Efficacy of *Bacillus atrophaeus* strain RS36 and *Priestia megaterium* strain RS91 with Partially Reduced Fertilization for Bacterial Leaf Blight Suppression in Rice Seedlings

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Abstract

Four plant growth-promoting rhizobacteria (PGPR) strains; *Bacillus atrophaeus* strain RS36, *Priestia koreensis* strain RS86, *Priestia megaterium* strain RS91 and *B. macauensis* strain RS100, were previously reported for their growth enhancement and anthracnose disease reduction in peppers. RS36 and RS86 do not produce siderophore, while RS91 and RS100 do. There is little evidence of using PGPR-mediated induced systemic resistance with reduced fertilization to control bacterial leaf blight (BLB) in rice seedlings. This study aimed to investigate the efficacy of those four individual PGPR strains and their co-inoculation with 75% recommended chemical fertilizer rate (RFR) against BLB disease in rice seedlings. Non-siderophore-producing PGPR strains experiment and siderophore-producing PGPR strains experiment were tested separately. Each experiment was conducted twice and contained a single strain and their mixtures with 75%RFR. Nonbacterized treatment with 100%RFR served as a control in each experiment. A completely randomized design was set up with 4 replications per treatment. Results demonstrated that rice seedlings treated with a single PGPR strain and their mixtures with 75% RFR generally had a lower percentage of disease severity than rice seedlings in the control treatment. Nevertheless, only rice seedlings treated with a single strain of RS36 in the non-siderophore PGPR experiment and a single strain of RS91 in the siderophore-producing PGPR experiment provided a significantly lower percentage of disease severity ($P \leq 0.05$) than the control of each experiment. No synergistic effect of disease suppression occurred when using PGPR mixtures. In conclusion, certain individual PGPR strains together with reduced fertilizer amount significantly suppressed BLB disease in rice seedlings.

Keywords: plant growth-promoting rhizobacteria, induced systemic resistance, bacterial leaf blight, reduced fertilization

Introduction

Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo.), is recognized worldwide as one of the major diseases in rice-producing areas. In Southeast Asia, including Thailand, crop loss ranges from 20%-70% depending on the cultivating areas. In general, susceptible rice seedlings are severely damaged or may be killed by this pathogen within two to three weeks (a syndrome known as "kresek") when transferred to a heavily infested area (Pacific Pests and Pathogens, 2019).

There have been many attempts to control BLB in mature rice plants such as rice-resistant cultivars by conventional and biotechnological means (Duy et al., 2021; Li et al., 2022; Wang et al., 2020), chemical control (Nasir et al., 2019; Sharma et al., 2022), and biological control using beneficial microorganisms including plant growth-promoting rhizobacteria; PGPR (Hop et al., 2014; Rahma et al., 2019; Rajer et al., 2022; Yasmin et al., 2016; Yasmin et al., 2017). Nevertheless, studies of biocontrol utilizing beneficial

microorganisms to control rice bacterial leaf blight in the past mainly focused on the mechanism of antibiosis, especially secondary metabolite released from microorganisms directly effecting on the Xoo. pathogen.

Several bacterial determinants of PGPR-mediated induced systemic resistance (PGPR-mediated ISR) mechanism have been reported as plants' immune inducers against pathogen invasion. Those determinants included lipopolysaccharide, flagellins, siderophores, biosurfactant, antibiotics, and volatile organic compounds (Ali et al., 2022; De Vleesschauwer et al., 2006; Jetiyanon & Plianbangchang, 2013; Meziane et al., 2005; Ryu et al., 2004; Weller et al., 2004).

Jetiyanon and Wittaya-areekul (2009) previously reported that four PGPR strains including *Bacillus atrophaeus* strain RS36, *Priestia koreensis* strain RS86, *P. megaterium* strain RS91 and *B. macauensis* strain RS100 induced pepper against anthracnose disease caused by *Colletotrichum gloeosporioides*. The four PGPR strains mentioned above promoted pepper growth when no pathogen inoculation was applied. As the mechanism of plant growth promotion, those four PGPR strains fixed nitrogen, solubilized phosphate, and produced indole-3-acetic acid (IAA). Only RS86 could solubilize potassium. Both RS91 and RS100 produced siderophore, which was one of the bacterial determinants for ISR activity. RS36 and RS86 did not produce siderophore. Their exact mechanism involving their ISR activity was still uninvestigated.

