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Efficiency of new plant growth promoting rhizobacteria on corn diseases control

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The efficacy of two plant growth promoting rhizobacteria (PGPR) strains including *Bacillus subtilis* TU-Orga1 and *Pseudomonas fluorescens* TU-Orga2 obtained from rice rhizosphere against naturally occurring diseases as well as their capacity to improve crop yield of sweet corn cv. Insee2 was studied. TU-Orga1 was significantly greater in inhibition of *Acidovorax avenae* subsp. *avenae* (*Aaa*) and *Erwinia chrysanthemi* pv. *zeae* (*Ecz*), caused bacterial leaf streak and stalk rot of corn, respectively by antibiosis. Greenhouse experiments using TU-Orga1 and TU-Orga2 treatments increased highest salicylic acid accumulation in corn leaves with 7.85 and 6.98 μg g^{-1} fresh weight, respectively to protect *Ecz* infection. Each PGPR strain was single applied in the field through seed treatment and 3-foliar-spray-intervals at 14, 21, and 28 days after planting. Two PGPR treatments resulted in reduced severity of all diseases and increased yields when compared with the control treatment. There were differences among the treatments in that the highest level of disease suppression of bacterial stalk rot resulted with treatments TU-Orga1 (P = 0.05), whereas TU-Orga2 showed the highest level of disease suppression of bacterial leaf streak and sugarcane mosaic virus and provided significantly greater marketable yield increases than the other treatments. This illustrates the potential of these new biocontrol agents to suppress multiple diseases. They could become a component of an integrated program or an organic farming for corn disease management.

Key words: Plant growth promoting rhizobacteria (PGPR), *Acidovorax avenae* subsp. *avenae, Erwinia chrysanthemi* pv. *zeae*, SCMV, systemic acquired resistant.

INTRODUCTION

Corn is an economically important crop and many disease-causing organisms including several fungi, viruses, bacteria, and nematodes disseminated in the corn production system. Increased production of corn has led to emerging disease problems of various diseases including seed and seedling diseases, downy mildew, Northern corn leaf blight, Southern corn leaf blight, Stewartii's wilt, bacterial leaf streak, bacterial stalk rot, and sugarcane mosaic virus (SCMV). Seed and seedling diseases caused by *Pythium* sp., *Rhizoctonia* sp. and *Fusarium* sp., downy mildew caused by *Peronosclerospora sorghi*, Northern corn leaf blight caused by *Setosphaeria turcica*, Southern corn leaf blight caused by *Bipolaris maydis*, bacterial leaf streak caused by *Acidovorax avenae* subsp. *avenae* (*Aaa*), and bacterial stalk rot caused by *Erwinia chrysanthemi* pv. *zeae* (*Ecz*) have occurred in many corn growing areas in Thailand (Prathuangwong et al., 2004). Also, SCMV is one of the most important viruses

