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EFFICACY OF *Bacillus siamensis* STRAIN IN MANAGING SHEATH BLIGHT, ENHANCING GRAIN YIELDS AND DECOMPOSING RICE STUBBLE AND STRAW

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ABSTRACT

Rice sheath blight (RSB) caused by *Rhizoctoni solani* is an economically significant disease worldwide. As an alternative to chemical fungicides due to increased concerns on environmental safety, the present study was conducted by screening certain plant growth-promoting rhizobacteria (PGPR). Bacteria isolates with high potential to inhibit mycelial growth of *R. solani*, were evaluated for their efficacy to promote growth of rice seedling in August 2014 at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. Rice seeds (var. Chai Nat1) were soaked in bacterial cell suspension (10^8 CFU/ml) for 24 h, then incubated for another 24 h before planting in circular cement well (80 cm - diameter, 45 cm - height) contained with paddy field soil. Isolate RRK1 provided the highest percent seed germination, longest root length of 21-d-old seedlings, effectively solubilized phosphate and provided high cellulase enzyme activity. After inoculation of 60-d-old rice plants with *R. solani* colonized paddy seeds, each plant was sprayed with 15 ml of bacterial cell suspension (10^8 CFU/ml) for three times at 7 d interval. Isolates RRK4 and RRK1 gave high efficacy to reduce RSB incidences by 54.89 and 32.71%, respectively, at 21 d after pathogen inoculation. Isolate RRK1 promoted plant growth and yield by increasing number per plant of tillers (21.31%), panicles (16.23%) and yield (28.92%).This isolate also increased rice stubble and straw decomposition efficiencies and significantly reduced number of sclerotia in soil. The strain RRK1 showed a 16S rDNA sequence similarity of 100% with *Bacillus siamensis* and also formed a distinct phylogenetic line with a clade encompassed by this species.

Key words: biological control, rice disease, biodegradation, yield enhancer, antagonistic bacteria

INTRODUCTION

Rice (*Oryza sativa* L.) is an important food crop for human consumption worldwide. Of different biotic stresses affecting rice cultivation, rice sheath blight (RSB) is an economically significant one causing considerable grain yield losses (Feng *et al*. 2008, 2017; Prasad and Eizenga, 2008). The RSB is caused by *Rhizoctonia solani* Kuhn and is problematic in all rice growing areas of the world. This disease is particularly severe when susceptible varieties are grown (Prasad and Eizenga, 2008). It

is a devastating disease of upland and rainfed lowland rice in Asia (Sariano and Reversat, 2003). Management of RSB is currently through application of chemical fungicides (Savary and Willocque, 2000; Pal et al., 2005). However, the environmental problems, pathogen resistance and harmful effects against beneficial microflora as well as producers and consumers due to indiscriminate use of chemicals is of huge concern in present day agriculture. In this context, use of an alternatives to fungicidal management of plant diseases is gaining significance. Plant Growth-Promoting Rhizobacteria (PGPR) are widely used as an alternative or supplement to chemical fungicides in managing plant diseases of rice (Kumar et al., 2009, 2012; Lucas et al., 2009). In rice, PGPR application has earlier proved to be highly beneficial both in terms of disease reduction and in enhancing grain yields (Kumar et al., 2012)

The gram positive bacteria, *Bacillus* spp. have been widely used as biocontrol agents against RSB. *Bacillus* spp. not only inhibit *R. solani* but also tolerate high temperature, desiccation, oxidizing agents and ultraviolet radiation (Jeyarajan and Nakkeeran, 2000). Certain strains of *B. subtilis* and *B. megaterium* provided significant inhibition of mycelial growth and sclerotial germination of *R. solani* (Luo et al., 2005; Kumar et al., 2012), whereas plant growth-promotion and yield enhancement by *Bacillus* spp. were also reported (Raja et al., 2006; Al-Taweil et al. 2009; Wang et al., 2009)

Sclerotia and mycelial fragments in plant debris and on weeds produced in previous rice crops are important primary sources of inocula for RSB outbreak in the following season (Kobayashi *et al*., 1997). The RSB inocula will eventually spread in the water, attach to the leaf sheath and blades and cause disease infection. Reduction of RSB inoculum through biological control by *Bacillus* spp. will alleviate the disease occurrence in rice fields.

For biological control of RSB, several *Bacillus* formulations were used with bacterial cell suspension (Wang *et al*., 2009); water soluble granules; floating pellets (Kanjanamaneesathian *et al*., 2007); powder formulations and empty fruit bunch powders (Al-Taweil *et al*., 2009). Mew and Rosales (1992) applied bacteria to rice seeds (seed bacterization), and soil (root) applications (Al-Taweil *et al*., 2009) were carried out to control RSB. Combined applications of bacterial antagonists or PGPR to seeds, roots and foliage may provide synergistic effects in RSB management.

Farmers usually burn rice stubble and straw remained in the rice fields after harvest. Preparation of planting areas as soon as possible for sowing new crops is a main reason for burning rice fields. However, burning of rice stubble and straw cause deterioration of soil structure, beneficial soil microflora, balance of mineral nutrients in soil as well as soil ecosystem. Alternatively, rapid fermentation process of rice stubble and straw by using beneficial bacteria may be a sustainable approach for environmental protection. Moreover, sclerotial numbers produced on rice stubble and straw may be reduced due to the antagonistic activities of beneficial bacteria. The present study sought to screen various bacterial isolates isolated from rice rhizosphere soil to control RSB and promote growth and grain yield of rice, and to test the efficacy of these bacteria to accelerate the decomposition process and reduce the survival ability of sclerotia in rice fields.