From our preliminary study, all four PGPR strains (RS36, RS86, RS91, RS100) and their mixtures promoted better rice growth and fresh weight as compared to the non-bacterized control. Most bacterized treatments also exhibited a somewhat higher number of tillers than non-bacterized control. Moreover, the investigators observed that leaves treated with PGPR were greener than the non-bacterized control (personal communication with Jetiyanon, K.), which might be due to nitrogen fixing ability of those four PGPR strains, one of the plant growth promotion effects. Reddy et al. (1979) reported that BLB severity in susceptible rice varieties was correlated with the increased nitrogen amount that plants received. Since nitrogen amount is one of the factors that could stimulate BLB development in rice, the use of PGPR-mediated ISR strains which also enhance nitrogen fixing ability might interfere with plants' immunity against BLB disease. Until now, unfortunately, there has been no study on this issue. The hypothesis of this study, therefore, was the using PGPR-mediated ISR strain possessing nitrogen fixing ability in combination with partially reduced fertilization could suppress BLB disease and compensate for rice growth equivalent to plants receiving full fertilization. This study aimed to investigate the efficacy of four individual PGPR strains and their mixtures with a 75% recommended chemical fertilizer rate (RFR) in rice seedlings against bacterial leaf blight disease under greenhouse conditions.

Materials and Methods

Sources of PGPR, maintenance and culture conditions, and compatibility test

B. atrophaeus strain RS36, *P. koreensis* strain RS86, *P. megaterium* strain RS91 and *B. macauensis* strain RS100 were obtained from the culture collection of the first author's laboratory at the Department of Agricultural Science, Faculty of Agriculture, Natural Resources, and Environment, Naresuan University, Thailand. RS36 was isolated from the rhizosphere of waxy corn (*Zea mays* var. *ceratina*) at Bang Rakam subdistrict, Phitsaulok Province, Thailand. RS86 was isolated from the rhizosphere of cassava (*Manihot esculenta*) at Ban-Krang subdistrict, Phitsanulok Province, Thailand. RS91 was isolated from the rhizosphere of Chinese cabbage (*Brassica*

rapa L.) at Bueng Phra subdistrict, Phitsanulok Province, Thailand. RS100 was isolated from the rhizosphere of Chinese kale (*Br. oleracea* var. *alboglabra*) at Bueng Phra subdistrict, Phitsanulok Province, Thailand.

The bacterial cultures were maintained in tryptic soy broth (TSB) (HiMedia, Laboratories Limited, Mumbai, India) supplemented with 30% glycerol at -80°C for long-term storage. One loop of each frozen PGPR strain culture was transferred to each 500mL Erlenmeyer flask containing 200mL of TSB and incubated on an open-air shaker (Amerex Instruments, Inc., Concord, CA, USA) with a speed 100 rpm/min at room temperature for 24h. Each PGPR strain was adjusted to 10^8 colony forming unit (CFU)/mL before use.

Testing compatibility between PGPR strains using the disk diffusion test method adopted by Beijerinck (1898) was aimed at ensuring that the bacteria did not produce any substance against each other before using as a mixture. For non-siderophore producing PGPR strains (RS36 and RS86), a hundred microliters of 10^5 CFU/mL suspension of RS36 (the tested strain) were dropped and spread over tryptic soy agar (TSA; Himedia) surface until dried. A sterile assay disc (6mm in diameter, Macherey-Nagel GmbH & Co. Germany) was dipped into RS86 bacterial suspension (10^8 CFU/mL), the challenged strain, and placed onto the agar already spread with the tested strain. Each plate contained four discs of strain RS86. Plates were incubated at 30°C for 24h. The result of the compatibility was examined the following day. The tests were conducted twice. For compatibility test between siderophore-producing PGPR strains (RS91 and RS100), the tests were performed the same procedure as mentioned above.

