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BIOCONTROL AND PLANT GROWTH PROMOTION IN RICE PLANT (*Oryza sativa* L.) BY ENDOPHYTIC BACTERIA

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Abstract

Endophytic bacteria are bacteria that have intimate relationship with their host without inducing any pathogenic symptom. It has an ability to protect their host against phytopathogens and promote plant growth. Present study was designed to isolate and characterize bacterial endophytes from root and stem of rice plant for their potential as biocontrol agents against rice bacterial leaf blight disease (*Xanthomonas oryzae pv Oryzae* (*Xoo*)) and plant growth promoting (PGP) traits. A total of two hundred and twelve endophytic bacteria were successfully isolated from surface sterilized healthy rice samples collected from different rice cultivation fields across Peninsular Malaysia. Seventeen isolates (7.3%) were found to inhibit the growth of *Xoo* by using disc diffusion assay. *In vitro* test demonstrated the ability of 19 (8.15%) strain to produce indole-3-acetic acid (IAA), 9 (3.86%) fixed nitrogen, 30 (12.86%) to produce phosphate solubilization and 16 (6.87%) to produce siderophores. Molecular identification by 16S rRNA amplification successfully identified the potential endophytic bacteria as *Enterobacter sp., Geobacillus thermoparaffinivorans, Gamma proteobacterium, Pseudomonas fluorescent, Bacillus subtilis* and *Bacillus cereus*. Under greenhouse conditions, high root and shoot length were recorded in rice inoculated with *Bacillus subtilis* with 69.87±3.56 cm and 29.73±1.55 cm respectively. *Geobacillus thermoparaffinivorans* exhibited the highest disease suppressing activity against *Xoo* and grain yield per panicle (34.23±7.79 g) on rice. In the light of these findings, *Geobacillus thermoparaffinivorans* may be considered as a source of biocontrol agent and plant growth promoter to boost rice productivity.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the leading food crops in the world and feeds more than half the world's population [1]. However, the crops are exposed to various types of biotic and abiotic which causes of yield losses. Rice seedling blight caused by *Xanthomonas oryzae pv. oryzae* (*Xoo*) is the common diseases causing severe crop damage and losses in rice yield [2]. Annually, the average yield losses due to bacteria seedling blight diseases were accounted to be more than 70% in Asian countries [3]. Occurrence of bacterial leaf blight disease during the main and off seasons in rice growing regions in Malaysia had been reported at Padang Besar, Perlis that affected more than 4,440 rice hectares of rice field [4]. Besides that, a major outbreak was also reported in Selangor, Kedah, Kelantan, as well as Perlis in 2014 and the yield loss was estimated more than 60,000 metric tonnes of rice [5] [6].

Several chemicals and cultural methods have been used by researchers for the management of the disease and plant development, but the results are not effective due to resistance of pathogen to different antibiotics and many other environmental factors [7]. The growing concern about human and animal health as well as environment clean forced researcher to carry out more research and technological advancement in providing national food security [8]. Previous research has found that the application of endophytic bacteria could suppress the bacterial disease and at the same time could enhance the health of plant development in agriculture sector [7].

Endophytic bacteria defined as bacteria that inhibit the internal part of plant tissue without causing any negative effect or cause any harms to the host [9]. The population density of endophytes is highly variable, depending mainly on the bacterial species and host genotypes but also on the host developmental stage, inoculum density, and environmental conditions. The distribution of the endophytes is observed more in root compared to the other plant's part and these phenomena indicating that endophytes enter the plant's tissues through roots and then migrates to others plant system [10]. Some of the endophytes act as biocontrol agents while others increase plant growth [11]. Plant growth activities have been shown for many endophytic bacteria by providing phytohormones and biologically fixing nitrogen. Keeping in view the above-mentioned important points, the aim of the present study was to isolate and characterize bacterial endophytes from rice leaves and root tissues according to its morphology and molecular characteristics. The isolates were also evaluated for their ability to suppress bacterial leaf blight disease.