MATERIALS AND METHODS

Isolation of *Rhizoctonia solani.* *R. solani* was originally isolated from RSB infected seedlings by tissue transplanting technique (modified from Agrios, 2005). The pure culture was grown on potato dextrose agar (PDA) slant at room temperature (28-30˚C) for 3 d and kept at 4˚C until further use.

Screening of antagonistic bacteria. Rhizospheric soil samples were collected from rice plants obtained from the farmer's paddy fields at Moeng District, Kanchanaburi Province, Thailand. Bacteria were isolated from these soil samples by using serial dilution spread plate on nutrient glucose agar (NGA) medium. All bacterial isolates were tested for the inhibition of mycelial growth of *R. solani* by dual culture technique on PDA medium (Rabindran and Vidhyasekaran, 1996). The dual culture plates

were further incubated at 28°C for 3-5 d. Radial fungal growth of *R. solani* growing toward the bacterial colony was measured and compared with the control. The percent reduction in colony radius compared to the control was calculated and reported as an inhibition percentage using the formula: inhibition (%) = $100(R_1-R_2)/R_1$ where R_1 = colony radius of *R. solani* in the control (without bacterial colony) and R_2 = colony radius of *R. solani* in dual culture plate towards the bacterial colony. The width of clear (inhibition) zone was calculated using the formula: clear zone = $D_1-D_2/2$, where D_1 = average diameter of clear (inhibition) zone and D_2 = average diameter of bacterial colony.

Assay for cellulase enzyme activity. Assay for cellulase enzyme activities was performed by growing bacterial isolates on carboxy methyl cellulose agar (CMCA) at 28ºC for 3 d. The activity was assayed by using 1% Congo Red solution spread over surface of bacterial colonies for 30 min before pouring out and washing twice with 1M NaCl. Diameter of clear (inhibition) zone was recorded (Yung et al. 2009).

Phosphate solubilization test. Bacterial isolates were cultured in nutrient glucose broth (NGB) for 36-48 h. By using micropipette, 50 µl of bacterial culture was placed into each of four holes (5 mm in diameter) in an agar plate contained with Pikovskaya (PVK) agar (PVK ingredients per litre : 10g glucose; 5 g $Ca_3(PO4)_2$; 0.2g KCl; 0.5g $(NH4)_2SO_4$; 0.2g NaCl; 0.002g $MnSO_4.H_2O$; 0.002g $FeSO_4.7H_2O$ and 15g agar, pH 7.0) (Surange et al. 1971). All plates were incubated at 28ºC for 7 d, then the halo or clear zone diameter was measured.

Effects of bacteria in promoting rice seedling growth. Rice seeds (var. Chainat 1) were placed in double layers of cheese cloth and soaked in bacterial cell suspension (10^8 CFU/ml) for 24 h. After the cell suspension was drained off, soaked seeds were kept moist and incubated for 24 h before sowing in the circular cement well (80 cm - diameter and 45 cm – height) contained with paddy field soil. At 21 d after planting (DAP), root length and seedling height (shoot length) were recorded.

Rice sheath blight pathogen inoculation. Pure culture of *R. solani* isolated from infected leaf sheath with typical RSB symptom was used for rice plant inoculation. Mycelial plugs (5 mm-diameter) cut from a 3-d-old *R. solani* colony grown on PDA medium were inoculated onto autoclaved paddy seeds, five plugs per 150 g paddy seeds contained in heat tolerant plastic bag. All inoculated bags were incubated at 28°C for 7 d before use. Ten grams of mycelia colonized paddy seeds were placed in each porous paper fiber sachet or tea bag (7.5x7.5 cm) for using as pathogen inoculant. For pathogen inoculation, the 60-d-old rice plants were inoculated by inserting a sachet of *R. solani* colonized paddy seeds between the tillers (1 sachet per plant), attached to the water line. Three days after pathogen inoculation (DAPI), all sachets were removed from the rice tillers.

Effects of bacteria on sheath blight incidence and yield of rice. The effects of bacterial isolates on RSB incidence and yield of rice were tested under net house conditions. Rice seeds (var. Chainat 1) were soaked in bacterial cell suspension and sown in the circular cement well as mentioned above. The 60-d-old rice plants were inoculated with *R. solani* colonized paddy seeds. After pathogen inoculation, the whole plants were sprayed for three times at 7 d interval with 15 ml of bacterial cell suspension (10^8 CFU/ml) for each plant.

A randomized complete block design (RCBD) was used with eleven treatments, four replications for each treatment and 15 plants per replication (circular cement well). The first eight treatments were comprised of rice seed soaking and plant spraying with each of eight bacterial isolates. The ninth treatment was rice seed soaking with water and plant spraying with a fungicide (validamycin 3% W/VSL) at 20 ml/ 20 L and this served as the standard chemical control. The tenth treatment was rice seeds soaked with clean water and the plants were inoculated with *R. solani*, served as the untreated control, while the eleventh treatment was rice seeds soaked with clean water and uninoculated with *R. solani*, served as uninoculated or healthy control. Chemical fertilizers were applied three times to each

circular cement well as recommended by the Rice Department (Bureau of Rice Research and Development, 2018). These included N-P-K formulations of 16-20-0 (156.25 kg/ha), 46-0-0 (125.00 kg/ha) and 46-0-0 (62.50 kg/ha) which were applied at 15, 45 and 55 d after sowing (DAS), respectively.