Sources of rice seeds and clay soils

Thai rice seed (*Oryza sativa* L.) cultivar Phitsanulok 2 was tested in this study. This cultivar is photoperiod insensitive and susceptible to bacterial leaf blight (BLB) pathogen. Seeds were kindly provided by the Phitsanulok Rice Research Center, Rice Department, Ministry of Agriculture and Cooperatives, Thailand.

Clay soils used in the greenhouse experiment were collected from the grower's rice paddy fields adjacent to Naresuan University and were autoclaved before use. Approximately 1 kilogram of clay soil was transferred to each pot (12 centimeters in diameter), and water was added to saturate the soil before planting.

Source of BLB pathogen, maintenance, culture conditions

Xanthomonas oryzae pv. *oryzae* strain 21PL013 (159) or Xoo-159 was obtained from Department of Agricultural Science, Faculty of Agriculture, Natural Resources, and Environment, Naresuan University, Thailand. The pathogen was isolated from infected BLB disease of Thai rice cultivar RD79 at Phitsanulok Rice Research Center, Wang Thong District, Phitsanulok Province, Thailand. Xoo-159 was maintained in TSB supplemented with 30% glycerol at -20°C before using for culture preparation.

For culture preparation, one loop of frozen Xoo-159 culture was transferred to a 250mL Erlenmeyer flask containing 100mL of TSB and incubated under an Environmental Shaker-Incubator (BIOSAN, Riga, Latvia) at a speed of 150 rpm/min under 28 °C for 24h. The cell suspension grew quickly within 24 h. Then, the Xoo-159 concentration was adjusted to 10^9 CFU/mL before use.

Testing *in vitro* antibiotic activity of PGPR strains on Xoo-159

The test was applied by Jetiyanon and Kloepper (2002). A hundred microliters of Xoo-159 suspension (10^9 CFU/mL) were spread over the TSA surface until dried. Each sterile assay disc was dipped into PGPR suspension (10^8 CFU/mL) of each PGPR strain and placed onto the agar already spread with the Xoo-159.

For non-siderophore producing PGPR strains, each plate contained two discs of RS36 and RS86. For siderophore producing PGPR strains, each plate contained two discs of RS91 and RS100. Plates were incubated at 28°C for 24h. The results were observed the following day. The tests were conducted twice.

Greenhouse Experiments

Two separate experiments, one with non-siderophore producing PGPR strains and another with siderophore-producing PGPR strains. The average temperature in greenhouse conditions was 34°C/27°C day/night. The experimental design of each experiment was completely randomized. For non-siderophore producing PGPR strains experiment, there were four treatments including; 1) non-bacterized control with 100% recommended fertilizer rate (RFR), 2) *B. atrophaeus* strain RS36 (10^8 CFU/ml) with 75%RFR, 3) *P. koreensis* strain RS86 (10^8 CFU/ml) with 75%RFR, and 4) mixtures of RS36 and RS86 (10^8 CFU/ml) with 75%RFR. For siderophore producing PGPR strains experiment, there were four treatments including; 1) non-bacterized control with 100%RFR, 2) *P. megaterium* strain RS91 (10^8 CFU/ml) with 75%RFR, 3) *B. macauensis* strain RS100 (10^8 CFU/ml) with 75%RFR, and 4) mixtures of RS36 and RS86 (10^8 CFU/ml) with 75%RFR. The mixtures of PGPR strains were prepared by combining equal proportions of each strain before application. Each experiment had four replications (pots) per treatment. Each pot contained one plant. Both experiments were independently conducted twice. The RFR of complex commercial fertilizer (15-15-15) was applied 38 kg N, 16 kg P, 32 kg K ha^{-1}.

Rice seeds were surface sterilized with 3% NaOCl for 10 min and rinsed three times with sterile dH_2O according to Jetiyanon and Plianbangchang (2002). Seeds were incubated at 30°C for 24h and were seeded in sterile clay soils containing in a plastic tray. Nine-day-old seedlings were transferred to pots containing sterile clay soils (one seedling/pot). Two days later, pots were drenched with 50mL of bacterial suspension, either individual PGPR strains or PGPR mixtures according to the treatment mentioned above. The non-bacterized control treatment was drenched with water. On the following day, rice plants in each treatment were fertilized according to the aforementioned rate. Pots were flooded starting from the day of transplanting, and the water was maintained about 2 centimeters above the soil line in each pot throughout the experiment.