MATERIALS AND METHODS

Materials

Universal markers, 518F (5′-CCAGCAGCCGCGGTAATACG-3′) and 800R (5′TACCAGGGTATCTAATCC-3′) were purchased from Sigma-Aldrich Inc., USA.

Methods

Sample Collection and Isolation of Endophytes

Source of rice plants from Kedah, Pulau Pinang and Kelantan were uprooted manually and brought to the laboratory. Root and leaf sections were cut to 2 cm long by using sterile scalpel. The surface of the leaves and roots was surface sterilized by using 10% (v/v) of Sodium Hypochlorite and a few drops of Tween-20 were added. Sterilized distilled water was used to rinse the samples and tissues was ground with mortar and a pestle to obtain the bacterial suspension. Serial dilutions were performed up to 10^{-3}, then the sample was streaked on nutrient agar and incubated at 30°C for 7 days. Control plate was prepared using the water from final rinse on NA [12].

Bacterial Pathogen

Leaves of rice plant from MARDI Seberang Prai's rice field that showed typical bacterial leaf blight symptoms were collected to isolate the *Xanthomonas oryzae pv. oryzae* (*Xoo*). The infected leaves were ground with sterilized pestle and mortar and was suspended for 2 hours in 200 ml of sterile saline. The suspensions were performed serial dilution up 10^{-4} in test tubes. Aliquots of 0.1 ml were spreaded on PSA media and incubated at 30°C for 5 days. The pathogenicity of the isolated *Xoo* was confirmed by clip inoculation method. *Xoo* suspension of 10^8 cfu ml^{-1} were clip inoculated on the thirty-day old rice plant that was grown in greenhouse [13].

Screening of Isolates for Biocontrol Activity

The antagonism of bacterial isolates against *Xoo* were screened *in vitro* by disc diffusion method as described by [14]. The cultures of 24 hours old grown nutrient broth (NB) were used for inoculation of bacterial strain on trypticase soy agar plates (TSA). Pour plate technique was applied to pour TSA medium into a petri dish. The antagonistic activity was estimated by measuring clear zone of bacterial growth surrounding the disc in mm and the experiment was carried out in triplicates.

In vitro Screening of Endophytic Bacteria for Plant Growth Promoting Properties

Quantitative and qualitative test were conducted to determine important PGP traits in endophytic bacteria. These included the detection of IAA production, ACC Deaminase production, siderophore production and Phosphate solubilizing ability.

Evaluation of Endophytes for Idole-3-acetic Acid (IAA) Production

The potential endophytic bacteria were grown in the 100 ml of LB broth and incubated at 30°C for 24h. The culture then was centrifuged at 10,000 g for 15 minutes and the supernatant was collected. After that, 2 ml of the supernatant and 2 ml of Salkowsky reagent was allowed to react for 30 minutes at 28°C. The development of pink color indicating the production of indole-3-acetic acid was recorded [15]. The density of pink color was then measured by using UV spectrophotometer at 530 nm wavelength [16]. The IAA quantities in samples were determined based on a calibration standard curve of pure IAA as a standard [17].

Nitrogen Fixation Activity

The potential endophytic bacteria were dispensed by 10 µl onto Nitrogen free media and was incubated for 7 days at 28°C. The positive result was recorded when the media changed from green to blue [16]. Nitrogen free media containing limiting amount of carbon source shows an increase in the pH when strain N3 is grown in it. The pH increase in the medium was visualized by the color change of the medium from green to blue, because Nitrogen free media contains pH sensitive dye bromothymol blue [18].

Siderophores Production

Chrome azurol S (CAS) agar plates was used to plate on potential endophytic bacteria and incubated at 30ºC for 24 hours. The result was recorded when yellow or orange halo around bacteria was detected [12]. Development of yellow or orange color halo zone due to iron removing from dye complex by siderophore [19].

Phosphate Solubilizing Production

Potential endophytic bacteria were grown on Pikovskaya's agar and incubated at 28±2°C for 48- 72 hours. Phosphate solubilizing ability of the cultures was observed when the clearance zone appeared [20]. Phosphate enzyme produce by potential endophytic bacteria converted tricalcium phosphate in the medium from insoluble to soluble forms [21].

