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Screening and Selection of Elite Plant Growth Promoting Rhizobacteria (PGPR) For Suppression of *Rhizoctonia solani* and Enhancement of Rice Seedling Vigor

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Sheath blight (ShB) of rice, caused by *Rhizoctonia solani* is an economically significant disease throughout the world. Management of ShB using plant growth-promoting rhizobacteria (PGPR) is an ecologically-friendly and viable alternative to current use of chemical fungicides. To select efficacious PGPR strains, approximately 70 isolates were screened for their antagonistic activity on vegetative growth and sclerotial germination of *R. solani* using dual in-vitro antibiosis assays. The strains were also screened for enhancement of rice seedling emergence under in vitro conditions. Ten PGPR strains were screened in each assay. In dual culture assays, mycelial plug of actively growing culture of *R. solani* was grown in Petri dishes containing 10% TSA and the PGPR strain was streak inoculated at 2 CM away on opposite sides. Plates were incubated at 250 C for 5 days and observations on mycelial inhibition of *R. solani* by PGPR were taken. Surface sterilized sclerotia of *R. solani* were treated with liquid PGPR inoculum for 24 h and later plated onto Petri dishes containing PDA. There were five replications for each PGPR strain. Sclerotial germination and growth inhibition were observed after incubating for 5 days at 250 C. To study the effect of PGPR strains on rice seedling vigor, surface sterilized rice seeds of Cv. Cocodrie in bacterial suspensions at a concentration of 4 x 108 cfu/ml for 24 h. The seeds were later dried and incubated for 7 days. There were 3 replications and 25 seeds for each replication. In vitro antibiosis assays showed that the majority of the strains significantly suppressed the mycelial growth of R. solani compared to the control. Similarly, four out of 70 strains tested significantly suppressed sclerotial germination when the sclerotia were treated with PGPR suspensions. Majority of the strains significantly increased seedling vigor when tested as seed treatments under in vitro conditions. The strains *Bacillus amyloliquefaciens* AP 219, *B. subtilis* strains MBI 600 and AP 52 performed the best in these assays.

Key words: Rice, Sheath blight, *Rhizoctonia solani*, Biocontrol, PGPR, Plant growth promotion.

A number of fungi, bacteria, virus, nematode and mycoplasma-like organisms cause disease on rice plants. Among the fungal diseases, sheath blight (ShB) caused by *Rhizoctonia solani* reduces yields up to 50% under optimum growing conditions[22] and is considered a serious constraint

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for rice production. The pathogen survives in the form of the dormant mycelia in previous crop stubble, as sclerotia in the soil for several years and on weeds[13,14]. Pathogen survival is mainly through sclerotia in rice fields. These sclerotia are irregularly shaped, brown to black colored, and are able to survive in soil for several years. The succeeding transplanting crop will be infected when these sclerotia float in the water and accumulate around plant bases under conditions of crop submergence. Infectivity of sclerotia is positively correlated to their size[16]. Initial infections occur on sheaths of rice seedlings near the water line and subsequently the ShB lesions spreads vertically. Under favorable conditions, these lesions coalesce and in some cases, lodging of seedlings occur especially in taller cultivars.

Rice cultivars' resistance to ShB ranges from very susceptible to moderately susceptible[6]. Currently, ShB is managed through systemic and non-systemic fungicides[19]. Management strategies mainly aimed at prevention of outbreak or epidemics through the use of both host plant resistance and chemical pesticides. The persistent, injudicious use of chemicals has toxic effects on non-target organisms and can cause undesirable changes in the environment. Many of these chemicals are too expensive for use by the farmers of Asia, where 90% of the world's rice is grown. Large-scale and long-term use of resistant cultivars is likely to result in shifts in the virulence characteristics of pathogens, culminating in resistance breakdown. Research during the previous two decades indicates another potential option for rice disease management, which is the use of plant growth-promoting rhizobacteria (PGPR). Use of PGPR assumes special significance as it is an ecologically-friendly and cost effective strategy which can be used in integration with other techniques for a greater level of protection with sustained rice yields.