Four weeks after rice seeding, all plants were artificially inoculation with infected scissors of the Xoo-159 suspension (10^9 CFU/ml) by cutting off the leaf tip (≈3 cm from the tip) at a fully expanded fifth leaf from the main rice stem as described by Ke and Yuan (2017). Disease development was monitored daily. Eight days after pathogen inoculation, the infected leaf was carefully cut by a clean scissor at the leaf base. Disease measurement was conducted by 1) measuring the lesion length from the inoculation site to the edge of disease invasion toward the leaf base and 2) measuring the percentage disease severity of infected leaf using a program Image Analysis Software for Plant Disease Quantification (ASSESS version 1, 2002) by Lakhdar Lamari, The American Phytopathological Society, St Paul, Minnesota, USA. Because the infected area of rice seedlings exhibited pale grey-green streak with little yellowish, a red marker was used to paint the infected area to differentiate it from the healthy green tissues as shown in Fig. 1. Then, each leaf was scanned under a Flatbed Scanner (Scanjet G2410, Hewlett-Packard Co., California, USA). Each scanned leaf was processed through the ASSESS program to calculate the percentage of disease severity. The percentage of disease severity is calculated based on the formula shown below.

$$\% \text{ disease severity} = \frac{\text{(digital value of infected area processed through ASSESS)}}{\text{(digital value of the whole leaf area processed through ASSESS)}} \times 100$$

Figure 1 Rice leaf (upper leaf side) illustrating faint grey-green streak in the middle leaf with a little yellowish color along the infected area from the cutting inoculation site downward to the leaf base (a) compared with red color after painting with a marker at the infected area (lower leaf side) to differentiate the green healthy tissues (b) before processing through ASSESS program

For measuring lesion length, each lesion was measured from the inoculating site to the longest invasive lesion edge toward the leaf base.

Since only one infected leaf from the whole plant in each treatment was prosecuted through disease development assessment, the rest of the plant was still available for the growth components evaluation, including plant height, plant fresh weight, root length, and root fresh weight. Plant height was measured from the soil surface up to the highest leaf. Plant fresh weight was evaluated by cutting the stem about 1 cm above the soil surface and weighing the whole plant on a two-digit weighing machine. Root examination was carried out by taking the whole roots out of the pot, then gently washing soils from roots with water until cleaned. The length from intact cores to the longest root of each washed root plant was directly measured. Excessed water was absorbed before weighing the root's fresh weight.

All data were analyzed by analysis of variance (ANOVA), and the treatment means were compared using Fisher's protected least significant difference (LSD) test at ($P \leq 0.05$) using SAS software (Statistical Analysis System Institute [SAS]).

Results

In vitro antibiosis of four PGPR stains against the BLB pathogen

After 24-h incubation, there was a faint growth of Xoo-159 occurring on the agar surface, while four PGPR strains developed a colony around a sterile disc. On the following day, a yellow straw-colored colony of Xoo-159 was evident all over the agar surface. RS36 had the greatest growth expanding out of a sterile disc edge about 10-12 mm. RS91 and RS100 expended approximately 3.5 mm from a sterile disc edge. Among PGPR

strains, RS86 had the least growth (≈1-2 mm from a sterile disc edge) compared to PGPR strains. Nevertheless, there was no antibiosis of those four PGPR strains against the Xoo-159 (Fig. 2).