Glasshouse Study

In Planta Evaluation of the Suppression of Bacterial Leaf Blight

The seeds were treated with the potential antagonistic bacteria for 2 hours and then were planted in a pot. The experiment was carried out by using completely randomized block design (RCBD). There were three replicated for each treatment with 3 seeds per pot. After 45 days of seedling, the plant was sprayed with antagonistic bacteria that was prepared with sterilized distilled water. Plants that were sprayed with sterilized distilled water only were acted as negative control plants while tetracycline antibiotic was used as positive control. The clip inoculation of leaves for negative control was done by applying sterilized distilled water whereas the infected control and inoculated treatments were clip inoculated by using scissor with *Xoo*'s culture on 23rd day of sowing. On the 7th, 14th and 21st day of *Xoo* inoculation, the lesion length was measured and the data for one treatment was obtained from 15 inoculated leaves [8].

The disease severity after leaves was inoculated by *Xoo* and the biocontrol efficacy was calculated using the following formula [21]:

$$Disease\ Severity\ (\%) = \frac{\text{No.of units X disease grade}}{\text{Total leaves observe X Max grade}} \text{ X } 100$$

$$Bio\ efficacy\ (\%) = \frac{\text{Relative disease severity of control treatment} - \text{disease severity of treatment}}{\text{Disease severity of control}} \text{ X } 100$$

In Planta Evaluation for Plant Growth Promotion of Endophytes

A greenhouse experiment was conducted to measure the plant growth-promoting effects of the isolated bacterial endophytes on rice seedlings varieties, MR284, which are commercially cultivated in Malaysia. Normal and healthy-looking, rice seeds were used and washed thoroughly with soap and rinsed under tap water. Subsequently, 50 mL of sterilized distilled water was used to soak in the seeds with 1ml of 10% providone- iodine for 2 hours and re-rinsed with sterilized distilled water. The most promising isolated endophytic bacteria was grown on nutrient agar plates and incubated at 30ºC for two days. Bacterial cells on nutrient agar plate was scraped from the medium and suspended in sterilized distilled water. Each bacterial suspension was inoculated with ten seeds for 24 hours and then was transferred in sterile soil in pots that will be placed under same conditions to allow the growth of the seeds. Completely randomized design (CRD) was applied with two repetition and each treatment contained 5 pots [22]. An equal amount of tap water was used with regular intervals to water the pots in plastic trays. The soil was steam sterilized at 100°C to eliminate the existing soil microbes. The noninoculated plants grown on sterile soil were used as controls. Phosphate and nitrogen fertilizer were applied during planting and 10 days after planting, respectively. Rice seed grown on sterile virgin soil, distilled water and without fertilizer act as negative control. The rice plants were watered daily. Morphological data such as plant height, root length, shoot length, grain yield per panicle were measure at 100 days of seedling. The seed germination rate was recorded by the formula [23]:

$$\text{Germination Percentage} = \frac{\text{The total seeds germinated at end trial}}{\text{number of initial seeds used}} \text{ X } 100$$

Identification of Antagonistic Bacteria using Molecular Method

Potential endophytic bacteria were incubated in Nutrient broth for 24 hours at room temperature. Then, 1 ml of overnight growth endophytic bacteria was centrifuge at 15,000 rpm for 3 minutes. Fresh endophytic bacteria pellet was used to extract the bacteria DNA by using Genomic DNA Mini Kit (RBC). Amplification of the 16S rRNA was performed by Polymerase Chain Reaction (PCR) with universal primer 518F (5′-

CCAGCAGCCGCGGTAATACG3′) and 800R (5′TACCAGGGTATCTAATCC-3′) [11]. Amplifications of gene was performed for 30 cycles in Eppendorf gradient thermal cyclers programmed [24]. The reaction was proceeded as 1 cycle for 3 minutes at 94°C (Denature), 40 cycles for 1 minute at 94°C (Denature), followed by 50°C for 1 minute (Anneal) and 1 minute at 72°C (Elongation). The PCR product were analysed on a 1.0% of agarose gel along with 500 bp ladder and was stained with 0.5 µg/ml of gel star. After that, the gels were visualized under UV light and photographed subsequently. The product of PCR was sent to MYTACG Bioscience Enterprise for sequencing process. The pairwise alignment analysis of partial 16S rRNA gene sequences was carried out on the MEGA X database [25]. The 16S rRNA gene sequences of known bacterial species were subjected to Basic Local Alignment Search Tool (BLAST) for identification and all obtained sequence were submitted to NCBI GenBank database [16].