Assessment of plant growth, disease incidence and yield. At 60 DAS, the number of tillers per plant was recorded, while RSB disease incidences were assessed at 14 and 21 DAPI. Plant height (the length from the base of tiller to the terminal of the flag leaf) and RSB lesion height were measured and disease incidence was calculated as percentage of relative lesion height (%RLH) using the following formula (Anh et al. 1986).

$$\text{Sheath blight incidence (\% RLH)} = \frac{\text{sheath blight lesion height} \times 100}{\text{plant height}}$$

For the yield assessment, rice panicles from each plant were collected and recorded as number of panicles per plant. All seeds were manually detached from the harvested panicles. The moisture content of the rice seeds was reduced to 14%, then total weight of the whole rice yield of all treatments were recorded.

Decomposition of rice stubble and straw (RSS). After harvesting, RSS were collected and dried before cutting into pieces (10-15 cm). These pieces were placed in the bag (5x7 inch) made from nylon screen (16x16 holes/inch^2) at 100 g per bag. The bioproduct prepared from each bacterial isolate as wettable powder formulation (10^8CFU/g) was added into each circular cement well at a rate 0.06 g/m^2 and thoroughly mixed with water in the well. The bags contained with pieces of RSS were buried under muddy soil (10-cm depth), four bags per circular cement well, four wells (replications) per treatment. At 7 and 14 d after burying, all bags were taken from the wells and subjected to strong water stream (3 min for each bag) in order to remove all decomposed tissues of RSS. The retained materials in the bag were dried and. weighed. Percentage of decomposition efficiency was calculated using the formula; Decomposition efficiency (%) = $100(W_1 – W_2)/W_1$, where W_1 = Dry weight of RSS in the bag before burying and W_2 = Dry weight of retained materials in the bag after decomposed tissues of RSS were removed. Sclerotia produced on or in partially decomposed RSS retained in each bag were recorded.

Identification of bacteria. The most promising beneficial bacterial isolate was identified at molecular level through 16S rDNA sequencing. For PCR amplification of 16S rDNA, DNA templates for PCR amplification were prepared by using "Genomic DNA mini kit (Blood/culture cell)" (Geneaid Biotech Ltd., Taiwan). DNA coding for 16S rRNA regions was amplified through PCR with Taq polymerase, as described by Kawasaki *et al*. (1993), Yamada *et al*. (2000) and Katsura *et al*. (2001). A PCR product for sequencing 16S rDNA regions was prepared by using two primers, 20F (5'-GAG TTT GAT CCT GGC TCA G-3', positions 9-27 on 16S rDNA by the *E. coli* numbering system; Brosius *et al*., 1981) and 1500R (5'-GTT ACC TTG TTA CGA CTT-3', position 1509-1492 on 16S rDNA by the *E. coli* numbering system; Brosius *et al*., 1981). The PCR amplification was taken with DNA Engine Dyad® Thermal Cycler (Bio-Rad Laboratories). Direct sequencing of the single-banded and purified PCR products (ca. 1500 bases, on 16S rDNA by the *E. coli* numbering system; Brosius *et al*., 1981) was proceeded. Then, sequencing of the purified PCR products was carried on an ABI Prism® 3730XL DNA Sequence (Applied Biosystems, Foster City, California, USA) by sequencing service provider. The two primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') or 800R (5'-TAC CAG GGT ATC TAA TCC-3') and 518F (5'-CCA GCA GCC GCG GTA ATA CG-3') or 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3') for single strand 16S rDNA sequencing, and 4 primers of 27F, 518F, 800R and 1492R for double strands 16S rDNA sequencing were used. The nucleotide sequences derived from all primers were assembled using Cap contig assembly program, an accessory application in BioEdit (Biological sequence alignment editor) Program (http://www.mbio.ncsu.edu/ BioEdit/BioEdit.html). The identification of phylogenetic neighbors was initially perfromd by the BLASTN (Altschul *et al*., 1997) program against the database containing type strains with validly published prokaryotic names

(Kim *et al*., 2012). Eight sequences with high scores were then used for calculating pairwise sequence similarity using global alignment algorithm (Myer and Miller, 1988), which was implemented at the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al. 2012).

Statistical analyses. All data were statistically analysed using ANOVA. The significance of differences between the treatment means was determined using Duncan's Multiple Range Test. All statistical analyses were performed using SPSS version 16.0 (SPSS Inc.; Chicago, IL, USA). The significant level was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Inhibition of mycelial growth of *Rhizoctonia solani* by bacteria

Total of 80 isolates of bacteria were obtained from rhizosphere soil of rice plants. These included 57 and 23 isolates of Gram positive and Gram negative bacteria, respectively. From dual culture test, nine isolates (RRK1 – RRK9) inhibited the mycelial growth of *R. solani* by 30.62 – 75.00% as compared to the control. The width of clear (inhibition) zone was in the range of 2.0 – 18.6 mm. Isolate RRK1 grew and spread rapidly toward the colony of *R. solani*. This isolate provided the largest size of colony (13.8 mm) without the obvious clear zone (2.0 mm), while isolate RRK9 had the smallest size of colony (0.2 mm) but with large inhibition zone (10.0 mm) (Table 1). The most promising mycelial growth suppression of *R. solani* by RRK1 might be attributed to the strong competition for nutrient and space, whereas RRK9 provided strong antibiosis mechanism by producing highly effective antibiotics to inhibit the growth of *R. solani* mycelia. However, detailed investigations are to be carried out to ascertain the specific antibiotic production of this RRK9 strain and its quantification before exploiting its use in the biological control of RSB under field conditions.