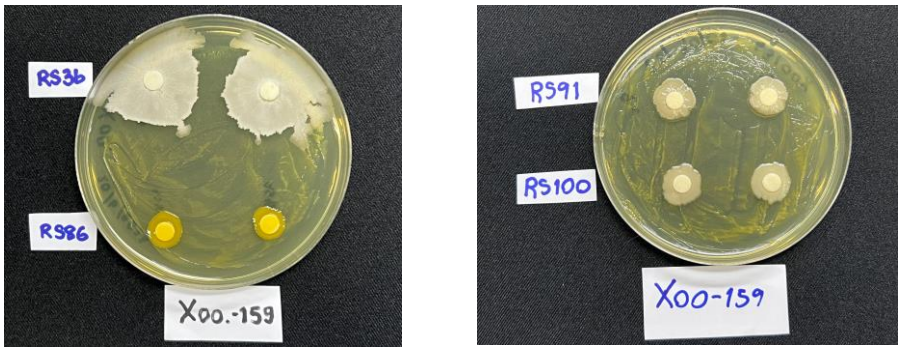

Figure 2 Testing *in vitro* antibiosis of non-siderophore-producing PGPR strains (RS36 and RS86; left) and siderophore-producing PGPR strains (RS91 and RS100; right) on *Xanthomonas oryzae* pv. *oryzae* strain 159 (Xoo-159) 48 hours after incubation

PGPR compatibility

No antibiosis was exhibited between non-siderophore-producing PGPR strains (RS36 and RS86) and between siderophore-producing PGPR strains (RS91 and RS100). The colony of the challenged strain, growing out of a sterile disc, was attached to the colony of the tested strain (Fig. 3).

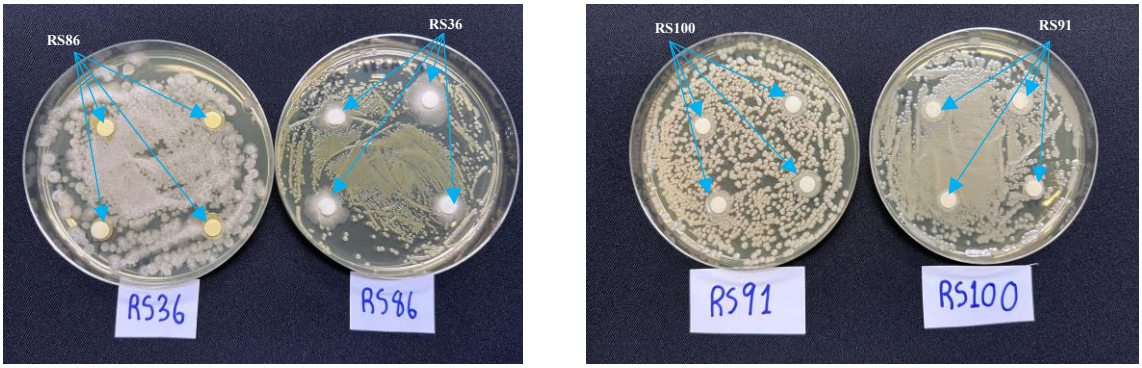

Figure 3 Compatibility between non-siderophore-producing PGPR strains (RS36 and RS86; left) and between siderophore-producing PGPR strains (RS91 and RS100; right) 24 hours after incubation

BLB disease suppression under greenhouse conditions

One day after challenged inoculation with the pathogen, little necrosis occurred at the infection site in all inoculated leaves. Slow disease progression generally occurred within 2-4 days after inoculation. On the fifth day after inoculation, dry leaf tissues with wavy edges appeared quickly along the sides of the infected area on the susceptible leaf, while the resistant leaf showed only a small amount of necrosis at the tips where inoculation occurred (Fig. 4). After day 5, susceptible leaves presented grey-green streaks starting from the inoculation site, with wavy edges and dry tissue extending toward the leaf base, while resistant leaves showed slower disease progression.