Statistical Analysis

Analysis of variance (ANOVA) was performed based on the data obtained from the greenhouse experiment. Mean differences among treatment were compared using Tukey's Test at probability level of 0.05 by using SPSS Statistics Version 23.

RESULTS AND DISCUSSION

Addressing future food insecurity requires action on producing a better crop protection from the disease-causing phytopathogens. For this reason, sustainable agriculture has emerged as one of the efforts made by researcher using environmentally friendly biological agents [26]. Plant-bacteria associations has gained considerable attention by numerous researchers for their abilities to promote plant growth and control plant pathogens [27]. Endophytic bacteria are having the potential due to their ability to suppress disease as well as enhance the plant health and it seem to be economical because of lower cost [28]. The present study was carried out to isolate and characterize the endophytic bacteria from leaves and root for their abilities as biocontrol (antagonistic) agent against rice plant disease and to enhance plant growth under *in vitro* and *in vivo* conditions.

Results obtained in this study revealed that rice plant harbour large culturable endophytic bacteria. Two hundred and twelve endophytic bacteria with six distinct bacterial strains were identified from the roots and leaves (Figure 1). Endophytes in this study was confirmed with the absence of bacterial colony on control plates after the collected leaves and roots were sterilized as described by [29]. Most endophytic bacteria isolated in this study were obtained from roots. Endophytic bacteria are distributing heterogeneously within their host plants part. However, most endophytic bacteria can be found more in root rather than in leaf. Similar study was also reported by [30]. [31] reported the findings of endophytic bacteria isolated from rice root in China where majority of the bacteria (59.28%) belonged to genera Gallonella and Burkholderia of the Phylum Proteobacteria. Soil is the major source for bacteria that become endophytic inhabitant within the plant. Bacteria are able to invade the plant tissue through the roots opening before started to colonize the root. It is then move upward toward stem, leaf sheath and finally leaf blade [32]. Therefore, it was not surprising to find out that the isolated endophytic bacteria mostly came from root. Previous study suggested that plant species and cultivar including environmental conditions and also phytopathogens contributed to the frequency and compositions of occurring endophytic bacteria in plants [33].