Table 1. *In vitro* effects of bacterial isolates derived from rice rhizosphere soil on mycelial growth suppression of *Rhizoctonia solani*, sizes of bacterial colonies and the widths of clear zones at 4 days after incubation

Isolate	Inhibition of mycelial growth(%)	Width of bacterial colony (mm)	Clear zone (mm)
RRK1	75.00 a[1/]	13.8 a	2.0 c
RRK2	63.75 b	7.0 b	6.5 b
RRK3	58.12 bc	5.3 c	18.6 a
RRK4	57.50 bc	2.1 d	6.0 bc
RRK5	55.62 bc	1.1 e	12.3 ab
RRK6	55.00 bc	0.9 ef	8.5 b
RRK7	51.25 c	0.7 ef	7.2 b
RRK8	38.75 d	0.4 f	2.5 c
RRK9	30.62 d	0.2 f	10.0 ab
Control	00.00 e	-	-
C.V. (%)	14.35	14.74	13.38

[1/] Means followed by the same letter (s) within each column are not significant difference according to Duncan's Multiple Range Test ($P \leq 0.05$).

Cellulase activity of bacterial isolates

RRK1 was the most promising isolate which produced high cellulases for degrading cellulose in CMCA medium. The observed cellulose clearance zone was 1.37 cm which was significantly higher than all tested isolates. Isolates RRK4, RRK9 and RRK10 produced 1.02, 1.01 and 0.91 cm of clear zones, while isolates RRK2 and RRK8 provided the smallest clear zones (0.43 and 0.58, respectively).

However, another four isolates (RRK3, RRK5, RRK6 and RRK7) failed to produce clear zone on CMCA medium (Table 2).

Table 2. Clear zones derived from cellulase production on carboxy methyl cellulose agar (CMCA) and phosphate solubilization on Pikovskaya (PVK) agar by beneficial bacteria

Isolate	Clear zones (cm) of cellulase enzymes	Clear zones (cm) of phosphate solubilization
RRK1	1.37 a[1/]	0.63 b
RRK2	0.43 c	0.23 d
RRK3	0.00 d	ND[2/]
RRK4	1.02 b	0.30 cd
RRK5	0.00 d	ND
RRK6	0.00 d	ND
RRK7	0.00 d	1.07 a
RRK8	0.58 c	0.77 b
RRK9	1.01 b	0.42 c
C.V. (%)	24.60	19.71

[1/] Means in a column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

[2/] Not determined

The hydrolysis zone on CMCA medium agreed with the reports of Yung *et al*. (2009) and Behera *et al*. (2014). The width of clear zone correlated with the ability of cellulolytic microorganisms for producing cellulases to hydrolyse cellulosic compounds (Cowling 1976). Therefore, RRK1 which was the most effective cellulase secretor could be expected to accelerate the degradation of straw and stubble in the rice field. Moreover, utilization of these substrates also provided potential to increase the population of RRK1 while reducing the survival ability of pathogen's propagules produced and attached or embedded in rice straw and stubble residues.

Phosphoruous solubilizing activity

The ability of bacterial isolates to solubilize phosphorus tested on Pikovskaya (PVK) agar revealed that RRK7 was the most promising isolate to solubilize P with a solubilization zone or clear zone of 1.07 cm. Isolates RRK1 and RRK8 provided 0.63 and 0.77 cm of phosphate solubilization zones, while RRK2 and RRK 4 gave low phosphorous solubilizing activities with 0.23 and 0.30 cm of clear zones, respectively (Table 2). The production of clearing zones around the colonies of the tested colonies is an indication of the presence of phosphate solubilizing capabilities of RRK isolates. Clear zone on a plate indicates the production of organic acids into the surrounding medium (Katznelson et al. 1962). This study indicated that RRK1 may have an important role in increasing soil phosphorous availability by improving solubilization of fixed soil phosphorous and applied phosphates, resulting in higher crop yields (Hameeda et al. 2008; Khan et al. 2007; Rodriguez et al. 2006; Zaidi et al. 2009).

Efficacy of bacteria on sheath blight incidence

All beneficial bacteria isolates significantly reduced RSB incidences both at 14 (29.57-54.89%) and 21 (19.16-32.71%) days after pathogen inoculation (DAPI). Disease reductions derived from bacterial isolates RRK1 (54.89%), RRK4-7 (51.41, 40.56, 48.28, 50.45%) and RRK9 (47.61%) were not significant difference at 14 DAPI, but these were significantly higher than the reduced disease incidence obtained from the use of chemical fungicide (validamycin). Among bacterial treatments, RRK1 gave the lowest RSB incidence (9.35%) as shown in Table 3. At 21 DAPI, all bacterial treatments significantly reduced RSB incidences (19.16-32.71%) as compared to the untreated control, while these disease reduced efficacies were comparable to the use of chemical fungicide (validamycin) (18.91%). The lowest RSB incidence was obtained from the treatment RRK1 (16.04%) which was significantly

lower than the fungicide treatment (19.33%) (Table 3). These results suggest the high potential to use beneficial bacteria derived from rice rhizosphere soil to soak seeds and spray the plant for reducing RSB incidence in organic rice production.

