IDENTIFICATION OF PLANT GROWTH PROMOTING ANTAGONISTIC BACTERIA AGAINST BLAST DISEASE OF RICE

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Abstract

This study aimed to isolate and identify plant growth-promoting bacteria from rhizosphere of rice plants that exhibit antagonistic properties against *Magnaporthe oryzae*, the causal agent of blast disease. Bacteria were isolated from the surface of rice leaves, stems, and soil attached to the roots. The antagonistic activity of the isolated bacteria was assessed using a dual culture method, and identities were determined through 16S rDNA sequencing. To evaluate their growth-promoting abilities, assays were conducted to measure indole acetic acid (IAA) production, siderophore secretion, hydrogen cyanide (HCN) production, and phosphate solubilization. The results revealed that *Bacillus subtilis*, identified as BDISO_01R, exhibited antagonistic behavior and the maximum inhibition of *M. oryzae* (81.00%) was obtained by bacterial isolate BDISO-01R. Additionally, eight bacterial isolates demonstrated IAA production, sixteen isolates produced siderophores, and nine isolates displayed phosphate solubilization capability. This research sheds light on the diverse microbial arsenal that can potentially promote rice growth while combating blast disease.

Keywords: Plant growth, antagonistic bacteria, *Magnaporthe oryzae*

Introduction

Rice (*Oryza sativa* L.) is a genus of perennial grass in the Poaceae (grass family), generally familiar as Asian rice. Rural and urban people mostly depend on rice for calories intake and over half of the world's population widely consumed it as staple food (Khush, 2005). Asia is the top growing and consuming (around 90%) continent in the world (Salim *et al.*, 2003). Following acreage and production, Bangladesh secured fourth position next to China, India, and Indonesia among the rice-producing countries of the world (BBS, 2017). As many as 43 rice diseases are reported in Bangladesh and vulnerability to these diseases caused low yield of rice (Fakir, 2000). Rice (Oryza sativa) is an indispensable staple crop worldwide, ensuring food security for a significant global population. However, the persistent threat of blast disease caused by Magnaporthe oryzae severely impacts rice production, leading to substantial yield losses and economic setbacks (Jones and Dangl, 2006). Rice diseases, blast caused by *Pyricularia oryzae* is the most explosive and potentially damaging disease. The disease affects the crop at all stages. The average loss due to blast has been reported to be around 28-36%, and in certain areas yield losses could be as high as 80-100% (Kato, 2001; Hossain *et al.*, 2017). So it is the potential threat for crop failure from this disease that it has been ranked among the most important plant diseases of

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them all. Conventional control methods relying on chemical pesticides face challenges, prompting the search for sustainable alternatives. Plant growth-promoting bacteria (PGPB) have emerged as promising biocontrol agents, capable of enhancing plant growth and suppressing pathogens through diverse mechanisms (Compant *et al.*, 2005). Harnessing the antagonistic potential of PGPB offers a viable approach to combat rice blast disease. The phylloplane (leaf surface) and rhizosphere (root zone) of rice plants host a rich microbial community, including bacteria with biocontrol potential against blast disease (Berendsen *et al.*, 2012; Mendes *et al.*, 2013). Targeting these microbial niches provides an opportunity to discover naturally occurring antagonistic bacteria for disease management. In this study, our objective was to isolate and identify PGPB from the rice phylloplane and rhizosphere that demonstrate antagonistic activity against M. oryzae. Bacteria were isolated from rice leaves, stems, and the soil attached to the roots.