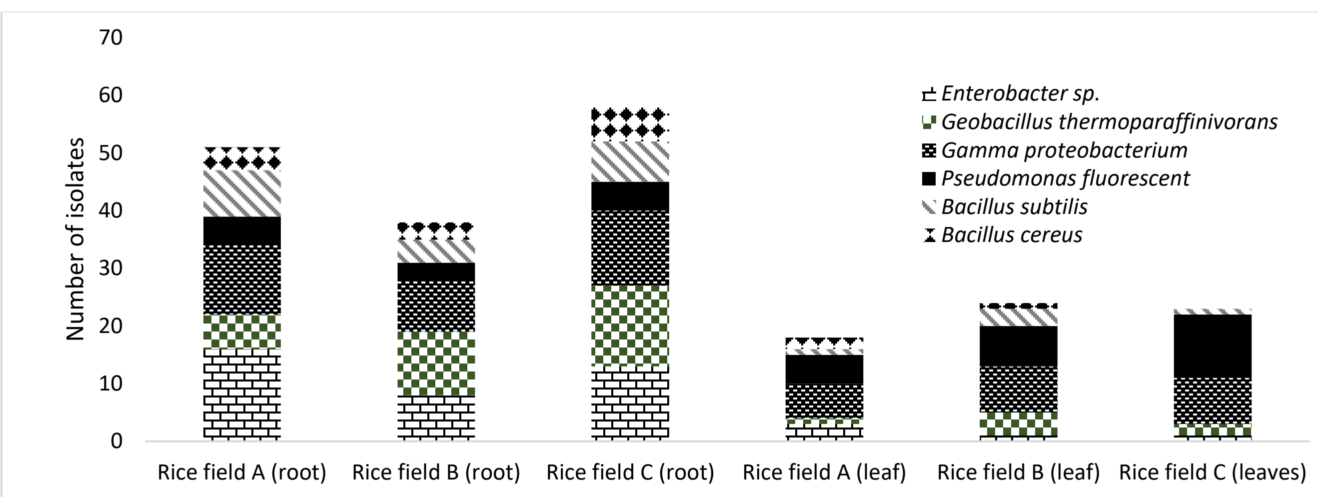

Figure 1. The distribution of the different bacterial genera of endophytes isolated from three rice cultivated fields across Peninsular Malaysia

Sequences analysis of the 16S rRNA gene generated by PCR was furthered characterized to identify the endophytic isolates. From the 16S rRNA nucleotide sequencing, all isolates displayed 99-100% similarity with a species already found in the GenBank database (Figure 1) including *Enterobacter sp., Geobacillus thermoparaffinivorans, Gamma proteobacterium, Pseudomonas fluorescent, Bacillus subtilis* and *Bacillus cereus.* Most of the isolated bacterial endophytes in this study were also reported in other studies on rice endophytes, though they were not isolated in similar rice cultivar, suggesting that these bacteria are widespread in the rice and that they are well adapted to the rice niche [29, 34]. We surmised that the differences of endophytic bacteria composition found in previous and present study was influenced by the environmental conditions due to the geographically different countries. Endophytic isolates were evaluated *in vitro* for their potential to inhibit the growth of bacterial plant pathogens, *Xanthomonas oryzae pv. oryzae*. The isolates suppressed the growth of the pathogens, demonstrated by different size of inhibition zone. The antagonistic activity of different bacterial isolates was significantly different. The inhibition zone recorded by bacterial endophytes ranged between 30 to 35 mm in radius (Table 1). Amongst the isolates tested *Geobacillus thermoparaffinivorans* and *Pseudomonas fluorescent* demonstrated the largest inhibition effect on the pathogens (Figure 2). *Enterobacter sp.* had the lowest inhibition effect against pathogens.

Table 1. Antibacterial activity of Endophytes against Bacterial Leaf Blight.

Endophytes	Diameter of Inhibition Zone (mm)
Enterobacter sp.	30.5 ^{a} ± 0.58
Geobacillus thermoparaffinivorans	35.0 ^{b} ± 0.00
Gamma proteobacterium	31.3 ^{c} ± 0.58
Pseudomonas fluorescent	35.0 ^{d} ± 0.00
Bacillus subtilis	33.3 ^{e} ± 0.58
Bacillus cereus	32.3 ^{f} ± 0.58

*Mean of three replications. Means in a column followed by different superscript letters are significantly different according to Tukey's test.

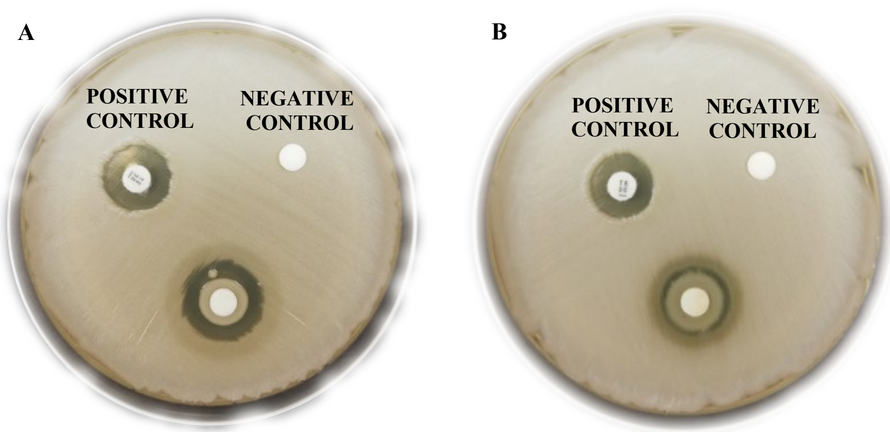

Figure 2. The inhibition zone produced by endophytic bacterial strains against. (A) *Geobacillus thermoparaffinivorans* and (B) *Pseudomonas fluorescent*