Due to pesticide resistance, environmental pollution, and escalated crop protection costs, research on alternative disease management strategies is increasing. Research on PGPR for use on rice pathogens started in the 1980s. Research is still concentrated on the identification, evaluation and formulation of potential PGPR strains for deployment. For industry, meanwhile, ShB is economically important. It is one of the few crop diseases that justify the development of single-target fungicides. However, chemical fungicides present hazards to human health and the environment, and farmers in Asia are already rejecting them in favor of more sustainable approaches. But the Asian market is open to a range of chemicals, some of them so hazardous that their sales are restricted by several governments.

When fungicides are used intensively, they place enormous selection pressure on ShB, and the pathogen can develop resistance. Farmers are therefore left with the choice of using fungicides in moderation, which leaves the crop vulnerable to ShB, or beginning a cycle of heavier dosages of chemicals. Neither option is sustainable. Not only is chemical protection too expensive but, even in moderation, there are indirect costs from the use of fungicides to the health of the farming family and the surrounding ecosystem, which put great strain on the grower's limited resources. As these problems have become more widely recognized, the international agriculture research institutions have responded by shifting their focus to biological agents.

One strategy for biological control of ShB is the use of PGPR. In previous studies, some of the PGPR provided significant suppression of *R. solani* and increased seedling growth when compared to other fungal antagonists. Rice plants and ecosystem are the natural habitat of many bacteria and rice ecosystem is rich in bacteria that are beneficial in pathogen control and in plant growth promotion[16]. The populations of these bacteria however depend on the seed source, seed health and also on sowing of germinated or pre-germinated seed[4]. In tropical rice fields, some bacteria are considered responsible for fungistatic of *R. solani* sclerotia, as evidenced by the finding that more than 80% of the non viable sclerotia were colonized by bacteria (S. Z. Yin and T. W. Mew, *unpublished data*). Of these, gram positive bacilli such as *B. subtilis*, *B. laterosporus* and *B. pumilus* were prevalent in rice ecosystems with potential antagonistic activity on rice pathogens besides plant growth promotion[23]. Inoculation of rice seeds with bacteria can bring about positive changes in seedling growth and yield increase besides controlling plant pathogens[3]. Seed bacterization with *B. vallismortis* EXTN-1 resulted in seedling

growth promotion and suppression of sheath blight, blast and brown spot diseases[21]. *Bacillus* spp. produces endospores that withstand desiccation, heat, oxidizing agents, and UV radiations[10]. Earlier reports indicated that *Bacillus* spp. exhibited effective antagonism on ShB pathogen[26]. They also produce certain antibiotics[5] and enzymes such as phenylalanine ammonia-lyase (PAL), peroxidases, and pathogenesis-related proteins (PR) in rice leaves when applied against ShB disease[9].

To date, there are no published reports of the effect of PGPR on germination of sclerotia of *R. solani*. Because sclerotial germination is a key part of disease occurrence and rapid disease spread, any potential inhibition of sclerotial germination by PGPR would be helpful in disease management. Hence, the aim of the present study was to screen several PGPR strains with known plant growth-promoting and antagonistic activities in other crops and pathosystems for their antagonism to mycelial growth, and sclerotial germination of *R. solani,* as well as their influence on rice seedling vigor under *in vitro* conditions.

MATERIAL AND METHODS

Source of *Rhizoctonia solani*

A multinucleate and virulent isolate of *R. solani* anastomosis groupAG-1 IA was obtained from the culture collection of Dr. D. E. Groth, Rice Research Station, LSU AgCenter, Crowley, Louisiana, USA. The isolate was originally isolated from ShB infected rice seedlings. The culture was maintained on potato dextrose agar (PDA) or on rye kernels for further use.

Source of PGPR strains

Approximately 70 PGPR strains were obtained from Dr. J. W. Kloepper, Department of Entomology and Plant Pathology, Auburn University, AL, USA, and used in this study. The selected strains possessed one or several of the following characteristics: (i) *in-vitro* antibiosis against various fungal pathogens, (ii) promotion of rhizobial root nodulation, (iii) enhancement of root and shoot growth of various crops and vegetables, and (iv) capacity to produce plant growth regulators. Purified and identified strains were grown for 48 h at 25° C in 20 ml sterile tryptic soy broth (TSB) (Difco, Detroit, Michigan, USA) on a reciprocating shaker (80 rpm). Bacteria were pelletted by centrifugation for 20 min at 10,000 x g. Bacterial cells were then washed (twice) in 0.1 M phosphate buffer (PB) (pH 6.8), resuspended in TSB amended with 20% sterile glycerol, and frozen in vials at -80^0 C for long term storage. In each screening assay a new vial of PGPR was used.

