IJMR
International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975–9174, Vol. 3, Issue 2, 2011, pp-120-130
Available online at http://www.bioinfo.in/contents.php?id=27

PLANT GROWTH-PROMOTING ACTIVITIES OF *BACILLUS SUBTILIS* MBI 600 (INTEGRAL®) AND ITS COMPATIBILITY WITH COMMONLY USED FUNGICIDES IN RICE SHEATH BLIGHT MANAGEMENT

VIJAY KRISHNA KUMAR K.[1*], REDDY M.S.[2], KLOEPPER J.W.[2], YELLAREDDYGARI S.KR.[2], LAWRENCE K.S.[2], ZHOU X.G.[3], SUDINI H.[4], MILLER M.E.[5], APPA RAO PODILE[6], SURENDRANATHA REDDY E.C.[7], NIRANJANA S.R.[8] AND CHANDRA NAYAKA S.[8]

[1]Department of Entomology & Plant Pathology, Auburn University, Auburn, AL, USA & Acharya N G Ranga Agricultural University, Hyderabad, INDIA
[2]Department of Entomology & Plant Pathology, Auburn University, Auburn, AL, USA
[3]Texas A & M University, Agri-Life Research & Extension Center, Texas, USA
[4]International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, INDIA
[5]Department of Biological Sciences, Auburn University, Auburn, AL, USA
[6]Department of Plant Sciences, University of Hyderabad, AP, INDIA
[7]Department of Genetics and Genomics, Yogi Vemana University, Kadapa, AP, INDIA
[8]Department of Applied Botany and Biotechnology, University of Mysore, Mysore, INDIA
*Corresponding Author: Email-kotamvk@gmail.com

Received: January 01, 2011; Accepted: September 06, 2011

Abstract: Sheath blight (ShB) of rice caused by *Rhizoctonia solani* is an economically important disease, causing significant yield losses. In this study, the growth promoting activities of commercial formulation of a bioagent, *Bacillus subtilis* MBI 600 (Integral®) and its compatibility with rice fungicides were evaluated. Integral was evaluated for growth promotion in rice on four cultivars (Cocodrie, Catahoula, Neptune, and Trenasse) under *in vitro* conditions. Treated rice seeds were incubated for 7 days, and the shoot and root lengths were measured. Rice cv. Cocodrie seeds were treated with strain MBI 600 at various concentrations and seeded in pots containing field soil in GH in a randomized complete block design. Germination and seedling lengths were measured at 7 and 15 days after sowing (DAS). The strain MBI 600 was found to produce siderophores. Seed treatment with Integral significantly increased shoot and root lengths at all concentrations in cvs. Cocodrie, Catahoula, and Trenasse under *in vitro* conditions. The shoot lengths ranged from 39 to 42 mm at a concentration of 2.20 x 10^9 cfu/ml in all CV's. At 2.20 x 10^9 cfu/ml, the root lengths ranged from 47 to 69 mm. The shoot and root lengths of control seedlings were each up to 20 mm. Seed treatment with 2.20 x 10^8 and 2.20 x 10^9 cfu/ml significantly increased seedling emergence (81 to 89%) compared to 2.20 x 10^6 and 2.20 x 10^7 cfu/ml, and control (61%) under GH conditions. Similarly, seed treatment with 2.20 x 10^9 cfu/ml of MBI 600 resulted in the highest shoot and root lengths (335 and 166 mm respectively). Integral has good tolerance to hexaconazole, propiconazole, and validamycin; moderate tolerance to tricyclazole; and poor tolerance to benomyl and mancozeb at 1000 ppm. Integral showed compatibility to carbendazim and azoxystrobin up to 400 ppm. Overall, our results suggest that Integral produces siderophores, promoted rice seedling emergence and growth, and is compatible with rice fungicides.
Keywords- Rice, Sheath blight, *Rhizoctonia solani*, biocontrol, *Bacillus subtilis*, growth promotion, fungicidal compatibility.

Introduction

Rice is the major staple food crop for the majority of humans. However, production levels are reduced due to various fungal diseases. Among these diseases, sheath blight (ShB) caused by *Rhizoctonia solani* Kuhn. is a major production constraint causing significant yield losses under high input and high production environments worldwide [40]. In U. S. rice growing regions of the Midsouth, ShB is the most devastating disease on rice [15, 23, 25]. Conventional use of chemical fungicides for ShB management has negative effects on soil fertility, the ecosystem, and increases crop production costs [9]. Biocontrol of ShB using plant growth-promoting rhizobacteria (PGPR) offers a promising means of ShB management. PGPR strains are known to colonize and survive both in the rhizosphere and on the phyllosphere [21]. In previous studies, use of PGPR has significantly improved growth and yields of rice [27]. Their application promotes plant growth by direct and indirect mechanisms. Direct growth promotion is due to production of phytohormones, solubilization of phosphates [2, 20], increased uptake of iron through production of siderophores [9, 16], and

volatile metabolites. Indirect methods of plant growth promotion are due to antibiosis, HCN [12], competition for space and nutrients, parasitism or lysis of pathogen hyphae, inhibition of pathogen-produced enzymes or toxins, and through induced systemic resistance (ISR) [31].

For a PGPR to be effective under field conditions, the key is to characterize the strain for plant growth-promoting and disease suppressing features. Moreover, knowledge on the exact mode of action is essential for devising effective disease management strategies [36]. Research on rice ShB management through use of fresh cells [26, 47] or formulations of bacterial antagonists has been attempted [10, 49, 19]. Seed emergence, plant growth promotion and increase in crop yields are the other attributes of a superior PGPR strain besides disease suppression. Earlier reports confirmed the enhancement of seed germination, seedling length, and dry matter production of roots and shoots of rice seedlings by PGPR [3].