The glasshouse screening was performed to all the potential endophytes and it showed different effect on development of bacterial leaf blight disease in rice (Table 2). Generally, all potential endophytes demonstrated a substantial reduction of bacterial leaf blight disease compared to control. *Geobacillus thermoparaffinivorans* and *Bacillus sp* showed the highest disease control efficacy, 83.49% and 81.56% respectively. Similar study has reported that *Geobacillus thermoparaffinivorans* and *Bacillus* strain have broad spectrum of antagonistic activity against bacterial phytopathogens [35, 36]. The lowest suppression efficiency was observed in *Enterobacter sp.* with 24.27%. This indicated that the isolates might produce secondary metabolites that able to inhibit the growth of *Xoo*. Endophytic bacteria are reported able to produce enzymes and secondary metabolites such as antibiotics or chitinase enzyme, hydrogen cyanide phenazines phloroglucinols, pyrrolnitrin and pyoluteorin [37, 38, 39] which can supress growth of plant pathogens [40, 41]. Previous study had reported the genera of Bacillus, Pseudomonas and phylum

Proteobacteria as frequently occurring bacteria in agricultural crops [42, 43]. [44] found that *Pseudomonas fluorescens* strain PDY7 was able to suppress bacterial blight under glass house and field condition with production of 2,4-diacetylphloroglucinol (DAPG). As far as our concern, this is the first study reported the efficiency of *Geobacillus sp* against X*oo*. According to [45], *Geobacillus sp* are grouped together as *Bacillus sp* but then was reclassified based on thermophile's characteristic to a separate genus, *Geobacillus sp*. In another study, [46] reported the use of *Geobacillus sp* in controlling and protecting plants against Fusarium wilt, the widespread plant pathogen. Therefore, the result in present work suggested *Geobacillus thermoparaffinivorans* together with *Bacillus sp* to be developed and commercialize as promising biocontrol agent in agriculture sector in future.

Table 2. Effects of potential endophytes in controlling bacterial leaf blight disease in glasshouse experiment.

Treatment	Disease Severity (%)	Suppression efficacy (%)
Xoo	76.30	-
Xoo + Chemical bactericide	10.23	90.43
Xoo + *Enterobacter* sp	57.78	24.27
Xoo + *Bacillus subtilis*	14.07	81.56
Xoo + *Bacillus cereus*	36.30	52.42
Xoo + *Gamma proteobacterium*	43.70	42.73
Xoo + *Pseudomonas fluorescent*	51.11	33.01
Xoo + *Geobacillus thermoparaffinivorans*	12.60	83.49

Plant growth promoting bacteria gained popularity as biocontrol agents for their role in combating plant disease though their full potential has not yet been explored. *In vitro* tests found that all six strains were significantly able to produce siderophores and IAA, solubilize phosphate and could fix nitrogen.

Production of IAA by endophytes is another valuable trait that influences plant growth directly [47]. *Pseudomonas fluorescens* produce the highest amount of indole acetic acid; 8.69± 0.01 mg/L while *Bacillus subtilis* produce the lowest; 1.67±0.02 mg/L (Table 3). From previous study, it showed that most of the endophytic bacteria that produce IAA take part at the plant roots to improve the water and nutrients intake from the soil [16]. The production of IAA by these bacteria encourage the strength of the root, shoots weight and have more active metabolism [48]. The result of present strains with significant amount of IAA production are align to investigation made by [49] which reported the ability of *Bacillus sp* to produce IAA, siderophore and proven improve plant growth in paddy and many tropical plants. All the six strain were found positive for their ability to fix atmospheric nitrogen. Since plants are non-sessile and could not afford to take nitrogen directly from atmosphere, endophytes may help by fixing atmospheric nitrogen and providing it to the plants [50].

