31.0**
INR7 alone	58.3	75.0	70.8	75.0	69.8**
$LSD_{0.05}$					8.4
$LSD_{0.01}$					10.4

[a] The experimental design was a randomized complete block with three replications of each treatment. Disease severity was rated 9 days after inoculation with *C. gloeosporioides*.

[b] Strain IN937a = *Bacillus amyloliquefaciens*, IN937b, SE34, SE49, T4, and INR7 = *B. pumilus*, and SE56 = *B. sphaericus*.

[c] * and ** indicate significant treatment effects compared to the control at $P = 0.05$ and $P = 0.01$, respectively.

bacterial pathogens, suggesting but not proving that the mechanism of control is not a direct antagonism. We have also demonstrated that one PGPR strain (IN937a) and four other compatible mixtures of PGPR treatments, including IN937a + IN937b, IN937b + SE34, IN937b + SE49, and T4 + INR7, induced systemic resistance against all four tested plant/pathogen combinations. These results showed that certain individual and compatible mixtures of PGPR strains could provide a broad spectrum of PGPR-mediated ISR activity against different pathogens. Further, certain compatible PGPR mixtures caused at least a 50% disease suppression in most plant/pathogen combinations, compared to the SPB control treatment, while a single PGPR strain could suppress at the same rate against only postemergent damping-off and bacterial wilt systems. This finding indicated that most compatible PGPR mixtures induced a higher level of protection than individual PGPR strains.

It has been reported that mixtures of PGPR strains either in a two-way or three-way combination gave a greater protection, compared to single-strain treatments, of cucumber angular leaf spot disease caused by *Pseudomonas syringae* pv. *lachrymans* under field conditions (Raupach and Kloepper, 2000). Certain compatible PGPR mixtures may enhance combinations of plant defense mechanisms such as peroxidase enzymes, lignification, superoxide dismutase, or phenolic compounds more than those treated with individual PGPR strains. Therefore, our study shows that the approach of using certain compatible mixtures of PGPR for controlling multiple diseases in different plant/pathogen combinations has potential benefits in practical agriculture.

Studies of physiological changes have shown that a particular PGPR strain, such as *B. pumilus* strain SE34, one of our tested strains, could induce callose and pectin in close association with phenolic compounds in the newly formed wall appositions in pea root, in response to attack by the fungus *Fusarium oxysporum* f. sp. *pisi* (Van Hall) Snyd. and Hans. (Benhamou et al., 1996). Another study reported an increase in peroxidase activity in pea roots colonized by rhizosphere bacteria (Albert and Anderson, 1987). Coincidentally, with our previous data, cucumber plants treated with *B. pumilus* strain SE49 resulted in a rapid lignification, in response to ingress of *C. orbiculare* and total peroxidase and superoxide dismutase (SOD) activities were increased to a greater extent than those in the buffer control treatment (Jetiyanon et al., 1997). Terpenoid synthesis in seedling cotton roots induced by *Trichoderma virens* (Miller, Giddens and Foster) von Arx has been reported to be a major mechanism of suppression of the disease caused by *R. solani* (Howell et al., 2000). In the case of a viral disease, it has been reported that no CMV antigen was detected, based on ELISA results, in any PGPR-treated cucumber plants, which were apparently symptomless after challenge inoculation with CMV (Raupach et al., 1996). However, it is unclear whether the induced resistance is directed at CMV replication or movement and by which defense mechanisms. In addition, some studies have reported that radish and *Arabidopsis* treated with *Pseudomonas fluorescens* strain WCS417 did not induce the accumulation of pathogenesis-related proteins against *F. oxysporum* Schlecht. f.sp. *raphani* J.B. Kendrick and W.C. Snyder (Hoffland et al., 1995; Pieterse et al., 1996). Therefore, further work is needed to compare the physiological response of plants induced to resistance by individual PGPR strains with those induced by PGPR strain mixtures.

Acknowledgment

This work was supported by grant no. PDF4380048 from Thailand Research Fund (TRF).

References

Albert, F., Anderson, A.J., 1987. The effect of *Pseudomonas putida* colonization on root surface peroxidase. Plant Physiol. 85, 537–541.

Benhamou, N., Kloepper, J.W., Quadt-Hallman, A., Tuzun, S., 1996. Induction of defense-related ultrastructural modifications in pea root tissues inoculated with endophytic bacteria. Plant Physiol. 112, 919–929.