Table 3. Efficacy of beneficial bacteria to control sheath blight disease at 14 and 21 days after pathogen inoculation (DAPI) of rice plants by *Rhizoctonia solani*

Isolate	Disease incidence 14 DAPI (%)	Disease reduction 14 DAPI (%)[1/]	Disease incidence 21 DAPI (%)	Disease reduction 21 DAPI (%)[1/]
RRK1	9.35 d[2/]	54.89	16.04 c	32.1
RRK4	10.07 d	51.41	18.05 bc	24.28
RRK5	12.32 bcd	40.56	19.27 b	19.16
RRK6	10.72 d	48.28	18.01 bc	24.45
RRK7	10.72 d	50.45	18.43 b	22.69
RRK8	14.45 b	30.29	19.05 b	20.09
RRK9	10.86 cd	47.61	18.53 b	22.27
RRK10	14.60 b	29.57	17.88 bc	25.00
Validamycin	14.21 bc	31.45	19.33 b	18.91
Control (+ *R. solani*)	20.73 a	-	23.84 a	-
C.V. (%)	13.79	-	6.31	-

[1/] Sheath blight disease reduction was obtained from the disease incidences in the beneficial bacteria and validamycin (20 cc/20 l) treatments as compared to the *R. solani* inoculated control.

[2/] Means in a column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Our results are in agreement with previous reports on the use of *Bacillus* spp. to control RSB (Kanjanamaneesathian et al. 2007; Al-Taweil et al. 2009; Wang et al. 2009; Kumar et al. 2012). The present investigation indicated the promising suppression of RSB through both seed and foliar applications with beneficial bacteria. RSB has been demonstrated to effectively suppress through seed treatment, soil application and foliar spraying (Rabindran and Vidhyasekaran (1996). Multiple applications of *B. subtilis* MBI 600 as seed treatment, seedling root dips and foliar spraying effectively suppressed RSB under greenhouse and field conditions (Kumar et al. 2012). The efficacy of beneficial bacteria to reduce RSB in the present study might be attributed to the antibiosis mediated inhibition of RSB pathogen by bacterial isolates (He et al. 2002). Induction of systemic resistance against foliar pathogens by rhizosphere bacteria have been widely established (Singh et al. 2016).

Efficacy of bacteria on growth and yield of rice

Among bacterial treatments, tiller and panicle numbers per plant in RRK1 treatments (22.77 and 20.63) were significantly higher than the untreated control (18.77 and 17.15) and chemical fungicide treatments (17.99 and 15.43). The rice yields (g/m^2) in the treatments RRK1 (624.10 g/m^2) and RRK7 (606.60 g/m^2) were significantly higher than the untreated control (484.10 g/m^2). The rice yields from these two treatments were not significantly different when compared with all other RRK isolates (519.10 – 600.70 g/m^2), but were significantly higher than the chemical fungicide treatment (495.70 g/m^2) (Table 4). A significant increase in rice yields were obtained from treatments RRK1 (28.92%) and RRK7 (25.23%) compared with the untreated control, while the yield in chemical fungicide treatment was increased by 2.40% (Table 4).

Table 4. Effects of beneficial bacteria on tillers and panicles per plant and yield weight of rice (var. Chainat 1) grown in the 0.5 m^2 circular cement well (80 cm in diameter)

Isolate	Tiller/plant	Panicle/plant	Rice yield (g/m^2)
RRK1	22.77 a[1/]	20.63 a	624.10 a (+28.92%)[2/]
RRK4	20.88 a-d	18.26 abc	600.70 ab (+24.08%)
RRK5	19.22 a-d	17.53 abc	560.00 abc (+15.67%)
RRK6	20.77 a-d	16.86 bcd	589.10 abc (+21.68%)
RRK7	21.55 abc	16.80 bcd	606.60 a (+25.23%)
RRK8	20.55 a-d	17.20 bcd	530.70 abc (+9.62%)
RRK9	20.22 a-d	16.80 bcd	595.00 abc (+22.91%)
RRK10	20.21 a-d	16.43 bcd	519.10 abc (+7.23%)
validamycin	17.99 cd	15.43 cd	495.70 bc (+2.40%)
Control (+*R. solani*)	18.77 bcd	17.15 bcd	484.10 bc -
Control (-*R. solani*)	18.77 bcd	19.40 ab	582.32 abc (+17.24%)
C.V. (%)	9.39	10.00	10.69

[1/] Means in a column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

[2/] Percentages of rice yield weight (g/m^2) were increased (+) when compared with the pathogen inoculated control (control+ *R. solani*).

Our study revealed that the biological control efficacy of RRK isolates correlating well with its *in vitro* antagonistic activity (Table 1), cellulase enzyme activity and phosphate solubilization (Table 2). Phosphate-solubilizing microorganisms improve growth and yield (Singh and Kapoor, 1999). Thrust for alternatives to chemical control of plant pathogens has been increasing of late due to concerns about safety of both growers and consumers and the environments. The application of bacterial isolates RRK as biocontrol agents against RSB has shown promising results. Efficacies for enhancing growth and yield of most RRK isolates were comparable to the use of fungicide (validamycin), while RRK1 provided significantly higher efficacy. Therefore, further investigations on the efficacies of these RRK isolates against RSB under field conditions are needed. The most promising beneficial bacterial isolate RRK1 was identified at molecular level. The strain showed a 16S rDNA sequence similarity of 100% with *B. siamensis*. Moreover, phylogenetic analysis based on the sequence presented that strain RRK1 formed a distinct clade within the genus *Bacillus* neighbor-joining tree and was closely related to the *B. siamensis.* (Fig.1).