Source of rice cultivar

A high-yielding, very early maturing long-grain cultivar of Cocodrie developed at Rice Research Station, LSU AgCenter, Crowley, Louisiana, USA was used throughout this study. The seeds were stored at 4° C prior to use.

Influence of PGPR for suppression of vegetative growth of *Rhizoctonia solani*

A series of bio-assays were conducted to screen the efficacy of PGPR strains to inhibit the vegetative growth of *R. solani*. In each bio-assay, there were 10 PGPR strains and a control treatment. Each treatment was replicated five times. For testing PGPR strains, strains were retrieved from -80° C freezer, thawed and streaked onto TSA and checked for purity after incubation for 24 h at 30^0 C. A screening assay was conducted on 10% TSA plates by adopting a dual culture plate technique[7] against *R. solani*. Plugs of mycelium (5 mm diameter) were cut from the edge of an actively growing fungal colony on PDA with a No. 2 cork borer, and one plug was placed in the center of each TSA plate (100 x 15 mm). Two parallel 3.5 cm long streaks of bacteria were then made 2 CM apart on opposite sides of the plug. The pathogen not inoculated with the selective PGPR isolate served as control. The plates were incubated at 25° C for 5 days in the dark. Five days after incubation, the inhibition of the mycelial growth of the pathogen was measured by using the formula:

$$I = \frac{100\ (C - T)}{C}$$

where I = inhibition of mycelial growth of pathogen (%), C = growth of pathogen in the control plate (mm) and T = growth of pathogen in plates challenged with PGPR (mm). The width of the inhibition zone between PGPR and pathogen was measured after 7 days.

Influence of PGPR on the germination of sclerotia of *R. solani*

The effect of PGPR on sclerotial germination of *R. solani* was assayed by adopting the following procedure[11]. For this assay, sclerotia of *R. solani* were produced on PDA at 25°C by incubating the inoculated plates for 10 days. Uniform sclerotia were collected from PDA plates and surface sterilized in 2.5% sodium hypochlorite solution for 2 min. In this assay, the same 70 PGPR strains used above were used. Seven screening assays were conducted. In each assay, there were 10 PGPR strains and an uninoculated control. As explained above, fresh culture of each PGPR strain was retrieved from -80° C and streaked on TSA plates. A loopful of 24h old culture was inoculated into 250 ml flasks containing 50 ml of 10% TSB and incubated for 24h on a rotary shaker at 175 rpm at room temperature (26±2° C). Five surface sterilized sclerotia were placed in each flask and incubated for another 24h on a rotary shaker under similar conditions as above. The sclerotia were later gently removed with sterile forceps and placed onto PDA plates and incubated for 5 days at 25° C in the dark. Germination rate of the sclerotia and mycelial growth from germinated sclerotia were measured. Sclerotia incubated in sterile TSB without PGPR served as control. There were five replications for each PGPR strain and control in each assay. The % inhibition of mycelial growth from sclerotia was calculated using the following formula:

$$I = \frac{100\ (C - T)}{C}$$

where I = inhibition of mycelial growth of pathogen (%), C = mycelial growth of sclerotia not challenged with PGPR (mm) and T = mycelial growth from sclerotia challenged with PGPR (mm).

Influence of PGPR on rice seedlings vigor index

Rice seeds (cv. Cocodrie) surface sterilized with 1% sodium hypochlorite solution for 2 min were used in seed germination assay. Surface sterilized seeds were treated with individual PGPR strains. For seed treatment, PGPR were produced in 10% TSB for 24 h on a rotary shaker at 26° C and a concentration of 4 x 10^8 cfu ml^{-1} was used. Seeds were soaked in PGPR suspension for 24 h and germination of PGPR treated seed was tested using standard Roll Towel method[8]. Seeds were later blot dried, and incubated in growth chamber for 7 days. There were 7 assays, and in each assay there were 10 PGPR strains and a control. There were three replications in each treatment, with 25 seeds per replication. Seeds soaked in sterile distilled water served as control. Percent germination of seeds, root and shoot lengths of seedlings were measured after 7 days and the seedling vigor index was calculated[1] as follows:

Vigor index = % Germination x Seedling growth (shoot length + root length)

Statistical Analysis

The data were analyzed using SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA) and the treatment means were differentiated by a least significant difference (LSD) at P=0.05 using PROC- GLM.