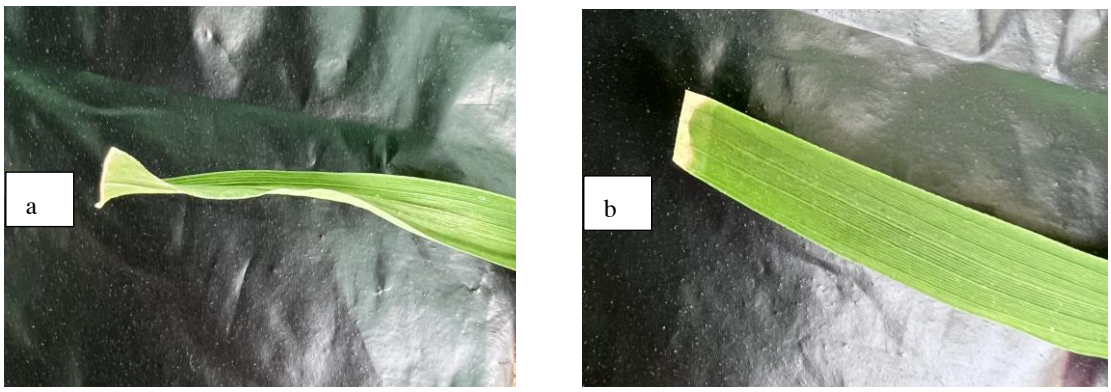

Figure 4 Susceptible rice leaf in non-bacterized control treatment showing dry-up tissues at the infected area with wavy edges (a) compared with resistant rice leaf in bacterized treatment treated with RS36 showing some necrosis at the infected tips (b) 5 days after BLB pathogen inoculation

For non-siderophore-producing PGPR experiment, leaves from all bacterized treatments with 75%RFR had shorter lesion lengths than leaves in the non-bacterized control treatment with 100%RFR alone. Rice induced with RS36 and 75%RFR had the shortest lesion length (≈4.4 cm) and significantly ($P \leq 0.05$) less about 37% shorter than that in the control treatment (≈7.0 cm). Rice leaf in the control treatment had the highest percentage of disease severity (≈21.11%) followed by treatment induced by RS86 with 75%RFR (≈19.78%), treatment induced by mixtures of non-siderophore producing strains with 75%RFR (≈15.40%), and treatment-induced by RS36 with 75%RFR (≈10.27%), respectively. Again, only rice induced by RS36 with 75%RFR had significantly about 51% lower disease severity than rice in the control treatment (Table 2). Considering the size of the infected leaf, all bacterized treatments with 75%RFR had greater leaf length and leaf area than the leaf in the control treatment. However, only treatment induced with mixtures of non-siderophore-producing strains had significantly longer lengths than the leaf in the control treatment ($P \leq 0.05$) (Table 1).

For the siderophore-producing PGPR experiment, disease severity in all bacterized treatments with 75%RFR was generally lower than rice in the control treatment. Only rice induced with RS91+75%RFR had significantly about 40% lower disease severity than rice in the control treatment ($P \leq 0.05$). The longest lesion length occurred in rice induced by PGPR mixtures (RS91+RS100) with 75%RFR (≈7.12 cm), followed by rice receiving 100%RFR alone (≈6.21 cm), rice induced by RS91 with 75%RFR (≈5.91 cm), and rice induced by RS100 with 75%RFR (≈4.77 cm), respectively. Both leaf length and leaf area of all bacterized treatments with 75%RFR generally were greater than the leaf in the control treatment. Only rice treated with a single PGPR strain had significantly longer leaf length ($P \leq 0.05$) than rice treated with 100%RFR alone (Table 1).

Growth promotion by PGPR strains

For non-siderophore producing PGPR experiment, the results showed that all rice growth components in bacterized treatments with 75%RFR, including plant height, plant fresh weight, root length and root fresh weight, were not significantly different as compared to the control treatment (Table 2).

For siderophore-producing PGPR experiment, the results demonstrated the same trend as those in the non-siderophore-producing PGPR strains. Generally, rice growth components in bacterized treatments with 75%RFR were not significant compared to the treatment receiving 100%RFR alone (Table 2).

Table 1 Efficacy of non-siderophore and siderophore-producing PGPR strains with 75% recommended fertilization rate (RFR) for the induction of systemic resistance in rice seedlings cultivar Phitsanulok 2 against bacterial leaf blight disease 8 days after challenge

Non-siderophore-producing strains experiment				
Treatment	**Mean lesion length (cm)**	**Mean leaf length (cm)**	**Mean percentage of disease severity**	**Mean entire leaf area (digital value)**
100% RFR alone	7.05a[y]	20.92b	21.11a	160,347a
RS36+75% RFR	4.48b	21.22ab	10.27b	171,451a
RS86+75% RFR	6.57a	21.61ab	19.78a	170,520a
RS36 and RS86 +75% RFR	6.21ab	22.68a	15.40ab	174,099a
$LSD_{0.05}$	2.02	1.67	5.79	19,805
Siderophore-producing strains experiment				
Treatment	**Mean lesion length (cm)**	**Mean leaf length (cm)**	**Mean percentage of disease severity**	**Mean entire leaf area (digital value)**
100% RFR alone	6.21ab[y]	21.22b	20.17a	161,410a
RS91+75% RFR	4.77b	22.57a	12.18b	176,608a
RS100+75% RFR	5.91ab	22.97a	15.25ab	177,446a
RS91 and RS100 +75% RFR	7.12a	22.10ab	17.15ab	175,305a
$LSD_{0.05}$	1.95	1.20	6.10	18,417