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infecting corn and the symptoms include necrosis and blight on leaf, stem, flower, and ear (Li et al., 2000). These diseases are causing severe economic loss of corn production in Thailand, so effective control measures are critical. Although chemicals are available for the management of corn diseases, inappropriate and non-discriminative use of chemicals is known to cause undesirable effects such as residual toxicity, development of resistance, environmental pollution, health hazards to humans and animals, increased expenditure for plant protection and are inefficient in controlling viral disease. Based on the fact that there are no direct control measures, management through induction of plants natural defense is important and sustainable. In recent years, plant defense induction have been extensively evaluated as a mean to defense themselves against pathogens based on the systemic acquired resistance (SAR) (Ryals et al., 1996; van Loon, 1997; Sticher et al., 1997; Vallad and Goodman, 2004). The inducers could be either synthetic compounds and biological agent provides an induced resistance to a broad range of pathogens. Plant defense induction by biological agent or plant growth promoting rhizobacteria (PGPR) has been established against Cucumber mosaic virus, Tobacco mosaic virus, and Tomato mottle virus (Maurhofer et al., 1998; Murphy et al., 2000; Raupach et al., 1996). Also, several PGPR strain including *Bacillus amyloliquefaciens* KPS46 and *Paenibacillus pabuli* SW01/4 have been reported as offering several functions including increased plant growth by indole-3-acetic acid induction and induced plant defense by SAR against multiple diseases (caused by fungi, bacteria, and viruses) in various crops such as soybean, rice, sesame, corn, sunflower, and vegetable (Prathuangwong and Kasem, 2004; Boonnadakul et al., 2012; Sathitthampana et al., 2012; Athinuwat, 2013). Existing control measures are inadequate for commercial production, while biological control using PGPR has become more important. The present study evaluated the efficacy of *Bacillus subtilis* TU-Orga1 and *P. fluorescens* TU-Orga2 isolated from rice rhizosphere to increase growth and vigor of the sweet corn plant and thereby control bacterial leaf streak, bacterial stalk rot, and other naturally disease infections via salicylic accumulation pathway. Biological control using PGPR in a system of integrated control measures may provide effective and sustainable management where chemical control is not available or practical.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Two new plant growth promoting rhizobacteria (PGPR) strains, *Bacillus subtilis* TU-Orga1 and *Pseudomonas fluorescens* TU-Orga2 isolated from rice rhizosphere and two commercial PGPR strains, *Bacillus amyloliquefaciens* KPS46 and *Paenibacillus pabuli* SW01/4 that have been isolated from soybean rhizosphere (Prathuangwong and Kasem, 2004) were evaluated. In addition, virulent strains of *Acidovorax avenae* subsp. *avenae* (*Aaa*), causal agent of bacterial leaf streak and *Erwinia chrysanthemi* pv. *zeae* (*Ecz*), causal agent of bacterial stalk rot were used in this study. Each strain stored in 50% glycerol at -80°C was reactivated by streaking onto nutrient agar (NA) plate (5 g of bacto-peptone, 3 g of beef extract, 15 g of agar, and 1 L of H_2O) and incubated at room temperature (28 ± 2°C) for 48 h. A loopful of each culture strain was transferred to nutrient broth (NB) containing 5 g of bacto-peptone, 3 g of beef extract, and 1 L of H_2O and incubated on a rotary shaker at 150 rpm and at room temperature (28±2°C) for 48 h. Cells were harvested by centrifugation for 20 min at 4,000 g washed by a second centrifugation in sterile water and finally resuspended in sterile water to a final concentration of 10^6 - 10^8 cfu/ml prior to application.

Efficacy of plant growth promoting rhizobacteria (PGPR) against bacterial leaf streak and bacterial stalk rot pathogens

Inhibition of *A. avenae* subsp. *avenae* and *E. chrysanthemi* pv. *zeae* caused bacterial leaf streak and bacterial stalk rot of corn, respectively by PGPR strains *B. subtilis* TU-Orga1, *P. fluorescens* TU-Orga2, *B. amyloliquefaciens* KPS46, and *P. pabuli* SW01/4, were performed by paper disc diffusion assay to verify whether 4 PGPR strains exhibited target disease suppression before further greenhouse and field studies. They were a total of 12 treatments arranged in completely randomized design (CRD) with 10 replications. The PGPR cell suspension was grown overnight in 50 ml NB at room temperature (28 ± 2°C) and adjusted in sterile distilled water to 10^8 cfu/ml. The 10 µl of each bacterial antagonist suspension was dropped onto 5 mm diameter of sterile filter paper disc and was plated on NA amended with 1 ml of each pathogen suspension (10^8 cfu/ml). The inhibition zone was determined after incubation for 48 h at room temperature (28 ± 2°C). The experiment was conducted three times. The data were subjected to analysis of variance with the general linear models procedure of SAS program. Treatment means were assessed using Duncan's Multiple Range Test (DMRT) and all tests of significance were conducted at P = 0.05.

Efficacy of plant growth promoting rhizobacteria (PGPR) against bacterial stalk rot under greenhouse conditions

This experiment was conducted to test the efficacy of PGPR strains for enhancement of corn seedling growth. Corn seeds cv. Insee2 were surface disinfected by treatment with 95% ethanol (v/v) for 2 min, and washed with sterile distilled water 5 times in order to remove the ethanol. Before planting, 20 g of corn seeds were mixed thoroughly with 1 ml of each PGPR suspension (10^6 cfu/ml). Seeds treated by distilled water or copper hydroxide served as the negative and positive controls, respectively. They were a total of 14 treatments arranged in randomized complete block design (RCBD) with five replications per treatment (Table 1). The experiment was conducted three times. Treated seeds were planted in 30 cm-diameter pots (two seeds per pot) containing steam-pasteurized potting media (silt clay loam soil and sand mixed in equal volumes). The pots were kept under greenhouse conditions and watered daily. At seven days after seedling emergence, seedlings were harvested for measurement of root and shoot lengths and seed germination. Fresh cultures of all 4 PGPR strains were used as the target microorganisms for controlling bacterial stalk rot. *E. chrysanthemi* pv. *zeae* challenged inoculation at seven days after planting by toothpick inoculation technique were used (Prathuangwong et al., 2004). The incidence and disease reduction of bacterial stalk rot infection was determined and recorded at 3 to seven days after inoculation (Prathuangwong et al., 2004). The data were subjected to analysis of variance with the general linear models procedure of SAS program. Treatment means were assessed using DMRT and all tests of significance were conducted

Table 1. Plant growth promoting rhizobacteria strains application to suppress naturally corn disease infections under field conditions.