RESULTS AND DISCUSSION

Influence of PGPR on suppression of vegetative growth of *R. solani*

Of the 70 PGPR strains screened, the majority significantly reduced the mycelial growth of the ShB pathogen with percent inhibition ranging from 0 to 83% (Tables 1-7). Ten strains exhibited 70% inhibition or more. These superior strains include *Bacillus subtilis* strains MBI 600 and AP 52; *B. amyloliquefaciens* strains AP 219, AP 136, AP 188, and AP 295; *B. mycoides* strains ABU 1240, ABU 1627 and ABU 3586; and one strain of *B. simplex* ABU 3296. Of these superior strains, the highest per cent inhibition was obtained with *B. subtilis* MBI 600 (Fig. 1.) The inhibition zones for these PGPR strains ranged from 0 to 5 mm.

Influence of PGPR on the germination of sclerotia of *Rhizoctonia solani*

Sclerotial germination of *R. solani* was completely inhibited by 4 of the 70 PGPR strains; MBI 600 (Fig. 2) (Table 2), AP 219, AP 52 (Table 1), and *B. simplex* strain ABU 1053 (Table 5). These strains also exhibited significant antagonism on mycelial growth of *R. solani*. Reduction in hyphal growth from sclerotia for other PGPR strains ranged from 6.7% to 42.7%.

Influence of PGPR on ice seedlings vigor index

The majority of the screened PGPR strains when applied to rice seeds were found to enhance germination, seedling length and thereby the vigor as observed on 7-day-old seedlings (Table 1-7). Highly effective strains in promoting seedling vigor include MBI 600 (Fig. 4), AP 219, AP 52, AP 295, ABU 1240, ABU 1627, ABU 3586,

Table 1. Effect of PGPR strains on vegetative growth and germination of sclerotia of *Rhizoctonia solani* and seedling vigor of rice (Assay 1)

Strain	Identification	% Inhibition of mycelial growth	Inhibition zone (mm)	% Inhibition of Sclerotial germination	Seedling Vigor Index
AP3	*Bacillus safensis*	33.1^{d}	0^{c}	20^{cd}	5964^{f}
AP7	*Bacillus safensis*	55.6^{c}	2^{bc}	30.6^{b}	6404^{cde}
AP18	*Bacillus pumilus*	35.2^{d}	2^{bc}	17.3^{d}	5171^{g}
AP40	*Bacillus anthracis*	60.7^{bc}	4.6^{a}	16^{d}	6283^{def}
AP52	*Bacillus subtilis subsp. subtilis*	73.3^{a}	4.6^{a}	100^{a}	8124^{b}
AP136	*Bacillus amyloliquefaciens*	74.5^{a}	5^{a}	34.6^{b}	6246^{ef}
AP188	*Bacillus amyloliquefaciens*	69.4^{ab}	4^{ab}	36^{b}	6614^{cd}
AP209	*Bacillus subtilis subsp. subtilis*	63.5^{abc}	4^{ab}	28^{bc}	6686^{c}
AP217	*Bacillus macauensis*	0^{e}	0^{c}	17.3^{d}	5961^{f}
AP219	*Bacillus amyloliquefaciens*	74.5^{a}	4^{ab}	100^{a}	8864^{a}
Control	-	-	-	-	5193^{g}

Means followed by a common letter in the columns are not significantly different at $p<0.05$.
[1]Mycelial growth was recorded at 5 days after incubation and % inhibition was calculated over control.
[2]Width of inhibition zone between pathogen and PGPR was measured at 7 days after incubation.
[3]Sclerotial growth was recorded at 5 days after incubation.
[4]Seedling vigor index was calculated at 7 days after incubation.