Table 3. *In vitro* of plant growth promotion activity

Isolates	IAA Production (mg/L)	Nitrogen Fixation	Siderophore Production (mm)	Phosphate Solubilization Activity (mm)
Bacillus cereus	$2.67^{a} \pm 0.03$	Positive	$4.33^{a} \pm 0.01$	$1.33^{a} \pm 0.03$
Bacillus subtilis	$1.67^{b} \pm 0.02$	Positive	$2.32^{b} \pm 0.02$	1.00b ± 0.02
Geobacillus thermoparaffinivorans	$3.00^{c} \pm 0.01$	Positive	$12.33^{c} \pm 0.03$	$5.67^{c} \pm 0.01$
Pseudomonas fluorescens	$8.69^{d} \pm 0.01$	Positive	$8.33^{d} \pm 0.02$	$1.67^{d} \pm 0.03$
Enterobacter sp.	$3.67^{e} \pm 0.03$	Positive	$3.99^{e} \pm 0.01$	$2.67^{e} \pm 0.02$
Gamma proteobacterium	$2.00^{f} \pm 0.01$	Positive	$4.67^{f} \pm 0.02$	$1.67^{f} \pm 0.01$

**Means in column followed with different letter (s) are significantly different*

Siderophore producing bacteria enable the plant growth promotion indirectly and suppress the growth of phytopathogens by sequestrating the limited iron [51]. Siderophore production by bacteria is crucial for photosynthesis process when the bioavailability of iron is low. According to [52], the lack of iron in plant will cause chlorosis. Chlorotic plant will grow unhealthy, produce less biomass and yield or in worse case will lead to complete crop loses [53]. Bacteria that have the potential to enhance plant nutrient acquisition are important in agriculture to transform the insoluble nutrients from soil for better plant absorption [54]. Siderophores have been suggested to be an environmentally friendly alternative to hazardous pesticides [55]. The present study demonstrated that the amount of siderophore produced varied between different endophytes. Significantly higher production of siderophores were shown by *Geobacillus thermoparaffinivorans* and *Pseudomonas fluorescens* isolates with 12.33±0.03 mm and 8.33±0.02 mm respectively. Different species of *Bacillus* and *Pseudomonas* have been described to have the ability to produce siderophore [56, 57, 58]. [59] stated the importance of phosphate solubilizing microorganism to improve fertility without causing any detrimental effect to health and environment compared to the usage of commercial fertilizers. Lately, the ability of bacteria to solubilize mineral phosphates gained an interest as it enhances the availability of inorganic phosphate for plant growth [60]. In this study, we found that *Geobacillus thermoparaffinivorans* strains exhibited the great potential for phosphate solubilization, both from inorganic and organic sources as compared to other strains. *Pseudomonas fluorescens* recorded among the lowest ability to solubilize phosphate solubilizing with 1.67±0.03 mm. However, previous reports demonstrated that phosphate is one of the significant traits of *Pseudomonas sp* and *Paenibacillus sp.* [61].

The existence of biofertilizer activities such as IAA production, nitrogen fixation and phosphate solubilization demonstrated that all six strains were capable to enhance plant growth. Hence, the ability to promote seedling growth were further explored among these strains. Surface sterilized rice seeds were treated with a few treatments as described in the experimental procedure. Rice seed demonstrated a significant increase in growth and bacterial inoculation (Table 4). Interestingly, all strain was found to induce significantly higher rate of germination percentage, increase plant height, root length, shoot length and grain yield per panicle as compared to the non-inoculated control plants.

Table 4. The *in vivo* study of growth promoting and disease suppressing activity of potential endophytic bacteria on rice.