In Asian countries, due to increased use of semi-dwarf, early-maturing, and high-yielding varieties, occurrence of ShB is common. The seriousness of ShB often warrants the use of chemical fungicides [45]. Currently, ShB management is mostly through the use of systemic and non-systemic fungicides [33]. Fungicides commonly used against ShB include Dithane M-45 [11], carbendazim [46], mancozeb [38], iprodione [18], and triazoles [44]. Other fungicides such as carbendazim and mancozeb as a mixture were also very effective [34]. Among a new group of fungicides, strobilurins was highly effective both in terms of ShB control and rice grain yield enhancement [5]. Application of fungicidal mixtures with more than one technical ingredient against multiple diseases is a common practice in rice production [43].

The compatibility of PGPR strains to fungicides is an important step for their use in ShB management. In earlier reports, use of Pseudomonads mixed with carbendazim and/or jinggangmycin, reduced ShB severity under greenhouse and field conditions [22, 52]. In our earlier studies, *Bacillus subtilis* strain MBI 600 significantly suppressed mycelial growth, sclerotial germination of *R. solani*, and reduced ShB symptoms in rice under controlled conditions. The objectives of these studies were i) to characterize the strain MBI 600 for growth promoting traits, ii) to determine its effect on seedling emergence and growth of rice cultivars under *in vitro* and greenhouse conditions, and iii) to study its compatibility with fungicides in rice. The information gathered from these studies will be useful in devising management strategies against rice ShB.

Materials and Methods

Source of rice cultivars

High yielding, conventional, long grain rice cultivars of Cocodrie, Neptune, Trenasse, and Catahoula developed at Rice Research Station, LSU AgCenter, Crowley, Louisiana, USA, were obtained and stored at 4^0 C prior to use.

Source and production of *B. subtilis* MBI 600 in liquid formulation

The strain MBI 600 was obtained from the Phytobacteriology Laboratory strain collection, Department of Entomology and Plant Pathology, Auburn University, AL, USA. For laboratory and greenhouse studies, the liquid formulation of *B. subtilis* strain MBI 600 was produced by Becker Underwood Inc., at their fermentation facilities located in Ames, Iowa, USA. The fermented product of MBI 600 was labeled as Integral®. The product contained a minimum of 2.2×10^{10} spores/ml. The product was packaged in 500 ml bottles and shipped to Department of Entomology and Plant Pathology, Auburn University, AL, USA to carry out the studies.

Purity check of *B. subtilis* strain MBI 600 in proprietary liquid formulation

To check for any cross contamination, the inoculum from bottles of Integral was streaked onto TSA plates and checked for growth and purity. This procedure was carried out in the Department of Plant Sciences, University of Hyderabad, Andhra Pradesh, India. To confirm the identity of MBI 600 strain, 16s rDNA sequence homology technique was used. Genomic DNA was isolated from the strain recovered from the product Integral by following standard procedures [1]. Approximately, 1409 bp of the 16S rDNA was amplified by polymerase chain reaction (PCR) using the following primers: 8F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-ACG GCT ACC TTG TTA CGA CTT-3'). The resultant amplicon was verified by agarose gel electrophoresis. After verification of proper amplification, the amplicon was purified using a Qiagen Kit. The purified product was sequenced and the sequences were compared with known sequences in the databases using BLAST (basic logical alignment search tool).

A loopful of strain of MBI 600 stored in bottles was grown for 48 h at 25^0 C in 20 ml sterile tryptic soy broth (TSB) (Difco, Detroit, Michigan, USA) on a reciprocating shaker (80 rpm). Bacterial suspension was centrifuged for 20 min at 10,000 x g. The resulting cell pellet was then washed two times in 0.1 M phosphate buffer (PB) (pH 6.8), re-suspended in TSB amended with 20% sterile glycerol, and stored in vials at -80^0 C prior to use. A new vial was used in each assay. The assays on characterization of MBI 600 strain for growth promotion were carried out by utilizing the facilities at Department of Applied Botany and Biotechnology, University of Mysore, India.

Production of Indole Acetic Acid (IAA)

Strain MBI 600 was retrieved from storage at - 80^0 C, thawed and used for production of IAA. A loopful of inoculum was streaked onto TSA and incubated for 24 h. Single colonies were then inoculated into 250 ml flasks containing TSB and grown on a rotary shaker for 72 h. Liquid bacterial suspension were centrifuged at 3000 rpm for 30 min. Approximately, 2 ml of supernatant was mixed with 2 drops of orthophosphoric acid and 4 ml of

Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5M $FeCl_3$ solution). Production of IAA was confirmed through color indication as described by Brick *et al.* [7].

Phosphate solubilization

For phosphate solubilization assays, a medium containing 2 g yeast extract, 20 g glucose, 2 g tri-calcium phosphate, 60 mg actidione, and 15 g agar mixed with 1000 ml water, adjusted to pH 7, was used. A loopful of inoculum of strain MBI 600 was streak inoculated in the center of Petri dishes containing the media described above and incubated at 28^0 C for 5 days and growth was observed. Bacterial colony forming clear zone was considered as phosphate solubilizer [37].

Production of siderophores

Chrome azurol S (CAS) assay was used to detect the production of siderophores by strain MBI 600. The composition of CAS agar was prepared by following the standard procedure [41]. Pure culture of MBI 600 was stab inoculated on CAS agar plates using sterile toothpicks and incubated at 28^0 C for 2 weeks in the dark. Development of an orange zone around bacterial growth was considered an indication of siderophore production. Reference bacterial strains with known siderophore production were used as positive controls. Plates of CAS-agar without strain of MBI 600 were incubated under the same conditions as described above served as a control. Change of color in the CAS media is an indication of production of siderophore [9].