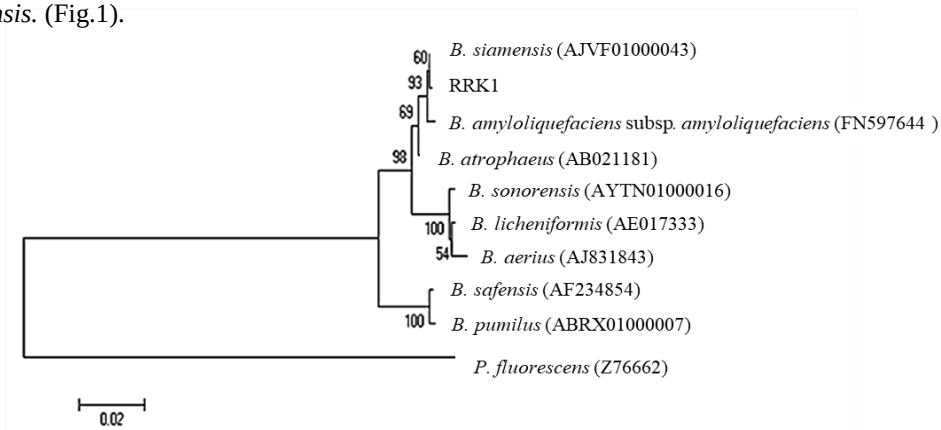

Fig. 1. Phylogenetic tree of *Bacillus* isolate RRK1 constructed by the neighbour-joining method based on the partial sequences of 16S rDNA.

Efficacy of bacteria for rice stubble and straw (RSS) decomposition

At 7 and 14 d after burying (DAB) RSS in the muddy soil, all RRK isolates excepted RRK10 significantly increased the percentages of decomposition efficiency by 69.96-137.15% and 88.46-144.95% as compared to the untreated control. RRK1 provided the highest increment of decomposition efficiency at 7 (137.15%) and 14 (144.95%) DAB, followed by RRK7 (120.45 and 114.33%, respectively). Low percent decomposition efficiency at 7 and 14 DAB was observed in chemical fungicide treatments (30.24 and 37.58%), the pathogen inoculated (control (+)) (24.31 and 20.34%) and the uninoculated control (28.32 and 23.58%) (Table 5). The fastest decomposition of RSS in RRK1 treatment may be attributed to the high cellulase activity which could be observed as large clear zone (1.37 cm) on CMCA medium (Table 2).

Table 5. Efficacy of beneficial bacteria for decomposing RSS (100 g) at 7 and 14 days after burying (DAB) in muddy soil and for reducing sclerotial numbers produced and survived in RSS

Treatment	Decomposition efficiency(%) 7 DAB	Decomposition efficiency(%) 14 DAB	Number of sclerotia in 100 g RSS
RRK1	48.00 a[1/] (+137.15)[2/]	57.76 a (+144.95)	56.00 h (-62.99%)[3/]
RRK4	38.06 a-d (+88.04)	44.44 bcd (+88.46)	86.33 d-g (-42.95%)
RRK5	37.58 a-d (+85.67)	43.66 bcd (+89.39)	108.67 cd (-28.19%)
RRK6	34.40 b-e (+69.96)	41.92 b-e (+77.77)	80.00 d-h (-47.13%)
RRK7	44.62 ab (+120.45)	50.54 ab (+114.33)	76.67 e-h (-49.33%)
RRK8	42.18 abc (+108.00)	49.10 abc (+108.35)	100.00 cde (-33.91%)
RRK9	42.30 abc (+108.99)	51.40 ab (+117.98)	61.33 gh (-59.47%)
RRK10	30.58 c-f (+51.08)	37.24 def (+57.93)	66.67 fgh (-55.94%)
Validamycin	30.24 def (+49.40)	37.58 c-f (+59.37)	119.33 bc (-21.14%)
Control (+)	24.31 ef (+20.10)	28.32 fg (+20.10)	151.33 a -
Control (-)	20.24 f -	23.58 g -	138.33 ab (-8.60%)
C.V. (%)	17.78	14.47	15.90

[1/] Means in a column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

[2/] Percentages of RSS decomposition efficiency were increased (+) when compared with the pathogen uninoculated control (Control-).

[3/] Percentages of sclerotia reduction when compared with the pathogen inoculated control (Control+).

Numbers of RSB sclerotia in 100 g RSS of all RRK treatments (56.00 – 108.67) were significantly lower than the untreated control (151.33). The RRK1 isolate provided the highest reduction of sclerotia (62.99%) produced in RSS, followed by RRK9 (59.47%), RRK10 (55.94%), RRK7 (49.33%), RRK6 (47.13%), RRK4 (42.95%) and RRK5 (28.19%) when compared with the RSB inoculated control. All RRK isolates except RRK5 and RRK8 provided significantly higher efficacy for reducing sclerotial numbers than the use of chemical fungicide (validamycin) treatment. (Table 5).

Sclerotia and diseased rice straw (debris) in the rice field are the primary infection sources responsible for the occurrence of RSB (Termorshuizen and Jeger, 2008; Tan *et al*. 2000; Feng *et al.* 2017). Sclerotia have high resistance and strong vitality in adverse environments and can survive for long time under diverse conditions (Li, 2004). Our study revealed that beneficial bacteria RRK isolates were responsible for the reduction of sclerotia retained in the RSS. Similar results were obtained by Feng *et al* (2008) who reported that some hyperparasitic or antagonistic fungal isolates belonging to *Trichoderma*, *Fusarium* and other fungal genera as well as some bacterial species were frequently isolated from the sclerotia artificially buried in dry paddy soil at normal room temperature. The survival

rates of sclerotia buried in paddy soil were significantly influenced by the moistures and microorganisms in soil (Feng *et al*. 2017).