Code	Treatment[1/]
T1	Seed treatment with *Bacillus amyloliquefaciens* KPS46 (A)
T2	Seed treatment with *Paenibacillus pabuli* SW01/4 (B)
T3	Seed treatment with *Pseudomonas fluorescens* TU-Orga2 (C)
T4	Seed treatment with *Bacillus subtilis* TU-Orga1 (D)
T5	3-foliar-spray-intervals with KPS46 at 14, 21, and 28 days after planting (E)
T6	3-foliar-spray-intervals with SW01/4 at 14, 21, and 28 days after planting (F)
T7	3-foliar-spray-intervals with TU-Orga2 at 14, 21, and 28 days after planting (G)
T8	3-foliar-spray-intervals with TU-Orga1 at 14, 21, and 28 days after planting (H)
T9	A + E
T10	B + F
T11	C + G
T12	D + H
T13	3-foliar-spray-intervals with copper hydroxide and insecticide at 14, 21, and 28 days after planting
T14	Nontreated

[1/] *Bacillus subtilis* TU-Orga1 and *Pseudomonas fluorescens* TU-Orga2 are new biological control agents isolated from rice rhizosphere. *Bacillus amyloliquefaciens* KPS46 and *Paenibacillus pabuli* SW01/4 are commercial strains isolated from soybean (Prathuangwong and Kasem, 2004).

at P = 0.05.

Plant biochemical response analysis

At 5, 6, 7, 8, 9, 10, 11, and 12 days after planting, the treated plant under greenhouse conditions, the lower and the upper leaves of sweet corn were separately collected from each treatment in each day as mention above for SAR relating biochemical analysis. After being detached, each leaf was cut into 2 halves and pooled as 1 sample. Subsequently, leaf tissues were analyzed for salicylic acid (SA) accumulation by the method as described by Raskin et al. (1989). Pooled leaf tissue (0.5 g) from each replication were randomly sampled, frozen with liquid nitrogen and macerated in a cold mortar with 1 ml of extraction solution (90:9:1 volume of absolute methanol, glacial acetic acid, and distillate water). The extract was subsequently centrifuged at 12,000 *g* and 4°C for 15 min. and the supernatant was collected for the analysis. To determine the SA content, 500 µl of the supernatant was mixed with an equal volume of 0.02 M ferric ammonium sulfate, incubated at 30°C for 5 min, and the absorbance at 530 nm was read by a spectrophotometer. The read absorbance was subsequently compared to those of the reference standard to obtain the actual amount of SA in the sample.

Efficacy of plant growth promoting rhizobacteria (PGPR) to suppression of naturally occurring diseases infection

Four PGPR strains, *B. subtilis* TU-Orga1, *P. fluorescens* TU-Orga2, *B. amyloliquefaciens* KPS46, and *P. pabuli* SW01/4, known to be effective were studied for their efficacy to suppress naturally occurring diseases infection. The investigation had been carried out in Pathumthani province with a history of bacterial stalk rot and bacterial leaf streak during July-October, 2012. There were a total of 14 treatments arranged in RCBD with three replicate plots (Table 1). Plots of 5 × 7 m^2 separated by 0.5 m paths were grown with sweet corn in 50 × 50 cm spacing. Corn seeds (cv. Insee2) were separately grown in seedling pots. They were irrigated by furrow system 1 time/week. Within the cropping season, the naturally infected diseases on tested plants were controlled by seed treatment alone with each PGPR strain, 3-foliar-spray-intervals alone with each PGPR strain at 14, 21, and 28 days after planting, and seed treatment plus 3-foliar-spray-intervals at 14, 21, and 28 days after planting with each PGPR strain as treatment described in Table 1. Seed treatment used PGPR suspension concentration at 10^6 cfu/ml and foliar spray used PGPR cell suspension concentration at 10^8 cfu/ml. The potential of disease control was compared with agrochemical treatment, copper hydroxide plus insecticide as positive control treatment and nontreated treatment.