Production of HCN

Production of HCN by strain MBI 600 was determined by a modified method of Miller and Higgins [29]. Pure culture of MBI 600 was streaked on to Petri dishes containing yeast extract mannitol agar (YEMA) amended with glycine (4.4 g/lit). Simultaneously, a filter paper soaked in 0.5% (w/v) picric acid in 1% Na_2CO_3 was placed in the upper lid of the Petri plate. After incubation at 28^0 C for 4 days, color changes were examined. Development of an orange red color in YEMA is a characteristic of HCN production. Petri dishes containing YEMA without strain of MBI 600 served as control.

Production of Cellulase

Production of cellulase by strain MBI 600 was assessed in M9 medium [28] amended with yeast extract (1.2 g/L) and cellulose (10 g L^{-1}) and congo red (0.02%). Strain MBI 600 was spot inoculated in the center of Petri dish containing M9 media, and incubated for one week at 28^0 C. Clear halos surrounding actively growing colonies are a positive sign for cellulose production [8].

Production of Chitinase

Chitinolytic ability of strain MBI 600 was assessed by streaking a loopful of 48-h-old culture of MBI 600 strain on water agar incorporated with 0.2% colloidal chitin [4]. The plates were incubated at room temperature for 4 days. Development of a hydrolytic zone (clearing zone) around the actively growing colonies is a sign for chitinase production [50].

Effect of *B. subtilis* MBI 600 on seedling growth of various rice cultivars under *in vitro* conditions

Rice seeds of cvs. Cocodrie, Catahoula, Neptune and Trenasse, as described above were used for the current study. Rice seeds of each cultivar were surface sterilized in 2% sodium hypochlorite for 10 minutes and then were washed twice with sterile distilled water and air dried. Two grams of surface sterilized rice seeds of each cultivar were soaked for 24 h in four different concentrations of strain MBI 600 produced in liquid formulation adjusted to 2.20×10^6, 2.20×10^7, 2.20×10^8, and 2.20×10^9 cfu/ml. Seeds were air-dried in a laminar flow-hood. Seeds of each rice cultivar soaked in sterile distilled water served as control. Air dried seeds were incubated in sterilized 250-ml beakers covered with aluminum foil to prevent hydration and incubated at room temperature for 7 days. Root and shoot development were monitored daily. There were four replications for each cv and for each concentration of bacterial inoculum. Ten seedlings from each replicated treatment were sampled for shoot and root lengths. The root length was measured from the germination site to the end of the main root, and the shoot length was measured from the germination site to the highest tip of the shoot of each seedling.

Effect of *B. subtilis* strain MBI 600 on seedling emergence and growth under greenhouse conditions

Four concentrations of strain MBI 600 produced in liquid formulation were used to evaluate increases in emergence and growth of rice under greenhouse conditions. The concentrations used were 2.20×10^6, 2.20×10^7, 2.20×10^8 and 2.20×10^9 cfu/ml. One CV of rice, Cocodrie, was evaluated. Four grams of seed were soaked in different concentrations separately for 24 h and then air dried. Rice seeds soaked in sterile distilled water served as the control. Plastic pots filled with field soil were used to grow seedlings. There were six replications for each treatment, one pot per replication and 15 seeds were seeded at equi-distance at 2 cm depth in each pot. Seeded pots were arranged on a bench in the GH in a randomized complete block. Pots were maintained at 26 ± 2 ^{0}C and a RH of 90%. Rate of seedling emergence was recorded every day for 7 days. Root and shoot lengths and root and shoot weights were recorded at 15 days after sowing (DAS). Individual seedlings were harvested and washed with tap water and air-dried. Shoot and root lengths and weights were measured.

Compatibility of *B. subtilis* strain MBI 600 to fungicides

Strain of MBI 600 produced in commercial liquid formulation was used for compatibility studies. The procedure described by Shanmugam and Narayanasamy [42] was implemented. Fungicides such as propiconazole (Tilt 250 EC), validamycin (Sheathmar 3L), benomyl (Benlate 50 WP), carbendazim (Bavistin 50

WP), tricyclazole (Beam 75 WP), mancozeb (80 WP), azoxystrobin (Heritage 50% WDG) and hexaconazole (Danzole 5 EC) were obtained from the manufacturers and were used for compatibility studies. Based on manufacturers' recommendations, the rates of 100, 200, 400, 600, 800, and 1000 ppm were selected. Nutrient agar (NA) plates amended with concentrations of fungicides were prepared by serial dilutions. Fresh culture of MBI 600 was retrieved from – 80^0 C freezer and streaked on TSA plates. A loopful of active culture was streaked on individual NA plates amended with appropriate concentrations of fungicides and incubated for 48 h. There were five replications for each fungicide and concentration and one plate per replication. To measure the compatibility, growth of strain MBI 600 on fungicide amended media was rated as +++ (Good); ++ (Moderate); + (Poor); and – (No growth) and compared with growth of strain MBI 600 on non-amended fungicide NA plates.

Compatibility of strain MBI 600 to azoxystrobin and carbendazim was assessed according to the procedure described by Omar *et al.* [32]. Fresh culture of strain MBI 600 was retrieved from -80^0 C freezer and streaked on TSA plates. Purified single colonies were streaked on NA slants and incubated for 24 h at 30^0 C. To this, 10 mL of sterile distilled water was added, and the bacterial culture was scraped from the agar surface with a sterile plastic loop. The bacterial suspension was homogenized by agitation using a vortex mixer. Sterilized YPG (yeast extract 5 g, bacterial peptone 5 g, glucose 20 g, 1000 ml H_2O, pH 6.8) liquid media were prepared and placed in 250 mL flasks. To these flasks, stock solutions of fungicides prepared in sterile distilled water at concentrations of 0, 200, and 400 ppm were added separately to make a final volume of 50 mL. The fungicide amended YPG media in flasks were later inoculated with 100µL of bacterial inoculum prepared as described above and incubated at 30^0C at 250 rpm. The flasks were sampled every 24 h for 72 h and number of colony forming units was determined on NA using serial dilution. There were five replications for each concentration of fungicide. Media without fungicides served as controls.