Table 2. Effect of PGPR strains on vegetative growth and germination of sclerotia of *Rhizoctonia solani* and seedling vigor of rice (Assay 2)

Strain	Identification	% Inhibition of mycelial growth	Inhibition zone (mm)	% Inhibition of Sclerotial germination	Seedling Vigor Index
AP278	*Bacillus subtilis subsp. subtilis*	61.1^{bc}	5^{a}	22.6^{bcd}	8145^{c}
AP279	*Bacillus subtilis subsp. subtilis*	56.0^{c}	2^{c}	24^{bcd}	5390^{f}
AP280	*Bacillus safensis*	27.4^{d}	0^{d}	25.3^{bcd}	6456^{d}
AP281	*Bacillus safensis*	20de	0^{d}	14.6^{d}	5610^{f}
AP282	*Lysinibacillus boronitolerans*	0^{e}	0^{d}	22.6^{bcd}	5561^{f}
AP283	*Bacillus safensis*	65.4^{abc}	3^{bc}	17.3^{cd}	7892^{c}
AP294	*Paenibacillus peoriae*	66.6^{abc}	4.3^{ab}	32^{b}	5985^{e}
AP295	*Bacillus amyloliquefaciens*	77.6^{ab}	4^{ab}	29.3^{bc}	13600^{a}
MBI 600	*Bacillus subtilis*	83.1^{a}	5^{a}	100^{a}	13192^{b}
AP302, 299	*Bacillus amyloliquefaciens*	50.1^{c}	1.3^{cd}	36^{b}	6644^{d}
Control	*Control*	-	-	-	5028^{g}

Means followed by a common letter in the columns are not significantly different at $p<0.05$.
[1]Mycelial growth was recorded at 5 days after incubation and % inhibition was calculated over control.
[2]Width of inhibition zone between pathogen and PGPR was measured at 7 days after incubation.
[3]Sclerotial growth was recorded at 5 days after incubation.
[4]Seedling vigor index was calculated at 7 days after incubation.

ABU 3296, and *Bacillus simplex* strain ABU 3099 with high seedling vigor (> 8,000) compared to control seedlings with seedling vigor in the range of 4867 to 5193 (Table 1-7). These superior strains were also found effective in reducing mycelial growth of ShB pathogen except for the strain ABU 3099 that showed moderate inhibition. Highest seedling vigor was obtained with PGPR strains AP

Table 3. Effect of PGPR strains on vegetative growth and germination of sclerotia of *Rhizoctonia solani* and seedling vigor of rice (Assay 3)

Strain	Identification	% Inhibition of mycelial growth	Inhibition zone (mm)	% Inhibition of Sclerotial germination	Seedling Vigor Index
AP304	*Bacillus amyloliquefaciens*	50.5^{ab}	2.6^{b}	28^{a}	6693^{de}
AP305	*Bacillus amyloliquefaciens*	56.4^{ab}	4.3^{a}	20^{ab}	7160^{c}
ABU 29	*Bacillus simplex*	47.8^{ab}	1.6^{bc}	14.6^{b}	6960^{cd}
ABU 89B	*Bacillus simplex*	23.9^{d}	0^{d}	21.3^{ab}	6327^{e}
ABU 161	*Bacillus megaterium*	50.9^{ab}	1.6^{bc}	18.6^{ab}	7998^{a}
ABU 169	*Bacillus megaterium*	33.7^{cd}	0.6^{cd}	18.6^{ab}	5522^{f}
ABU 279	*Bacillus cereus*	44.3^{bc}	1.3^{bcd}	22.6^{ab}	6630^{de}
ABU 288	*Bacillus megaterium*	24.7^{d}	0^{d}	22.6^{ab}	4810^{g}
ABU 334	*Bacillus simplex*	56.4^{ab}	2^{bc}	25.3^{a}	5461^{f}
ABU 354	*Bacillus cereus*	59.2^{a}	4.3^{a}	18.6^{ab}	7583^{b}
Control	-	-	-	-	4959^{g}

Means followed by a common letter in the columns are not significantly different at $p<0.05$.
[1]Mycelial growth was recorded at 5 days after incubation and % inhibition was calculated over control.
[2]Width of inhibition zone between pathogen and PGPR was measured at 7 days after incubation.
[3]Sclerotial growth was recorded at 5 days after incubation.
[4]Seedling vigor index was calculated at 7 days after incubation.