Data collection and analysis

The improvement of yield in term of quantity and quality and all disease incidences were measured in tested plants. Seed germination was evaluated at 14 days after planting. Yield production was measured at 90 days after planting with total and marketable yields by random sampling with 30 plants/ treatment. The incidence severity and reduction of all disease infection was determined and recorded at 60 days after planting.

RESULTS

Efficacy of plant growth promoting rhizobacteria (PGPR) against bacterial leaf streak and bacterial stalk rot pathogens

Relationship between *A. avenae* subsp. *avenae* and *E. chrysanthemi* pv. *zeae* suppression and production of antimicrobial compounds by *B. subtilis* TU-Orga1, *P. fluorescens* TU-Orga2, and *B. amyloliquefaciens* KPS46 was analyzed using a paper disc diffusion method where *P. pabuli* SW01/4 showed competition mode inhibition of

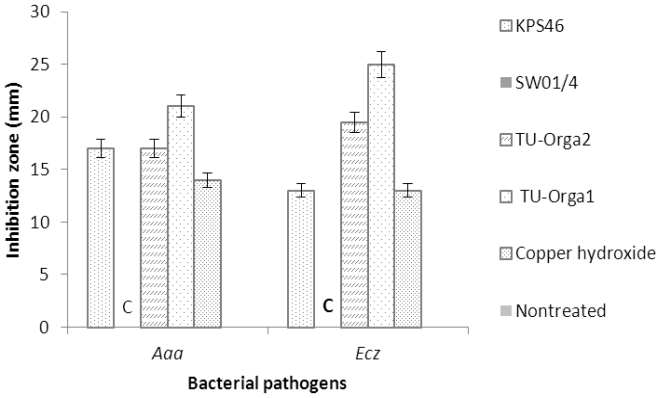

Figure 1. *Acidovorax avenae* subsp. *avenae* (*Aaa*) and *Erwinia chrysanthemi* pv. *zeae* (*Ecz*) growth inhibited by *Bacillus amyloliquefaciens* KPS46, *Paenibacillus pabuli* SW01/4, *Pseudomonas fluorescens* TU-Orga2, and *B. subtilis* TU-Orga1. C = competition, exhibited rapid growth, covered and inhibited the pathogen colonies.

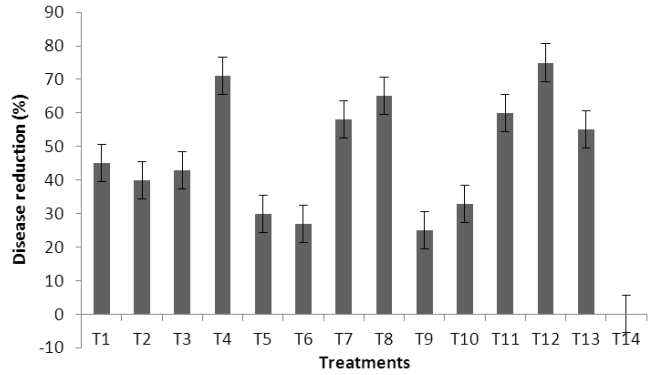

Figure 2. Efficacy of plant growth promoting rhizobacteria to control bacterial stalk rot of corn evaluated at 12 days after planting under greenhouse conditions. Codes T1 to T14 are shown in Table 1.

both pathogens by the same method (Figure 1). *B. subtilis* TU-Orga1 cells strongly suppressed growth of *A. avenae* subsp. *avenae* and *E. chrysanthemi* pv. *zeae* on NA plates, as revealed by a wide inhibition zone around the culture paper discs (Figure 1). For competition mode, *P. pabuli* SW01/4 exhibited rapid growth, covering and inhibiting the colonies of *A. avenae* subsp. *avenae* and *E. chrysanthemi* pv. *zeae.* These results demonstrate the different action modes and specificity modes of the 4 PGPR strains to bacterial leaf streak and bacterial stalk rot suppression.

Efficacy of plant growth promoting rhizobacteria (PGPR) against bacterial stalk rot under greenhouse conditions

We determine in this study, all of PGPR strains significantly increased seed germination, shoot, and root length better than the chemical treatment (copper hydroxide) and nontreated controls. Interestingly, seed treated with *B. subtilis* TU-Orga1 and *P. fluorescens* TU-Orga2 showed significantly highest increases in shoot and root length, and seed germination (Table 2).