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

Purity check of *B. subtilis* strain MBI 600 in proprietary liquid formulation

BLAST analysis of the 16s rDNA sequence of the strain MBI 600 generated from 1409 base pairs confirmed the purity and identity to the original identification of the parental strain prior to formulation in liquid.

Production of IAA, siderophores, cellulase, chitinase, HCN and phosphate solubilization by strain MBI 600

Strain MBI 600 was positive for siderophore production and negative for IAA, cellulase, chitinase, HCN and P solubilization (Table 1).

Effect of *B. subtilis* MBI 600 on seedling growth of various rice cultivars under *in vitro* conditions

Seed treatment with strain MBI 600 significantly increased shoot lengths compared to controls in cvs Cocodrie, Catahoula, and Trenasse (Table 2). At a concentration of 2.20 x 10^9 cfu/ml, shoot lengths were highest in cvs Cocodrie, Catahoula, and Neptune. Shoot lengths were not significantly different at 2.20 x 10^9 and 2.20 x 10^8 cfu/ml for the cvs Trenasse. Similarly, shoot lengths were not significantly different for cv. Neptune at 2.20 x 10^8 and 2.20 x 10^7 cfu/ml of strain MBI 600. The shoot lengths in all rice cvs ranged from 39.1 to 41.5 mm at 2.20 x 10^9 cfu/ml, whereas in the control, the shoot lengths ranged from 7.6 to 19.5 mm.

Seed treatment with MBI 600 at 2.20 x10^9, 2.20 x 10^8, and 2.20 x 10^7 cfu/ml significantly increased root lengths in all rice cvs over control (Table 3). At 2.20 x 10^9 cfu/ml, the root lengths in rice cvs ranged from 47.5 to 69.5 mm compared to control seedlings (8.3 to 19.9 mm). With increasing in concentrations of MBI 600, the root lengths were also increased in all four rice cvs. Development of mesocotyl roots and rootlets was prominent in all rice cvs at 7 days after incubation at 2.20 x 10^8 and 2.20 x 10^9 cfu/ml (Fig. 1).

Effect of *B. subtilis* strain MBI 600 on seedling emergence and growth under greenhouse conditions

Seed treatment with all concentrations of strain MBI 600 significantly increased emergence of seedlings over control in rice cv Cocodrie from 5 days after seeding under greenhouse conditions (Table 4). However, in seed treatments with 2.20 x 10^8 and 2.20 x 10^9 cfu/ml of strain MBI 600, the emergence was significantly greater over controls from day 2 after seeding. The highest rate of germination (89%) was recorded at a concentration of 2.20 x 10^9 cfu/ml of strain MBI 600 at 7 days after seeding (Fig 2). The percent germination in control was 61%.

Shoot and root lengths were significantly longer in seed treatment with strain MBI 600 at 2.20 x 10^7, 2.20 x 10^8, and 2.20 x 10^9 cfu/ml over control (Table 5). At 2.20 x 10^9 cfu/ml the shoot and root lengths (Fig 3 and 4) were greatest (335 and 166 mm respectively) over controls (222 and 73 mm respectively). Shoot and root weights were significantly greater at a concentration of 2.20 x 10^9 cfu/ml (0.23 and 0.10g). The shoot and root weights in control were 0.1 and 0.04 g, respectively.

Compatibility of *B. subtilis* strain MBI 600 to fungicides

Strain MBI 600 was compatible to 1000 ppm of hexaconazole, propiconazole, and validamycin based on its growth rated as good (Table 6). The strain was moderately compatible to tricyclazole and poorly compatible to benomyl and mancozeb at 1000 ppm. The strain has shown good compatibility up to 400 ppm when

grown on YPG media amended with carbendazim and azoxystrobin. The strain has good compatibility to carbendazim (Fig 5) and azoxystrobin (Fig 6) at 400 ppm. The growth of strain MBI 600 in YPG media amended with carbendazim and azoxystrobin individually at 200 and 400 ppm was same as that of controls at 72 h after incubation (Fig 5 and Fig 6).

Discussion

Various PGPR strains have been used to manage ShB disease and to enhance seedling growth and grain yields of rice [35, 48]. To date, there have been no studies on mode of action of any particular PGPR strain used against ShB or used to improve rice seedling growth or yields of rice. In our present study, the strain MBI was found to be positive for siderophore production. Siderophores are low molecular weight iron chelating compounds produced by PGPR in soil and are known to suppress rice pathogens through siderophore mediated antibiosis [9]. Under iron deprived conditions, *B. subtilis* secretes a catecholic siderophore termed as 2, 3-hydroxybenzoyl glycine that is similar to the precursor of *Escherichia coli* siderophore, enterobactin [14]. Siderophore producing rhizobacteria have exhibited strong antagonism towards several rice pathogenic fungi such as *Alternaria* sp., *Fusarium oxysporum*, *Pyricularia oryzae* and *Sclerotium* sp. [9]. Since iron is a limiting factor and is essential for the growth of microbes [17], rhizobacteria develop strategies to acquire iron. Earlier studies showed that siderophore production is a key factor for a PGPR strain to control plant pathogens such as *R. solani* [30].