Growth Promotion Activity						
No	**Treatment**	**Germination percentage (%)**	**Plant height cm/pot**	**Root length cm/pot**	**Shoot length cm/pot**	**Grain yield per panicle g/pot**
	Xoo (Negative control)	$77.67^{a} \pm 1.53$	$56.73^{a} \pm 8.44$	$13.83^{a} \pm 1.39$	$56.67^{a} \pm 3.79$	$22.45^{a} \pm 5.44$
	Xoo + chemical bactericide (Positive control)	$90.33^{b} \pm 0.58$	$68.13^{b} \pm 2.61$	$38.17^{b} \pm 2.02$	$62.00^{b} \pm 2.65$	$30.53^{b} \pm 4.02$
T1	*Xoo* + *Enterobacter* sp	$57.33^{c} \pm 1.15$	$66.93^{c} \pm 1.15$	$22.33^{c} \pm 2.08$	$55.33^{c} \pm 2.31$	$24.23^{c} \pm 1.20$
T2	*Xoo* + *Bacillus subtilis*	$81.33^{d} \pm 0.58$	$69.87^{d} \pm 3.56$	$29.73^{d} \pm 1.55$	$58.33^{d} \pm 2.52$	$30.47^{d} \pm 1.58$
T3	*Xoo* + *Bacillus cereus*	$62.67^{e} \pm 2.52$	$69.57^{e} \pm 7.42$	$20.00^{e} \pm 1.00$	$61.00^{e} \pm 2.00$	$18.30^{e} \pm 1.86$
T4	*Xoo* + *Gamma proteobacterium*	$75.33^{f} \pm 2.52$	$63.53^{f} \pm 7.06$	$27.47^{f} \pm 2.16$	$51.33^{f} \pm 2.08$	$20.93^{f} \pm 1.99$
T5	*Xoo* + *Pseudomonas fluorescent*	$72.00^{g} \pm 2.00$	$64.53^{g} \pm 1.14$	$24.00^{g} \pm 1.00$	$53.33^{g} \pm 1.53$	$30.89^{g} \pm 1.94$
T6	*Xoo* + *Geobacillus thermoparaffinivorans*	$93.33^{h} \pm 1.53$	$61.40^{h} \pm 1.56$	$26.00^{h} \pm 1.00$	$52.00^{h} \pm 3.00$	$34.23^{h} \pm 7.79$

*Means in column followed with different letter (s) are significantly different

The plants inoculated with of *Geobacillus thermoparaffinivoran* displayed significantly higher ($p \leq 0.05$) germination percentage (93.33±1.53%) as compared to that of non-inoculated control plants which showed 77.67±1.53% (Table 4). Plant height was also significantly different between the inoculated and control plants. High root and shoot length were recorded in rice inoculated with *Bacillus subtilis* with 69.87±3.56 cm and 29.73±1.55 cm respectively. According to [59], *Bacillus sp* was an endophytic bacterium most found in plant and plays role to stimulates plant growth. Inoculated plants with *Geobacillus thermoparaffinivorans* showed significantly higher ($p \leq 0.05$) grain yield per panicle i.e 34.23±7.79 g, as compared to 22.45±5.44 g in noninoculated control plants.

The capability of endophytic strains isolated in this study with plant growth activities confirm their symbiotic role in the rice host plant. Our findings suggested the potential endophytic strain could be directly and indirectly involved in promoting plant growth. The promising isolates should be further explored to identify the production of bioactive compounds conferring biocontrol activity for their performance at the field level.

CONCLUSION

The endophytic *Enterobacter sp., Geobacillus thermoparaffinivorans, Gamma proteobacterium, Pseudomonas fluorescent, Bacillus subtilis* and *Bacillus cereus* isolated from rice plant, exhibited antagonistic effects against rice bacterial leaf blight disease (*Xanthomonas oryzae pv Oryzae* (*Xoo*)) and plant growth promoting (PGP) traits. *Geobacillus thermoparaffinivorans* showed multiple plant growth-promoting effects that resulted in growth improvement of rice seedlings. Owing to these beneficial traits, the endophytic *Geobacillus thermoparaffinivorans* may play an important role in soil fertility, plant growth promotion, and disease control.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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