Furthermore, seed treated with two these PGPR cells were significantly reduced better than seed treated with copper hydroxide (Funguran®) in bacterial stalk rot suppression (Figure 2). Interestingly, seed treatment and 3-foliar-spray-intervals at 14, 21, and 28 days after planting with strain *B. subtilis* TU-Orga1 (T12) was significantly reduced higher than the other treatments in bacterial stalk rot suppression (P = 0.05) (Figure 2). The results were correlated with paper disc diffusion method, *B. subtilis* TU-Orga1 produces secondary metabolites which may have lead enhanced plant growth and induced systemic resistance against pathogens. When applied as a seed treatment, the antagonistic bacteria can also promote growth and induce resistance against several diseases of corn where foliar spray may directly kill the pathogens by antibiosis mode and/or induce resistance (Buensanteai et al., 2008a).

Extrapolating from the accumulated literature, these PGPR may have numerous mechanisms including antagonism against pathogens, alteration of nutrient availability, and direct interactions with plants (Buensanteai et al., 2008b). Therefore, the application method was a significant factor in biological control.

Plant biochemical response analysis

In this study, the PGPR strains were evaluated for their ability to induce defense responses and related chemicals to protect corn from *E. chrysanthemi* pv. *zeae*, bacterial stalk rot pathogen infection. Corn treatment with *B. subtilis* TU-Orga1 and *P. fluorescens* TU-Orga2 triggered increased accumulation of SA biochemical markers associated with induced resistance mainly after *E. chrysanthemi* pv. *zeae* inoculation. Our results indicate that in corn plants of cultivar Insee2 treated with *B. subtilis* TU-Orga1 and *P. fluorescens* TU-Orga2, SA level increased significantly five days after treatment and much more three days after challenge inoculation with *E. chrysanthemi* pv. *zeae*, reaching the maximum concentration of 6.98, and 7.85 µg g^{-1} fresh weight (Figure 3). By contrast, SA accumulation in nontreated and chemical-treated (T13), but pathogen-inoculated corn, was considerably lower (1.69 and 2.17 µg g^{-1} fresh weight).

Efficacy of plant growth promoting rhizobacteria (PGPR) to suppression of naturally occurring diseases infection

All PGPR treatments showed significant efficacy for

Table 2. Seedling vigor of corn seeds cv. Insee2 treated with plant growth promoting rhizobacteria under greenhouse conditions[1].

Treatment[2]	Plant growth parameter		
	Seed germination (%)	Root length (cm)	Shoot length (cm)
KPS46	89[b]	8.0[b]	4.5[c]
SW01/4	86[bc]	7.5b[c]	5.9[b]
TU-Orga2	100[a]	9.0[a]	5.8[b]
TU-Orga1	100[a]	9.5[a]	6.5[a]
Copper hydroxide	80[c]	7.0[c]	4.4[c]
Nontreated	80[c]	7.0[c]	4.5[c]

[1]Means in a column followed by the same letter are significantly different according to DMRT. Data collected 7 days after planting. [2]KPS46 = *Bacillus amyloliquefaciens*, SW01/4 = *Paenibacillus pabuli*, TU-Orga1 = *B. subtilis*, and TU-Orga2 = *Pseudomonas fluorescens*.