In these studies, the strain MBI 600 enhanced seedling emergence and growth of seedlings under laboratory and greenhouse conditions when used as seed treatment on various cultivars of rice. Significant enhancement of root and shoot growths was attributed to production of certain growth promoting substances and solubilization of elements such as phosphorus (Table 1). However, in our studies, the strain MBI 600 neither produced IAA nor solubilized phosphorus. Earlier reports showed that some strains of *B. subtilis* and *B. amyloliquefaciens* produced certain volatile compounds such as 2-3, butanediol and acetoin that stimulated plant growth [39]. Production of gibberellins and cytokinins was also responsible for the physiological basis of growth promotion in rice seedlings. Growth promotion can also be due to indirect mechanisms such as ethylene inhibition through ACC deaminase activity [13]. Further investigations are therefore needed in this direction to characterize the MBI 600 strain to identify the production of specific growth promoting substances involved in stimulating seed germination and promotion of rice seedling growth.

In our studies, the strain MBI 600 was highly tolerant to hexaconazole, propiconazole and validamycin; moderately tolerant to tricyclazole; and poorly tolerant to benomyl and mancozeb at 1000 ppm. The MBI 600 strain exhibited good tolerance at 400 ppm for carbendazim and azoxystrobin. Strains of *Bacillus* sp (B-44) were compatible to carbendazim at 500 and 1000 ppm respectively [22]. The strain 916 of *B. subtilis* was found to colonize the root system successfully without any population decline when combined with Jinggangmycin prior to application onto seed [52]. Compatibility of strains of *Bacillus* spp. to strobilurins group of fungicides was also reported. Also, combined applications of *B. subtilis* strain NJ-18 with 50% Kresoxim-methyl, strobilurin fungicide was very effective in suppressing rice ShB severity under field conditions [51]. Use of fungicide-compatible PGPR strains in conjunction with fungicides offers better control than non-compatible strains. For example, the integration of Kodiak® (*Bacillus subtilis*) with fungicides as seed treatment significantly controlled seed and soil borne diseases of cotton under field conditions [6]. Generally, seed bacterization with the higher inoculum concentrations yielded better growth promoting results than the lower inoculum concentrations on all the CVs of rice tested. In addition, strain MBI 600 showed compatibility to the majority of commonly used fungicides, which is a desired characteristic of PGPR strain. Hence, studies reported here suggest integration of strain MBI 600 with any of the fungicides that will have a commercial potential for management of ShB of rice under field conditions.

References

[1] Ahemad M. and Khan M. S. (2010) *EurAsia J Biosci,* 4, 88-95.

[2] Antoun H. and Kloepper J. W. (2001) *Plant Growth Promoting Rhizobacteria. Encyclopedia of Genetics. London: Academic Press.*

[3] Ashrafuzzaman M., Hossen F. A., Razi Ismail M., Anamul Hoque Md., Zahurul Islam M., Shahidullan S. M. and Sariah Meon. (2009) *Afr. J. Biotechnol*, 8, 1247-1252.

[4] Berger L. R. and Reynolds D.M. (1958) *Biochimica et Biophysica Acta*, 29, 522–534.

[5] Biswas A. (2006) *Environ Ecol*, 24S, 518-520.

[6] Brannen P. M. and Kenney D. S. (1997) J. *Ind. Microbiol. Biotechnol*, 19, 169-171.

[7] Brick J. M., Bostock R. M. and Silverstone S. E. (1991) *Appl Environ Microbiol*, 57, 535-538.

[8] Cattelan A. J., Hartel P. G. and Furhmann F. F. (1999) *Soil. Sci. Soc. Am. J*, 163, 1670-1680.

[9] Chaiharn M., Chunhaleuchanon S. and Lumyong S. (2009) *World J Microbiol Biotechnol,* 25, 1919-1928.

[10] Commare R. R, Nandakumar R., Kandan A., Suresh S., Bharathi M., Raguchander T. and Samiyappan R. (2002) *Crop Prot*, 21, 671-677.

[11] Das S. R. and Mishra B. (1990) *Indian Phytopath*, 43, 94-96.

[12] Dowling D. N. and O' Gara F. (1994) *Trends Biotechnol*, 12, 133-141.

[13] Glick B. R., Penrose D. M. and Li J. P. (1998) *J Theor Biol*, 190, 63-68.