Table 4. Effect of PGPR strains on vegetative growth and germination of sclerotia of *Rhizoctonia solani* and seedling vigor of rice (Assay 4)

Strain	Identification	% Inhibition of mycelial growth	Inhibition zone (mm)	% Inhibition of Sclerotial germination	Seedling Vigor Index
ABU 361	*Bacillus simplex*	3.1^{e}	0^{b}	12^{b}	5355^{cd}
ABU 371	*Bacillus megaterium*	40.7^{ab}	0^{b}	18.6^{ab}	5462^{c}
ABU 402	*Bacillus weihenstephanensis*	15.6^{cde}	0.6^{a}	21.3^{ab}	5020^{d}
ABU 457	*Bacillus simplex*	40^{ab}	0.6^{ab}	24^{a}	6120^{b}
ABU 524	*Bacillus simplex*	43.9^{a}	0.6^{ab}	20^{ab}	6780^{a}
ABU 871	*Bacillus simplex*	9.4^{de}	0^{b}	10.6^{b}	5453^{cd}
ABU 882	*Bacillus megaterium*	25.1^{bcd}	0^{b}	17.3^{ab}	6128^{b}
ABU 890	*Bacillus simplex*	45.1^{a}	1.3^{a}	26.6^{a}	6987^{a}
ABU 891	*Bacillus simplex*	20^{cd}	0^{b}	26.6^{a}	6269^{b}
ABU 1025	*Bacillus simplex*	27.8^{bc}	0^{b}	18.6^{ab}	5137^{cd}
Control	-	-	-	-	5064^{c}

Means followed by a common letter in the columns are not significantly different at $p<0.05$.
[1]Mycelial growth was recorded at 5 days after incubation and % inhibition was calculated over control.
[2]Width of inhibition zone between pathogen and PGPR was measured at 7 days after incubation.
[3]Sclerotial growth was recorded at 5 days after incubation.
[4]Seedling vigor index was calculated at 7 days after incubation.

295 (13,600), followed by MBI 600 (13,192) with significant differences between them.

Studies on the effect of PGPR in inhibiting vegetative growth and sclerotial germination of *R. solani* are important in identifying potential strains for ShB management.

Table 5. Effect of PGPR strains on vegetative growth and germination of sclerotia of *Rhizoctonia solani* and seedling vigor of rice (Assay 5)

Strain	Identification	% Inhibition of mycelial growth	Inhibition zone (mm)	% Inhibition of Sclerotial germination	Seedling Vigor Index
ABU 1053	*Bacillus simplex*	57.2^{b}	3^{a}	100^{a}	8218^{b}
ABU 1240	*Bacillus mycoides*	71.7^{a}	0^{b}	36^{b}	9224^{a}
ABU 1419A	*Bacillus simplex*	45.1^{c}	0^{b}	20de	4962^{e}
ABU 1627	*Bacillus mycoides*	74.5^{a}	0^{b}	25.3^{cd}	9400^{a}
ABU 1645	*Bacillus simplex*	0^{e}	0^{b}	29.3^{bc}	5136de
ABU 1687	*Bacillus simplex*	9.0^{d}	0^{b}	6.6^{g}	5805^{cd}
ABU 1930	*Bacillus simplex*	1.1^{e}	0^{b}	16^{ef}	5488^{cde}
ABU 1966	*Paenibacillus taichungensis*	9.8^{d}	0^{b}	10.6^{fg}	5220^{cde}
ABU 1970	*Bacillus simplex*	1.1^{e}	0^{b}	17.3^{def}	5822^{c}
ABU 2002	*Bacillus simplex*	2.7^{e}	0^{b}	16^{ef}	5454^{cde}
Control	-	-	-	-	4867^{e}

Means followed by a common letter in the columns are not significantly different at $p<0.05$.
[1]Mycelial growth was recorded at 5 days after incubation and % inhibition was calculated over control.
[2]Width of inhibition zone between pathogen and PGPR was measured at 7 days after incubation.
[3]Sclerotial growth was recorded at 5 days after incubation.
[4]Seedling vigor index was calculated at 7 days after incubation.

Table 6. Effect of PGPR strains on vegetative growth and germination of sclerotia of *Rhizoctonia solani* and seedling vigor of rice (Assay 6)