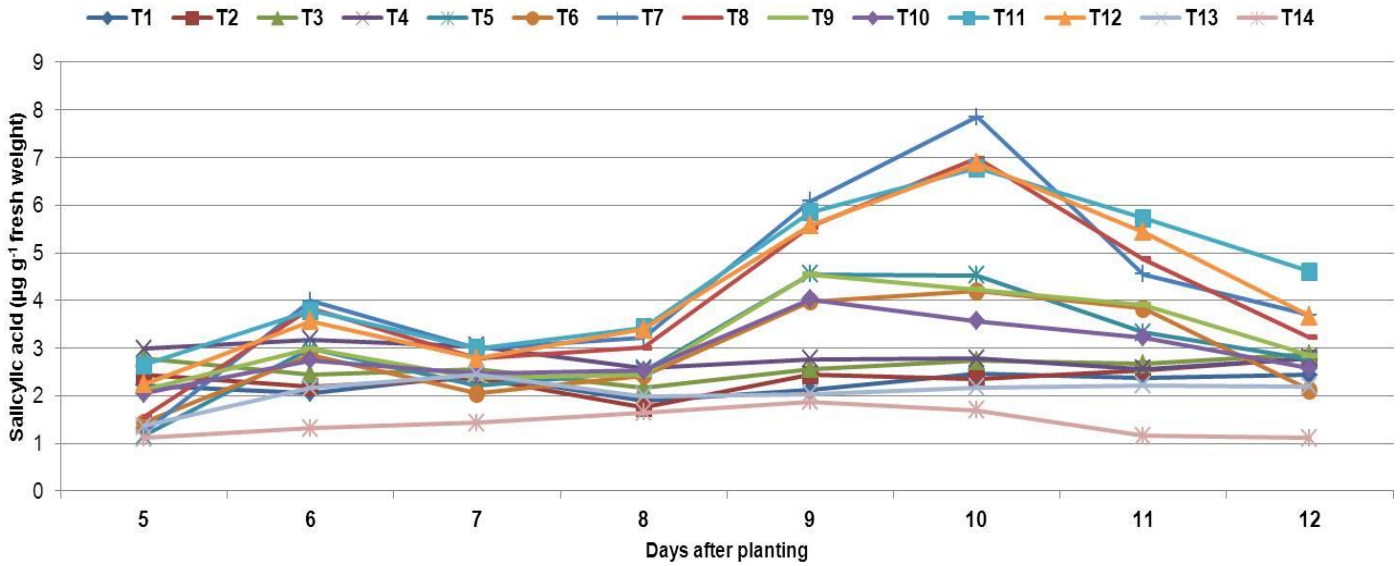

Figure 3. Accumulation of salicylic acid in leaves of sweet corn cultivars Insee2 pre- and post-challenge inoculation with *Erwinia chrysanthemi* pv. *zeae*. Codes T1 to T14 are shown in Table 1.

enhanced percentage of seed germination (Figure 4) and were significantly better than or equivalent to copper hydroxide (Funguran®) in reduced disease severity of bacterial leaf streak, bacterial stalk rot, and SCMV (Figure 5). There were differences among the treatments. The highest level of disease suppression of bacterial stalk rot resulted from treatments *B. subtilis* TU-Orga1 (T4, T8, T12) (P=0.05).

Interestingly, corn seeds treatments with *P. fluorescens* TU-Orga2 showed significantly highest increases in bacterial leaf streak and SCMV suppression (T3, T7, T11) (Figure 5). These PGPR strains appeared to have induced systemic resistance to various natural pathogens resulting in less infection compared with agrochemical, copper hydroxide (Funguran®) and nontreated control.

All treatments containing PGPR provided significant marketable yield increases (P=0.05) compared with that of chemical (Funguran®) and nontreated control (Figure 6). Interestingly, *P. fluorescens* TU-Orga2 provided greatest marketable yield increase and were significantly better in yield enhancement among the PGPR treatments (Figure 6). This indicates that PGPR enhanced plant growth, improved marketable yield, and induced resistance against bacterial stalk rot, bacterial leaf streak, and SCMV.

DISCUSSION

Many researchers have reported PGPR mediated plant protection against bacterial, fungal, and viral diseases of crop plants. In the present study, PGPR strains imparted beneficial properties in corn plants in terms of reduced disease severity and also resulted in significantly higher

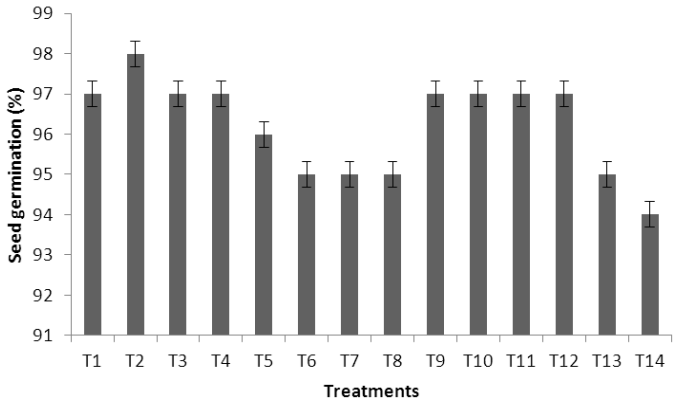

Figure 4. Efficacy of plant growth promoting rhizobacteria to enhances sweet corn cv. Insee2 seed germination at 14 days after planting under field conditions. Codes T1 to T14 are shown in Table 1.