[14] Grossman T. H., Tuckman M., Ellestad S. and Osburne M. S. (1993) *J. Bacteriol*, 175, 6203-6211.
[15] Groth D. E. and Lee F. N. (2002) *Rice: Origin, history, technology, and production. W. E. Smith and R. H. Dilday ed. John Wiley & Sons, Hoboken, NJ*, 413-416.
[16] Gupta C. P., Dubey R. C. and Maheshwari D. K. (2002) *Biol Fertil Soils*, 35, 399-405.
[17] Gurinot M. L. (1994) *Microbial Iron Transport. Annual Review of Microbiology. London: Academic Press.*
[18] Izadyar M. and Baradaran P. (1989) *Int. Rice Res. Notes,* 14, 25.
[19] Kanjanamaneesathian M., Pengnoo K. A. and Nilratana L. (1998) *Aust. Plant Pathol*, 27, 198-206.
[20] Katznelson H. and Bose B. (1959) *Can J Microbiol*, 5, 79-85.
[21] Krishnamurthy K. and Gnanamanickam S. S. (1998) *Biol Control*, 13, 158-165.
[22] Laha G. S. and Venkataraman S. (2001) *Indian Phytopath*, 54, 461-464.
[23] Lee F. N. and Rush M. C. (1983) *Plant Dis*, 6, 829-832.
[24] Lorck H. (1948) *Physiol. Plant*, 1, 142-146.
[25] Marchetti M. A. (1983) *Plant Dis*, 67, 162-165.
[26] Mew T. W. and Rosales A. M. (1986) *Phytopathol,* 76, 1260-1264.
[27] Mew T. W. and Rosales A. M. (1992) *Biological Control of Plant Diseases. E. S. Tjamos, G. C. Papavizas, and R. J. Cook, eds. Plenum Press, New York, USA*, 113-123
[28] Miller J. H. (1974) *Experiments in molecular genetics. 2nd ed. Cold Spring Harbor Lab., Cold Spring Harbor, NY.*
[29] Miller R. L. and Higgins V. J. (1970) *Phytopathol*, 60, 104-110.
[30] Nagarajkumar M., Bhaskaran R. and Velazhahan R. (2004) *Microbiol Res*, 159, 73-81.
[31] Nandakumar R., Babu S., Viswanathan R., Raguchander T. and Samiyappan R. (2001) *Soil Biol. Biochem*, 33, 603-612.
[32] Omar I., O'Neill T. M. and Rossall S. (2006) *Plant Pathol*, 55, 92-99.
[33] Pal R., Chakrabarti K., Chakraborty A. and Chowdhury A. (2005) *J Zhejiang Univ SCI 6B*, 8, 756-758.
[34] Prasad P. S., Naik M. K. and Nagaraju P. (2006) *Proc. Natl. Sem. On New Frontiers. Pl. Patho.* Pp. 139.
[35] Rabindran R. and Vidhyasekaran P. (1996) *Crop Prot*, 15, 715-721.
[36] Renwick A., Campbell R. and Coe S. (1991) *Plant Pathol*, 40, 524–532.
[37] Rosas S. B., Andres J. A., Rovera M. and Correa N. S. (2006) *Soil Biol. Biochem*, 38, 3502-3505.
[38] Roy A. K. and Saikia V. N. (1976) *Indian Phytopath*, 29, 354-356.
[39] Ryu C. M., Farag M. A., Hu C. H., Reddy M. S., Wei H. X., Pare P. W. and Kloepper J. W. (2003) *PNAS*, 100, 4927-4932.
[40] Savary S., Willocquet L., Elazegui F. A., Teng P. S., Du P. V., Zhu D., Tang Q., Huang S., Lin X., Singh H. M. and Srivastava R. K. (2000) *Plant Dis*, 84, 341-356.
[41] Schwyn B. and Neilands J. B. (1987) *Anal Biochem*, 160, 47-56.
[42] Shanmugam P. and Narayanasamy M. (2009) *The Internet Journal of Microbiology*, 6, 211-219.
[43] Stammler G., Itoh M., Hino I., Watanabe A., Kojima K., Motoyoshi M., Koch A. and Haden E. (2007) *J. Pestic. Sci,* 32, 10–15.
[44] Suryadi Y., Kadir T. S. and Sukumandi. (1989) *Int. Rice Res. Notes*, 14, 35.
[45] Swamy H. N., Sannaulla S. and Kumar D. M. (2009) *Karnataka Journal of Agricultural Science,* 22, 448-449.
[46] Thangasamy T. A. and Rangaswamy M. (1989) *Int. Rice Res. Notes*, 14, 24.
[47] Vasantha Devi T., Malarvizhi R., Sakthivel N. and Gnanamanickam S. S. (1989) *Plant Soil*, 119, 325-330.
[48] Vidhyasekaran P. and Muthamilan M. (1999) *Biocon. Sci. Technol*, 9, 67-74.
[49] Vijay Krishna Kumar K., Krishnam Raju S., Reddy M. S., Kloepper J. W., Lawrence K. S., Groth D. E., Miller M. E., Sudini H. and Binghai Du. (2009) *Journal of Pure and Applied Microbiology*, 3, 485-488.
[50] Viswanathan R., Sundar A. R. and Premkumari M. S. (2003) *World J Microbiol Biotechnol*, 19, 953-959.
[51] Yang D., Wang B., Wang J., Chen Y. and Zhou M. (2009) *Biol Control*, 51, 61-65.
[52] ZhiYi L. C., Feng Y. and Fan L. (2003) *Acta Phytophylacica Sinica*, 30, 429-434.

Table 1- Plant growth promoting characterization of Bacillus subtilis strain MBI 600.

Character	Result[1]
Chitinase	-
IAA	-
Cellulase	-
Siderophore	+
HCN	-
P solubilization	-

[1] + = Positive; and - = Negative

Table 2- Effect of various concentrations of Bacillus subtilis strain MBI 600 as seed treatment on seedling growth of various rice cultivars under in vitro conditions.

Treatment[1]	Seedling height (mm)[2]			
	Cocodrie	Catahoula	Neptune	Trenasse
Control	7.6^{e}	17.6^{e}	10.9^{c}	19.5^{d}
2.20 x 10^{6} cfu/ ml	20.6^{d}	25.5^{d}	11.7^{c}	28.1^{c}
2.20 x 10^{7} cfu/ml	25.3^{c}	35.1^{c}	19.6^{b}	32.6^{b}
2.20 x 10^{8} cfu/ml	29.8^{b}	47.4^{b}	22.9^{b}	38.9^{a}
2.20 x 10^{9} cfu/ml	39.1^{a}	63.6^{a}	43.3^{a}	41.5^{a}

.[1]Seeds of rice treated with strain MBI 600 produced in liquid formulations at 2.20 x10^{6} , 2.20 x 10^{7}, 2.20 x 10^{8}, and 2.20 x 10^{9} cfu/ml.