Datnoff, L.E., Nemec, S., Pernezny, K., 1995. Biological control of Fusarium crown and root rot of tomato in Florida using *Trichoderma harzianum* and *Glomus intraradices*. Biol. Control 5, 427–431.

Duffy, B.K., Weller, D.M., 1995. Use of *Gaeumannomyces graminis* var. *graminis* alone and in combination with fluorescent *Pseudomonas* spp. to suppress take-all of wheat. Plant Dis. 79, 907–911.

Duffy, B.K., Simon, A., Weller, D.M., 1996. Combination of *Trichoderma koningii* with fluorescent pseudomonads for control of take-all on wheat. Phytopathology 86, 188–194.

Hoffland, E., Pieterse, C.M.J., Bik, L., van Pelt, J.A., 1995. Induced systemic resistance in radish is not associated with accumulation of pathogenesis-related proteins. Physiol. Mol. Plant Pathol. 46, 309–320.

Hoffland, E., Hakulinen, J., van Pelt, J.A., 1996. Comparison of systemic resistance induced by avirulent and nonpathogenic *Pseudomonas* species. Phytopathology 86, 757–762.

Howell, C.R., Hanson, L.E., Stipanovic, R.D., Puckhaber, L.S., 2000. Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. Phytopathology 90, 248–252.

Janisiewicz, W.J., 1988. Biocontrol of postharvest diseases of apples with antagonist mixtures. Phytopathology 78, 194–198.

Janisiewicz, W.J., Bors, B., 1995. Development of a microbial community of bacterial and yeast antagonists to control wound-invading postharvest pathogens of fruits. Appl. Environ. Microbiol. 61, 3261–3267.

Jetiyanon, K., 1997. Interaction between PGPR and cucumber during induced systemic resistance: recognition and early host defense responses. Ph.D. dissertation, Auburn University, Auburn, AL, USA.

Jetiyanon, K., Tuzun, S., Kloepper, J.W., 1997. Lignification, peroxidase and superoxide dismutases as early plant defense reactions associated with PGPR-mediated induced systemic resistance. In: Ogoshi, A., Kobayashi, K., Homma, Y., Kodama, F., Kondo, N., Akino, S. (Eds.), Plant Growth-Promoting Rhizobacteria-Present Status and Future Prospects. Nakanishi Printing, Sapporo, Japan, pp. 265–268.

Liu, L., Kloepper, J.W., Tuzun, S., 1995a. Induction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. Phytopatholgy 85, 695–698.

Liu, L., Kloepper, J.W., Tuzun, S., 1995b. Induction of systemic resistance in cucumber against angular leaf spot by plant growth-promoting rhizobacteria. Phytopathology 85, 843–847.

Liu, L., Kloepper, J.W., Tuzun, S., 1995c. Induction of systemic resistance in cucumber by plant growth-promoting rhizobacteria: duration of protection and effect of host resistance on protection and root colonization. Phytopathology 85, 1064–1068.

Pierson, E.A., Weller, D.M., 1994. Use of mixture of fluorescent pseudomonads to suppress take-all and improve the growth of wheat. Phytopathology 84, 940–947.

Pieterse, C.M.J., van Wees, S.C.M., Hoffland, E., van Pelt, J.A., van Loon, L.C., 1996. Systemic resistance in Arabidopsis induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. Plant Cell 8, 1225–1237.

Raupach, G.S., Liu, L., Murphy, J., Tuzun, S., Kloepper, J.W., 1996. Induced systemic resistance in cucumber and tomato against cucumber mosaic cucumovirus using plant growth-promoting rhizobacteria (PGPR). Plant Dis. 80, 891–894.

Raupach, G.S., Kloepper, J.W., 1998. Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88, 1158–1164.

Raupach, G.S., Kloepper, J.W., 2000. Biocontrol of cucumber diseases in the field by plant growth-promoting rhizobacteria with and without methyl bromide fumigation. Plant Dis. 84, 1073–1075.

Wei, G., Kloepper, J.W., Tuzun, S., 1996. Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. Phytopathology 86, 221–224.

Zehnder, G.W., Yao, C., Murphy, J.F., Sikora, E.J., Kloepper, J.W., 2000. Induction of resistance in tomato against cucumber mosaic virus by plant growth-promoting rhizobacteria. Biol. Control 45, 127–137.