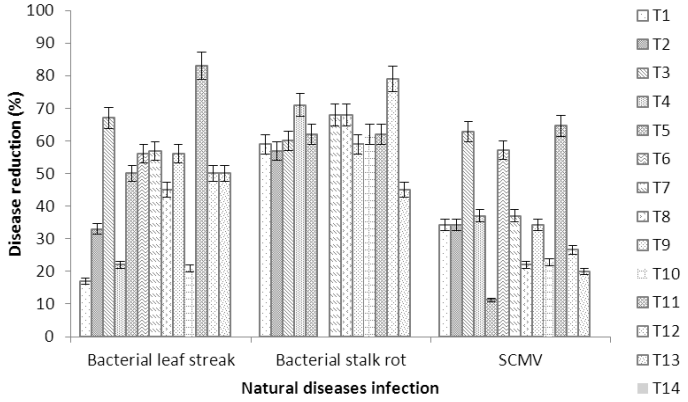

Figure 5. Efficacy of plant growth promoting rhizobacteria to reduction of bacterial leaf streak, stalk rot, and sugarcane mosaic virus diseases at 60 days after planting under field conditions. Codes T1 to T14 are shown in Table 1.

yield, when compared to the agrochemical (Funguran®) and nontreated treatment. Treatments with *B. subtilis* TU-Orga1, *P. fluorescens* TU-Orga2, *B. amyloliquefaciens* KPS46, and *P. pabuli* SW01/4 resulted in considerable disease suppression in corn plants.

The disease suppression observed in the PGPR treated plants is a product of antibiosis, enhanced plant growth, and induction of systemic resistance by the strains, as has been previously reported in many crops and also against many pathogen systems (Prathuangwong and Kasem, 2004; Sathitthampana et al., 2012). SAR by rhizobacteria has been proved against several bacterial, fungal, and viral plant-diseases (Alstrom, 1991; Leeman et al., 1995; Prathuangwong et al., 2005b; Sathitthampana et al., 2012).

B. amyloliquefaciens KPS46 and *P. pabuli* SW01/4 have been reported competitive with *Xanthomonas*

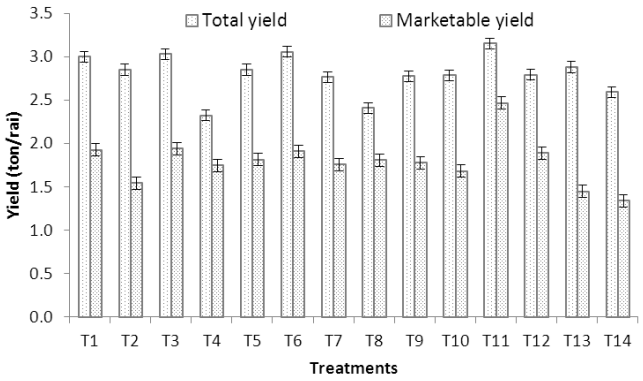

Figure 6. Efficacy of plant growth promoting rhizobacteria to enhances marketable yield of corn under field conditions. Codes T1 to T14 are shown in Table 1.

axonopodis pv. *glycines*, a soybean bacterial pustule pathogen. These two strains applied to seed treatment reduced various diseases of soybean in the field such as root rot (caused by *Fusarium* spp.), anthracnose (*Colletotrichum truncatum*), bacterial pustule (*X. axonopodis* pv. *glycines*), soybean crinkle leaf virus (SCLV), and soybean mosaic virus (SMV). They also increased yield quality and quantity through one or more mechanisms including competition (Prathuangwong and Kasem, 2004; Prathuangwong et al., 2005a; Buensanteai et al., 2009). Preecha et al. (2009) also reported that *B. amyloliquefaciens* KPS46 produced at least three effective antibiotics against *X. axonopodis* pv. *glycines*. Moreover, *B. amyloliquefaciens* KPS46 produced high levels of surfactin to inhibit *X. axonopodis* pv. *glycines*.

However, synthetic surfactants can stimulate plant growth by synergizing auxin action, activating certain plant enzyme systems, or affecting plant cell membrane permeability, thereby increasing water or nutrient uptake or excretion of plant factors such as riboflavin (Parr and Norman, 1965; Ernst et al., 1971).

B. subtilis TU-Orga1 has been reported competitive with *Xanthomonas oryzae* pv. *oryzae*, *Cercospora oryzae*, and *Bipolaris oryzae* the causal agent of bacterial blight, narrow brown spot, and brown spot diseases of rice, respectively (Boonnadakul et al., 2012; Sathitthampana et al., 2012). *B. subtilis* TU-Orga1 has been reported to secrete phytohormone like indole-3-acetic acid (IAA) for direct enhancement of rice growth (Sathitthampana et al., 2012). Therefore, secondary metabolites secreted by PGPR have a direct role in regulating plant growth, i.e., promote plant growth under optimal nutrient conditions and in the absence of plant deleterious organisms.