Further study will be focused on the development of application management of beneficial bacteria RRK isolates in order to increase seed germination, seedling growth, rice yield, RSS decomposition and to reduce the amount of RSB sclerotia left in the field after harvest of rice crop or before sowing the new crop. It is also important to understand how to use beneficial bacteria RRK isolates to reduce the survival abilities of RSB fungus mycelia and sclerotia in diseased RSS. The effects of *B. siamensis* RRK isolates as plant biofertilizers should be further investigated.

CONCLUSION

Multiple beneficial effects were derived from RRK isolates which were isolated from the rice soil rhizosphere. Beneficial bacteria RRK1 was the most promising isolate that provided significant benefits for rice plant which included increased seed germination, seedling growth promotion, RSB disease reduction, increased yield, increased RSS decomposition efficiency and reduced sclerotial number. Although, the disease protection trait, as well as plant growth and yield promotion capabilities were comparable to the use of chemical fungicide, most RRK isolates provided higher decomposition efficiency of RSS as well as greater reduction of sclerotial number. Overall, our results indicated that RRK1 and several other isolates have the potential as multi-beneficial bacteria which are suitable for use in both general agricultural practices and sustainable organic rice production.

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REFERENCES CITED

Agrios, G.N. 2005. Plant Pathology. (5thedition). Burlington, MA: Elsevier Academic Press. 952.

Al-Taweil, H.I., M.B. Osman, A.A. Hamid and W.M.W. Yusoff. 2009. Development of microbial inoculants and the impact of soil application on rice seedlings growth. Am. J. Agri. Biol. Sci. 4(1): 79-82.

Altschul, S. F., T. L. Madden, A. A. Schaeffer, J. Zhang, Z. Zhang, W. Miller and D.J. Lipman, D. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25: 3389-3402.

Anh, S.W., R.L. Pena, B.L. Candole and T.W. Mew. 1986. A new scale for rice sheath blight disease assessment. IRRN. 11(6): 17.

Behera, B.C., S. Parida, S.K. Dutta and H.N. Thatoi. 2014. Isolation and identification of cellulose degrading bacteria from mangrove soil of mahanadi river delta and their cellulase production ability. Am. J. Microbiol. Res. 2(1): 41-46.

Brosius, J., T. J. Dull, D. D Sleeter and H. F. Noller. 1981. Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. J. Mol. Biol. 148: 107-127.

Bureau of Rice Research and Development. 2018. Rice Knowledge Bank. Bangkok, Thailand. Retrieved on Jan 4, 2018 from http://www.ricethailand.go.th/Rkb/management/index.php-file=content.php&id=7.htm.

Feng, S.J., M. Yang, D.B. Zhou and E.X. Zhou. 2008. Fungal and bacterial species isolated from sclerotia of *Rhizoctonia solani* and their effects on the growth of *R. solani*. Acta Phytopathol. Sin. 38: 557–560.

Feng, S., C. Shu , C. Wang, S. Jiang and E. Zhou. 2017. Survival of *Rhizoctonia solani* AG-1 IA, the causal agent of rice sheath blight, under different environmental conditions. J. Phytopathol. 165: 44-52.

Hameeda, B., G. Harinib, O.P. Rupela, S.P. Wani and G. Reddya. 2008. Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. Microbiol. Res. 163: 234-242.

He, Z.L., W. Bian and J. Zhu. 2002. Screening and identification of microorganisms capable of utilizing phosphate adsorbed by goethite. Commun. Soil Sci. Plant Anal. 33(5&6): 647–663.

Jeyarajan, R and S. Nakkeeran. 2000. Exploitation of microorganisms and viruses as biocontrol agents for crop disease mangement. In: Upadhyay R K, Mukeri K G, Chamola M P, editors. Biocontrol potential and their exploitation in sustainable agriculture. USA: Kluwer Academic/ Plenum Publishers. 95-116.

Kanjanamaneesathian, M., R. Wiwattanapatapee, A. Pengnoo, K. Oungbho and K. Chumthong. 2007. Efficacy of novel formulations of *Bacillus megaterium* in suppressing sheath blight of rice caused by *Rhizoctonia solani*. Plant Pathol. J. 6(2): 195-201.

Katsura, K., H. Kawasaki, W. Potacharoen, S. Saono, T. Seki, Y. Yamada, T. Uchimura and K. Komagata. 2001. *Asaia siamensis* sp. nov., an acetic acid bacterium in the α-Proteobacteria. Int. J. Syst. Evol. Microbiol. 51: 559-563.

Katznelson, H., E. Peterson and J.W. Rouatt. 1962. Phosphate dissolving microorganisms on seed and in the root zone of plants. Can. J. Bot. 40: 1181–1186.

Kawasaki, H., Y.Hoshino, A. Hirata and K. Yamasato. 1993. Is intracytoplasmic membrane structure a generic criterion? It does not coincide with phylogenetic interrelationships among photosynthetic purple nonsulfur bacteria. Arch. Microbiol. 160: 358-362.

Khan M.S., A. Zaidi and P.A. Wani. 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture-A review. Agron. Sustain. 27: 29-43.

Kim, O.S., Y.J. Cho, K. Lee, S.H. Yoon, M. Kim, H. Na, S.C. Park, Y.S. Jeon, J.H. Lee, H. Yi, S. Won and J. Chun. 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. Int. J. Syst. Evol. Microbiol. 62: 716–721.

Kobayashi, T., T.W. Mew and T. Hashiba. 1997. Relationship between incidence of rice sheath blight and primary inoculums in the Philippines: Mycelia in plant debris and sclerotia. Ann. Phytopathol. Soc. Jpn. 63: 324-327.