[2] Means of four replications, 10 seedlings per replication

Means followed by a common letter in the columns are not significantly different according to LSD (at $p \leq 0.05$)

Table 3- Effect of various concentrations of Bacillus subtilis strain MBI600 as seed treatment on root development of various rice cultivars under in vitro conditions

Treatment[1]	Root length (mm)[2]			
	Cocodrie	Catahoula	Neptune	Trenasse
Control	14.3^{e}	19.9^{e}	8.3^{d}	17.7^{e}
2.20 x 10^{6} cfu/ml	31.0^{d}	33.1^{d}	10.2^{d}	23.8^{d}
2.20 x 10^{7} cfu/ml	36.1^{c}	45.4^{c}	36.1^{c}	35.0^{c}
2.20 x 10^{8} cfu/ml	41.2^{b}	52.5^{b}	49.8^{b}	42.0^{b}
2.20 x 10^{9} cfu/ml	47.5^{a}	69.5^{a}	54.1^{a}	50.5^{a}

[1]Seeds of rice treated with strain MBI 600 produced in liquid formulations at 2.20 x10^{6} , 2.20 x 10^{7}, 2.20 x 10^{8}, and 2.20 x 10^{9} cfu/ml.

[2] Means of four replications, 10 seedlings per replication

Means followed by a common letter in the columns are not significantly different according to LSD (at $p \leq 0.05$)

Table 4- Effect of various concentrations of Bacillus subtilis strain MBI 600 as seed treatment on seedling emergence of rice (Cv. Cocodrie) under greenhouse conditions.

Treatment[1]	Emergence of seeds (%)[2]					
	Day 2	Day 3	Day 4	Day 5	Day 6	
Control	44.5^{c}	45.6^{c}	51.1^{c}	52.2^{c}	61.1^{c}	
2.20 x 10^{6} cfu/ml	50.0^{bc}	50.0^{bc}	61.1^{bc}	65.6^{b}	73.3^{b}	
2.20 x 10^{7} cfu/ml	50.0^{bc}	50.0^{bc}	68.9^{ab}	75.6^{ab}	78.9^{b}	
2.20 x 10^{8} cfu/ml	54.4^{ab}	55.6^{b}	71.1^{ab}	75.6^{ab}	81.1^{ab}	
2.20 x 10^{9} cfu/ ml	60.0^{a}	64.5^{a}	80.0^{a}	85.5^{a}	88.9^{a}	

[1]Seeds of rice treated with strain MBI 600 produced in liquid formulations at 2.20 x10^{6} , 2.20 x 10^{7}, 2.20 x 10^{8}, and 2.20 x 10^{9} cfu/ml.

[2] Means of six replications, 15 seedlings per replication

Means followed by a common letter in the columns are not significantly different according to LSD (at $p \leq 0.05$)

Table 5- Effect of various concentrations of Bacillus subtilis MBI 600 as seed treatment on growth of rice seedlings (Cv. Cocodrie) under greenhouse conditions at 15 days after seeding.

Treatment	Shoot height (mm) [1]	Root length (mm)[1]	Shoot fresh weight (g) [1]	Root fresh weight (g) [1]
Control	222.0^{c}	72.7^{d}	0.10^{c}	0.04^{b}
2.20 x 10^{6} cfu/ml	234.3^{c}	95.7^{c}	0.10^{c}	0.06^{b}
2.20 x 10^{7} cfu/ml	289.0^{b}	119.0^{b}	0.14^{bc}	0.07^{ab}
2.20 x 10^{8} cfu/ml	298.7^{b}	132.3^{b}	0.16^{b}	0.07^{ab}
2.20 x 10^{9} cfu/ml	335.0^{a}	166.3^{a}	0.23^{a}	0.10^{a}

[1]-Seeds of rice treated with strain MBI 600 produced in liquid formulations at 2.20 x10^{6} , 2.20 x 10^{7}, 2.20 x 10^{8}, and 2.20 x 10^{9} cfu/ml.
[2] Means of six replications, 15 seedlings per replication
Means followed by a common letter in the columns are not significantly different according to LSD (at $p \leq 0.05$)

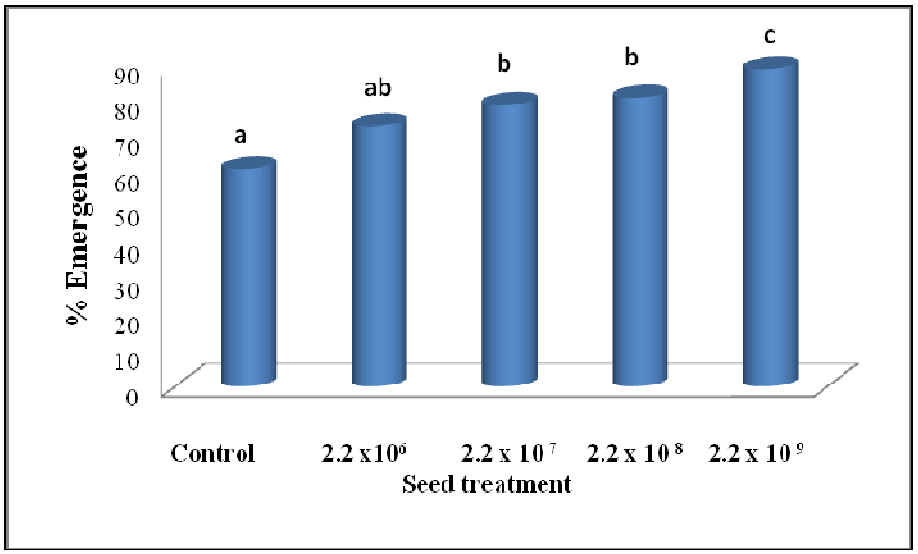
Table 6- Compatibility of B. subtilis strain MBI 600 with commonly used fungicides.

Fungicides	Fungicide concentrations (ppm)[1]					
	100	200	400	600	800	1000
Propiconazole	+++	+++	+++	+++	+++	+++
Validamycin	+++	+++	+++	+++	+++	+++
Benomyl	+++	+++	+++	+++	++	+
Carbendazim	+++	+++	+++	+	-	-
Tricyclazole	+++	+++	+++	+++	+++	++
Mancozeb	+++	+++	+++	++	+	+
Azoxystrobin	+++	+++	+++	+	-	-
Hexaconazole	+++	+++	+++	+++	+++	+++

[1]Rate of growth of strain MBI 600 in nutrient agar amended with various concentrations of fungicides: +++ = Good; ++ = Moderate; + = Poor; and - = No growth.