Strain	Identification	% Inhibition of mycelial growth	Inhibition zone (mm)	% Inhibition of Sclerotial germination	Seedling Vigor Index
ABU 2017	*Bacillus simplex*	64.7^{a}	4.6^{a}	24^{ab}	7047^{a}
ABU 2041A	*Bacillus simplex*	0^{h}	0^{c}	14.6^{cde}	5850^{d}
ABU 2099B	*Bacillus simplex*	18.0^{d}	0^{c}	28^{ab}	5823^{d}
ABU 2197	*Bacillus simplex*	6.6^{fg}	0^{c}	12de	5060^{e}
ABU 2213	*Bacillus simplex*	2.3^{gh}	0^{c}	6.6^{e}	6398^{b}
ABU 2252	*Bacillus megaterium*	11.3^{ef}	0^{c}	20^{bcd}	6255^{bc}
ABU 2424	*Bacillus simplex*	3.1^{gh}	0^{c}	21.6^{cde}	5647^{d}
ABU 2429B	*Bacillus megaterium*	12.5^{e}	0^{c}	14.6^{cde}	6213^{bc}
ABU 2549	*Bacillus mycoides*	52.9^{b}	1^{b}	21.3^{abc}	5946^{cd}
ABU 2644	*Bacillus simplex*	23.5^{c}	0^{c}	29.3^{a}	5956^{cd}
Control	—	—	—	—	4904^{e}

Means followed by a common letter in the columns are not significantly different at $p<0.05$.
[1]Mycelial growth was recorded at 5 days after incubation and % inhibition was calculated over control.
[2]Width of inhibition zone between pathogen and PGPR was measured at 7 days after incubation.
[3]Sclerotial growth was recorded at 5 days after incubation.
[4]Seedling vigor index was calculated at 7 days after incubation.

In our present study, two strains of *B. subtilis* (MBI 600 and AP 52) and one strain of *B. amyloliquefaciens* (AP 219) were found effective in suppressing the vegetative growth of the mycelium (73 to 83%), inhibits the sclerotial germination (100%) of *R. solani* and increased rice seedling vigor. PGPR offers a promising means of controlling plant diseases, besides contributing to resistance, growth and yield in rice[17]. Epiphytic and endophytic bacterial strains isolated from healthy rice seed have been found to inhibit the mycelial growth of ShB pathogen[27]. PGPR strains are known to produce siderophores, volatile metabolites, extra cellular secretions and antibiotics that are inhibitory to *R. solani*. Certain PGPR isolates also produce β 1, 3-glucanase, salicylic acid and HCN in inhibiting the mycelial growth of *R. solani*[18]. Reduction in germination and lysis of *R. solani* sclerotia by PGPR have also been reported[12]. Pre-treating the sclerotia with bacterial cell suspensions for 1 min to 4 weeks resulted in inactivation[20]. Suppression of rice ShB pathogen, *R. solani* by non-fluorescent PGPR like *Bacillus* spp was previously attributed to the production of chitinase[15] and other antifungal metabolites. A large number of bacterial strains were found to possess the ability to protect rice plants from ShB disease[25] and these were identified through dual plate assays.

Seed treatment with bacterial antagonists also resulted in increased shoot and root lengths of seedlings[24]. Use of diazotrophic bacteria in rice to promote growth and yields in rice is a common practice. Seed treatment with diazotrophs resulted in growth responses such as seedling emergence, radicle elongation, plumule length, cumulative leaf and root areas, grain and straw yields. The growth promotion in rice by diazotrophs is attributed to production of indole -3-acetic acid[2].

Effective inhibition of mycelial growth and sclerotial germination are pre-requisites for a candidate PGPR strain to be used for ShB management at field level. Since, sclerotial germination is a crucial step for ShB incidence, any strain that inhibits this process can be exploited for successful management of ShB. In addition, the selected strain should be able to enhance seedling growth and yields. Further studies were planned to determine the efficacy of the superior strains identified in this screening bio-assays for management of ShB under greenhouse and field conditions. The use of PGPR's for management

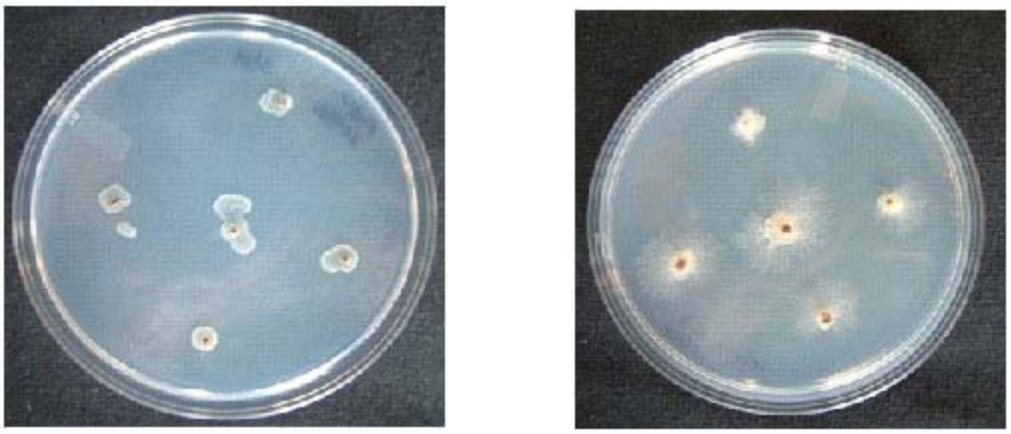
Table 7. Effect of PGPR strains on vegetative growth and germination of sclerotia of *Rhizoctonia solani* and seedling vigor of rice (Assay 7)