Kumar, K.V.K., S.K.R. Yellareddygari, M.S. Reddy, J.W. Kloepper, K.S Lawrence, X.G. Zhou, H. Sudini, D.E. Groth, S.K. Raju and M.E. Miller. 2012. Efficacy of *Bacillus subtilis* MBI 600 against sheath blight caused by *Rhizoctonia solani* and on growth and yield of rice. Rice Sci. 19(1): 55-63.

Kumar, K.V.K., S.K Raju, M.S. Reddy, J.W. Kloepper, K.S. Lawrence, D.E. Groth, M.E. Miller, H. Sudani and B. Du. 2009. Evaluation of commercially available PGPR for control of rice sheath blight caused by *Rhizoctonia solani*. J. Pure and Appl. Microbiol. 3(2): 485-488.

Li, S.D. 2004. Population dynamics and survival of *Rhizoctonia solani* AG-1 in field soil under rice wheat rotation. Agric. Sci. China. 3: 448–455.

Lucas, J.A., B. Ramos, F. Montes, J. Ojeda, M. Megias and F.J. Gutierrez. Manero. 2009. Use of two PGPR strains in the integrated management of blast disease in rice (*Oryza sativa*) in Southern Spain. Field Crops Res. 144(3): 404-410.

Luo, J.Y., G.L. Xie, B. Li, Y.C. Luo, L.H. Zhao, X. Wang, B. Liu and W. Li. 2005. Gram-positive bacteria associated with rice In China and their antagonists against the pathogens of sheath blight and bakanae disease in rice. Rice Sci. 12: 213-218.

Myers, E. W. and W. Miller. 1988. Optimal alignments in linear space. Comput. Appl. Biosci. 4: 11-17.

Pal, R., K. Chakrabarti, A. Chakraborty and A. Chowdhury. 2005. Dissipation of pencycuron in rice plant. J Zhejiang Univ SCI. 6(8): 756-758.

Prasad, B. and G.C. Eizenga. 2008. Rice sheath blight disease resistance identified in *Oryza* spp. accessions. Plant Dis. 92(11): 1503-1509.

Raja, P, S. Uma, H. Gopal and K. Govindarajan. 2006. Impact of bio inoculants consortium on rice root exudates, biological nitrogen fixation and plant growth. J. Biol. Sci. 6(5): 815-823.

Rodriguez, H., R. Fraga, T. Gonzalez and Y. Bashan. 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant and Soil. 287: 15-21.

Sariano, I.R.S. and G. Reversat. 2003. Management of *Meloidogyne graminicola* and yield of upland rice in South-Luzon. Phil. Nematol. 5: 879–885.

Savary, S. and L. Willocque. 2000. Rice pest constraints in tropical Asia: Characterization of injury profiles in relation to production situations. Plant Dis. 84(3): 341-356.

Singh, S. and K.K. Kapoor. 1999. Inoculation with phosphate-solubilizing microorganisms and a vesicular arbuscular mycorrhizal fungus improves dry matter yield and nutrient uptake by wheat grown in a sandy soil. Biol. Fertil. Soils. 28: 139-144.

Singh, U.B., D. Malviya, S. Singh, J.K. Pradhan, B.P. Singh, M. Roy, M. Imram, N. Pathak, B.M. Baisyal, J.P. Rai, B.K. Sarma, R.K. Singh, P.K. Sharma, S.D. Kaur, M.C. Manna, S.K. Sharma and A.K. Sharma. 2016. Bio-protective microbial agents from rhizosphere eco-systems trigger plant defense responses provide protection against sheath blight disease in rice (*Oryza sativa* L.). Microbiol. Res . 192: 300-312.

Surange, S., A.G. Wollum, N. Kumar and C.S. Nautiyal. 1971. Characterization of Rhizobium from root nodules of leguminous trees growing in alkaline soils. Can. J. Microbiol. 43: 891-894.

Tan, G.J., L. Chen, D.P.Hu and Y. Yang. 2000. Studies on viability and underground infectivity of *Rhizoctonia solani* sclerotia. Mycosystem. 19: 142–145.

Termorshuizen, A.J. and M.J. Jeger. 2008. Strategies of soilborne plant pathogenic fungi in relation to disease suppression. Fungal. Ecol. 1: 108–114.

Wang, H., K. Wen, X. Zhao, X. Wang, A. Li and H. Hong. 2009. The inhibitory activity of endophytic *Bacillus* sp. Strain CHM1 against plant pathogenic fungi and its plant growth-promoting effect. Crop Protect. 28: 634-639.

Yamada, Y., K. Katsura, H. Kawasaki, Y. Widyastuti, S. Saono, T. Seki, T. Uchimura and K. Komagata. 2000. *Asaia bogorensis* gen. nov., sp. nov., an unusual acetic acid bacterium in the α -Proteobacteria. Int. J. Syst. Evol. Microbiol. 50: 823-829.

Yung, C.L., D. Ganesh, D. Saratale, W.M. Chen, M.D. Bai and J.S. Chang. 2009. Isolation of cellulose-hydrolytic bacteria and applications of the cellulolytic enzymes for cellulosic biohydrogen production. Enzyme Microb. Technol. 44: 417–425.

Zaidi, A., M.S. Khan, M. Ahemad, M. Oves, and P.A. Wani. 2009. Recent advances in plant growth promotion by phosphate-solubilizing microbes. In: M.S. Khan, A. Zaidi, J. Musarrat (eds.). Microbial Strategies for Crop Improvement. Springer-Verlag. 23-50. Berlin Heidelberg.