Seed treatment with MBI 600 **Control**

Fig. 1- Influence of strain MBI 600 as seed treatment at 2.2 x 10^{9} cfu/ml on growth of mesocotyl roots and rootlets of rice seedlings of CV. Cocodrie at 7 days after seeding.

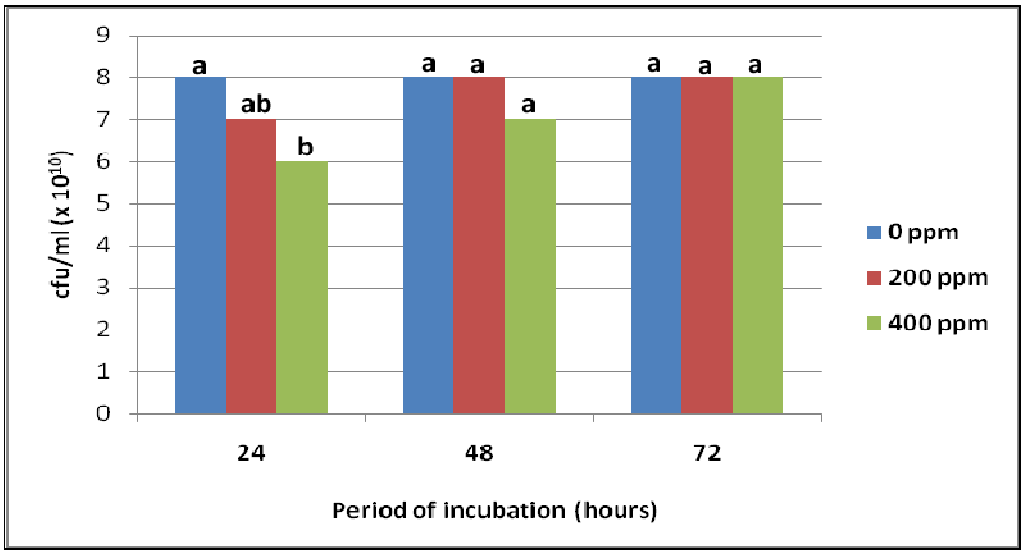


Values are means of six replications, 15 seeds per replication
Means followed by a common letter are not significantly different according to LSD (at $p \leq 0.05$)
Fig. 2- Influence of various concentrations of *Bacillus subtilis* strain MBI 600 as seed treatment on seed germination of rice, CV. Cocodrie, at 7 days after seeding under greenhouse conditions.

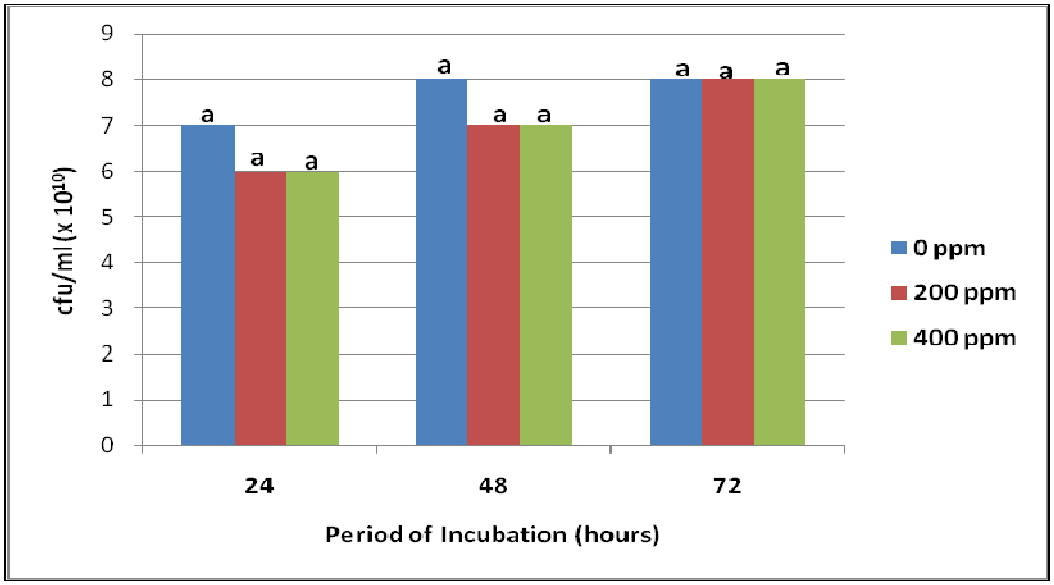
A=Control, B= 2.20 x 10^6 cfu/ml, C= 2.20 x 10^7 cfu/ml, D=2.20 x10^8 cfu/ml and E= 2.20 x 10^9 cfu/ml
Fig 3- Influence of various concentrations of *Bacillus subtilis* strain MBI 600 as seed treatment on root growth of rice under greenhouse conditions.

Control Seed treatment with MBI 600 at 2.20 x 10^9 cfu/ml

Fig. 4- Influence of various concentrations of *Bacillus subtilis* strain MBI 600 as seed treatment on seedling growth of rice under greenhouse conditions.



Values are means of five replications, one plate per replication
Means followed by a common letter are not significantly different according to LSD (at p≤0.05)
Fig. 5- Growth of strain MBI 600 on nutrient agar amended with various concentrations of carbendazim.



Values are means of five replications
Means followed by a common letter are not significantly different according to LSD (at p≤0.05)
Fig. 6- Growth of strain MBI 600 on nutrient agar amended with various concentrations of Azoxystrobin.