[y]Numbers with different letter(s) within a column show significant differences at $P \leq 0.05$ according to the least significant difference (LSD) test.

Table 2 Growth enhancement promoted by non-siderophore and siderophore producing PGPR strains with 75% recommended fertilization rate (RFR) in rice seedlings (cultivar Phitsanulok 2) 34 days after seeding[s]

Non-siderophore producing strains experiment				
Treatment	**Mean plant height[t] (cm)**	**Mean fresh weight[u] (gram)**	**Mean root length[v] (cm)**	**Mean root fresh weight[w] (gram)**
100% RFR alone	43.06a[x]	2.15a	16.22a	0.69a
RS36+75% RFR	43.07a	2.25a	17.67a	0.74a
RS86+75% RFR	44.89a	2.18a	17.43a	0.74a
RS36+RS86 +75% RFR	43.89a	2.32a	17.23a	0.73a
$LSD_{0.05}$	6.48	0.64	1.69	0.15

Table 2 (Cont.)

Siderophore producing strains experiment				
Treatment	**Mean plant height (cm)**	**Mean fresh weight (gram)**	**Mean root length (cm)**	**Mean root fresh weight (gram)**
100% RFR alone	40.13a[x]	1.48a	15.00a	0.48a
RS91+75% RFR	42.59a	1.64a	15.31a	0.58a
RS100+75% RFR	42.05a	1.74a	15.95a	0.56a
RS91+RS100 +75% RFR	42.58a	1.66a	16.36a	0.48a
$LSD_{0.05}$	5.89	0.42	1.96	0.12

[x] Numbers with the same letter within a column show not significant differences at $P \leq 0.05$ according to least significant difference (LSD) test.

Discussions

One of the principles of the PGPR-mediated ISR mechanism is that inducing microorganisms should not directly cause harm to the pathogen (Van Loon et al., 1998). A spatial separation between PGPR and pathogen is another consideration for ISR activity from PGPR act toward plant's immunity against pathogenic ingress. The absence of direct toxic effects of RS36, RS86, RS91, and RS100 on the Xoo. pathogen and different habitats between PGPR (applied as soil drenching) and bacterial leaf blight pathogen (airborne pathogen) were ISR mechanisms in this study. Our data were relevant to Jetiyanon and Kloepper (2002) study.

All bacterized treatments with 75%RFR generally showed lower bacterial leaf blight disease severity compared to the non-bacterized control with 100%RFR. Only rice treated with a single strain of *B. atrophaeus* strain RS36 and *P. megaterium* strain RS91 significantly exhibited inferior disease severity than the non-bacterized control. Moreover, either using mixtures of non-siderophore-producing strains or siderophore-producing strains did not show synergistic disease suppression on pathogen invasion. Salvo and Salamone (2019) reported that if veil-like pellicle development by bacteria occurs in a nitrogen-free medium such as JNFb semi-solid agar and then results in more alkalization of the culture medium changing color from green to blue due to bromothymol blue redox dye, it represents the more nitrogen fixation ability of the microorganisms. The Fig. 5 corresponded with Salvo and Salamone (2019) studies in which both RS86 and RS100 strains possessed greater ability of fixing nitrogen than RS36 and RS91. This result implied that rice induced by a single strain of either RS86 or RS100, and rice induced by mixtures of these two strains with RS36 or RS91 might receive higher nitrogen than that induced by a single strain of RS36 or RS91. There is one major consideration when using a PGPR mixture that exhibits both biocontrol and biofertilizer of nitrogen-fixing type. The excessive nitrogen production by the microorganism may result in impaired plant immunity against pathogens.