Bacteria and virus infection typically has a negative effect on photosynthesis and allocation of resources between organs, which leads to the characteristic chlorosis (Hull, 2002). A previously study has revealed the effect of the bacterial strain *B. amyloliquefaciens* KPS46 in bring-

ing out higher chlorophyll content in the leaves of the treated soybean plants (Buensanteai et al., 2009). This could be one of the reasons why there is a significantly higher yield in the *P. fluorescens* TU-Orga2 treated plants in the field. The present study shows 15 to 84% increases in the yield in the PGPR treated plants, when compared to the nontreated control.

The host resistance pathways involved in protection of crops from plant pathogen have been reported. Buensanteai et al. (2009) reported *B. amyloliquefaciens* KPS46 could express its function of salicylic (SA) and jasmonic acid (JA) signaling pathway to protect against *X. axonopodis* pv. *glycines*, soybean pathogen. This could be one of the reasons for *Bacillus* sp. significantly reduced *A. avenae* subsp. *avenae*, *E. chrysanthemi* pv. *zeae,* and SCMV infections. JA production by PGPR is responsible for the induction of systemic resistant in plants. SA is involved in systemic response related to defense processes, plays a key role in SAR response provoked by pathogen attack in many plant species, and treatment of SA will decrease the pathogen infection process (van Loon*,* 1997). Our study on the mechanism of biological control agents, two commercial strains *B. amyloliquefaciens* KPS46 and *P. pabuli* SW01/4 and new PGPR strains *B. subtilis* TU-Orga1 and *P. fluorescens* TU-Orga2 may be more than one mechanism to protect plant pathogen infection and enhance plant growth. With such information, we could potentially enhance the efficacy of biological control as a source of new bioproducts.

The host resistance pathways involved in protection of crops from viral diseases is unclear, even though there are various reports on systemic protection of plants from viral infection. Ahn et al. (2001) also suggest that there is simultaneous activation of both *PR-1a* and *PDF 1.2* genes in tobacco and *Arabidopsis* upon leaf-infiltration with EXTN-1. This indicated a SA and JA dependent pathway getting activated in crops with EXTN-1 as *PR-1a* gene is commonly used as an indicator of SA signaling and *PDF 1.2* of JA signaling (Reymond and Framer, 1998). Malamy et al. (1990) suggested SA is a likely endogenous signal in the resistance response of tobacco to viral infection.

Also, Ahn et al. (2001) demonstrated earlier the up regulation pathways of phenyl alanine ammonia lyase (PAL) and 3-hydroxy, 3-methylglutaryl CoA reductase (HMGR) in EXTN-1 treated tobacco plants upon challenge inoculation with Pepper mild mottle virus (PMMoV). Coordinated reduction of viral genome accumulation was clearly detected in the leaves of tobacco pretreated with EXTN-1. We have demonstrated earlier that *B. amyloliquefaciens* KPS46 has also induced production of SA, JA, PAL, peroxidase, and 1,3-β-glucanase indicating that these act as signals in the defense response pathway for the full expression of *PR-1a* and *PDF 1.2* genes and the other defense genes in *Arabidopsis* and soybean (Buensanteai et al., 2009). This could be one of the reasons why there is significantly lower SCMV infection in corn treated with PGPR strains. Furthermore, the mechanism of new PGPR strains *B. subtilis* TU-Orga1 and *P. fluorescens* TU-Orga2 need to be investigated.

This study reports on the use of PGPR with seed treatment and foliar spray for control of root and foliar diseases of corn. Crop management with an application of PGPR may provide longer term protection of plant development than that afforded by chemical (Funguran®) treatment alone. The data indicates that these PGPR strains have the potential for disease control and increasing plant growth and yield comparable to or superior to the pesticides and fertilizers for corn. All treatments using PGPR protected the plants satisfactorily against all natural disease infection, confirming the benefits of integrating control techniques. A biological control agent that can function in combination with other management tools to suppress diseases in the field might have value as a commercial product for disease control. Futuremore, for the Future perspective, the effect of environment, formulation and package on survival, stability and plant growth promoting ability of two new biological control agents will be studied.

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