Strain	Identification	% Inhibition of mycelial growth	Inhibition zone (mm)	% Inhibition of Sclerotial germination	Seedling Vigor Index
ABU 2772	*Bacillus subtilis*	54.9^{b}	3^{bc}	40^{a}	6237^{d}
ABU 3099	*Bacillus simplex*	48.2^{cd}	0^{d}	26.6^{abc}	8398^{b}
ABU 3118	*Bacillus simplex*	7.8^{e}	0^{d}	9.3^{c}	4998^{f}
ABU 3128	*Bacillus simplex*	48.6^{cd}	0^{d}	25.3^{abc}	7595^{c}
ABU 3135	*Bacillus weihenstephanensis*	49.8^{bcd}	0^{d}	33.3^{ab}	5434^{ef}
ABU 3296	*Bacillus simplex*	74.5^{a}	4^{a}	28^{ab}	9788^{a}
ABU 3421A	*Bacillus vallismortis*	5.1^{e}	0^{d}	34.6^{ab}	5289^{ef}
ABU 3454	*Bacillus weihenstephanensis*	50.9^{bc}	2.6^{c}	32^{ab}	5753^{de}
ABU 3586	*Bacillus mycoides*	70.1^{a}	3.6^{a}	42.6^{a}	8301^{bc}
ABU 3819	*Bacillus aerophilus*	45.1^{d}	0^{d}	18.6^{bc}	5289^{ef}
Control	-	-	-	-	4925^{f}

Means followed by a common letter in the columns are not significantly different at $p<0.05$.
[1]Mycelial growth was recorded at 5 days after incubation and % inhibition was calculated over control.
[2]Width of inhibition zone between pathogen and PGPR was measured at 7 days after incubation.
[3]Sclerotial growth was recorded at 5 days after incubation.
[4]Seedling vigor index was calculated at 7 days after incubation.

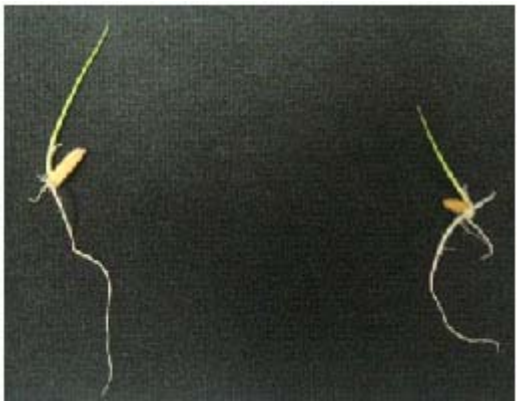
Challenged with MBI 600 Control

Fig. 1. Inhibition of vegetative growth of *R. solani* by *Bacillus subtilis* MBI 600 in a dual plate assay



Challenged with MBI 600 Control

Fig. 2. Inhibition of sclerotial germination of *R. solani* by *Bacillus subtilis* MBI 600 in a plate assay.



Challenged with MBI 600 Control

Fig. 3. Effect of seed treatment with *Bacillus subtilis* MBI 600 on rice seedling vigor index.

of ShB at field level at multiple locations can be explored. Understanding the mechanism of disease pathogen suppression is essential for the successful deployment of PGPR strains as a disease management strategy. Rice disease suppression by PGPR is governed by a multitude of factors. The success of a PGPR strain depends largely on the ability of the introduced agent to establish itself in the new environment and maintain a threshold population on the planting material or in the rhizosphere. The influence of these factors varies with the type of PGPR strain and the nature of the pathogen targeted for control. Future studies are directed in understanding the mechanism of ShB suppression by selective PGPR strains.

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