Several studies in the past used rice lesion length caused by bacterial leaf blight as a method for disease measurement (Ahsan et al., 2021; Duy et al., 2021; Wang et al., 2020). The lesion length, although easier for measurement, may easily cause interpretation errors for disease suppression since each entire leaf has a different length and the lesion does not consistently appear on each infected leaf (Fig. 6). Disease severity processed through ASSESS program investigated under greenhouse experiment in this study would be a more accurate representation of disease suppression than lesion length, since the entire leaf area is assessed against the infected area.

Even though rice seedlings in bacterized treatments received 25% less fertilizer than the control treatment, the study found that rice growth parameters in all bacterized treatments with 75%RFR are still comparable to the non-bacterized control with 100%RFR as shown in Tables 3. This indicated that all four PGPR strains applied either singly or as mixtures possessed the ability of at least 25% partial replacement of chemical fertilizer for rice growth. Our data agree with many studies that found similar plant growth by the use of PGPR with reduced chemical fertilizers and full rate of fertilizer alone (Adesemoye et al., 2009; Batool & Altaf, 2017; Jetiyanon & Plianbangchang, 2012).

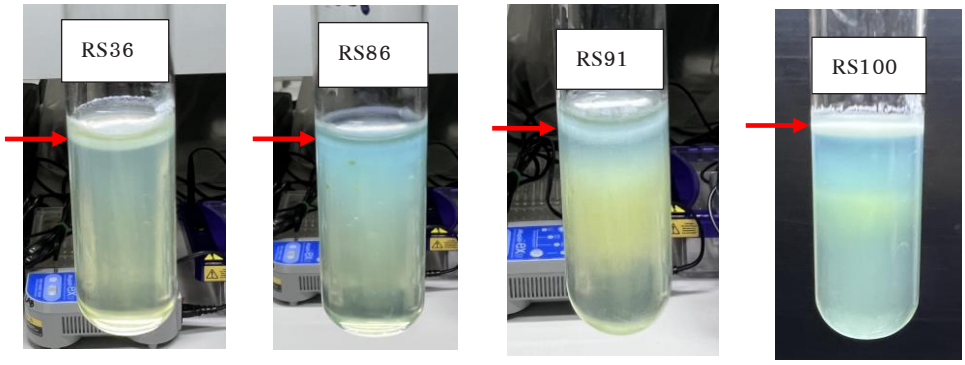

Figure 5 A typical veil-like pellicle development of *Bacillus atrophaeus* strain RS36, *Priestia koreensis* strain RS86, *Priestia megaterium* strain RS91, and *Bacillus macauensis* strain RS100 presented at the arrow under the medium JNFb semisolid surface 4 days after incubation period. The more alkalization of the culture medium changing color from green to blue, the more nitrogen fixation ability of the bacteria

Figure 6 Samples of rice leaves from treatment induced by mixtures of RS91 and RS100 with 75%RFR showing a non-uniform lesion from the starting clipping inoculation (a red marking representing a lesion caused by BLB disease development)

Conclusion and Suggestions

In conclusion, the application of certain individual PGPR strains, such as RS36 and RS91, with less nitrogen fixing activity and reduced fertilizer significantly suppressed bacterial leaf blight disease in rice seedlings. Further studies should focus on, monitoring rhizobacteria in plants, exploring appropriate rhizobacterial concentrations used to induce the plant's immune system, finding appropriate induction timing for maximum disease reduction, and investigating plant defense responses involved with disease suppression to answer critical questions for practical disease management.

Author Contributions

Author 1 (Kanchalee Jetiyanon): Conceptualization of the research, Development of methodology, Investigation and collecting data along with Author 2 and Author 3, Data analysis and interpretation, Manuscript writing.

Author 2 (Sasiwimon Boontawee) and Author 3 (Suttita Padawan): Investigation and collecting data.

Author 4 (Pinyupa Plianbangchang): Experimental design and statistic consultation, Manuscript review and editing.

Conflict of Interests

The authors declare no conflicts of interest. Some parts of this research work have been presented at the 20th Naresuan Research and Innovation Conference, July 11 - 12, 2024, Naresuan University, Phitsanulok, Thailand.

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