To determine the taxonomic identities of the antagonistic bacterial isolates, we employed sequencing of the 16S rDNA gene, a widely utilized genetic marker for bacterial classification (Woese, 1987). This approach enables accurate species-level identification, offering valuable insights into the diversity of potential biocontrol agents present in the rice phylloplane and rhizosphere. Additionally, we assessed the isolates' growth-promoting abilities by evaluating traits such as indole acetic acid (IAA) production, siderophore secretion, hydrogen cyanide (HCN) production, and phosphate solubilization (Vessey, 2003; Ahmad *et al.*, 2008; Khan *et al.*, 2018). These traits are known to contribute to improved nutrient uptake, hormonal regulation, and disease resistance in plants. By elucidating the antagonistic potential and growth-promoting capabilities of the isolated bacteria, our research aims to contribute to the development of sustainable and eco-friendly strategies for managing rice blast disease. Harnessing the power of plant growth-promoting antagonists not only offers economic relief to farmers but also helps reduce the environmental impact associated with conventional chemical control methods. Agrochemicals and their behavior of natural degradation harm the environment, causing major ecological and health problems. An eco-friendly and sustainable crop production in agriculture is possible by using plant growth promoting bacteria, which could be a great substitute for bio-fertilizers or biocontrol agents (Scavino and Pedraza, 2013). Moreover, the use of plant growth-promoting bacteria is on the top priority list for alternative biological control (Nelson, 2004). The main aim of our research was to identify and characterize naturally occurring antagonistic bacteria associated with rice plants which could effectively inhibit the growth of blast pathogen, *Magnaporthe oryzae in vitro* and to assess the plant growth promoting effects of these isolates.

Materials and Methods

Plant sample collection

The healthy rice plants with root system of different rice cultivars were collected from different agro-ecological zones (AEZs) of Bangladesh from the vicinity of Blast infected rice plants. The plant samples took to the Laboratory and used immediately after collection while in the refrigerator 4^0c/ roots system with soils were preserved for further isolation of antagonistic bacterial isolates.

Isolation and purification of bacteria

For bacteria isolation from rhizosphere, 1g of fresh roots with adhered soils were stirred in sterile distilled water for 10 min. Then 20 μL of serially diluted soil solution (10^{-5} or 10^{-6}) was spread in either LB agar or King's B agar plate sand the plates were put in an incubator adjusted at 28 °C until the bacterial colonies were grown. Individual bacterial colonies grew on plates and each colony have different morphological characteristics. These characteristics were obtained by sub-culturing and stored in peptone broth containing 20% glycerol at -80 °C for long term preservation.

Assessment of antagonistic activity

The plant growth promoting bacterial isolates which showing antagonistic activity determined by agar diffusion technique (Monteiro *et al.*, 2005) with some modifications. *M. oryzae* strain was grown in Potato sucrose agar plates for 48 h and after that *M. oryzae* strain was suspended in sterile distilled water up to cell density of 5×10^8 CFU mL^{-1}. Bacterial cell suspension was then spread in NBY agar plates using a cotton swab. The possible antagonistic bacterial cell suspension (5×10^8 CFU mL^{-1}) was then spot inoculated at three places. After drying, the plates were put in an incubator at 28 °C for 3-5 days. The radial growth inhibition of *M. oryzae* as indicated by clear halo zones were observed. Control trial was done by spot inoculation with a bacterium previously known as non-antagonistic. The percent growth inhibition of *M. oryzae* was estimated by using the formula of (Vincent, 1947) as given bellow:

$$I = \frac{T-C}{C} \times 100$$

where, I = percent inhibition,
T = colony diameter with clear halo zone (mm), and
C = antagonistic bacterial colony diameter (mm).

Molecular based identification

The isolates which showed maximum inhibition were used as representative antagonistic isolates of *Magnaporthe oryzae* and these isolates were identified by sequencing of 16S rDNA gene with the following steps:

Genomic DNA extraction

Bacterial culture from NA media was transferred in LB broth and shaken for 18 h at 28 °C. Then genomic DNA of antagonistic bacteria was extracted according to Wizard® Genomic DNA purification Kit (Promega, Madison, USA). Obtaining the DNA pellet was rehydrated by adding 25 μL DNA rehydration solution and kept it overnight at 4°C. Finally, the genomic DNA samples of the isolates were preserved at −20°C for further use.

Primers and PCR conditions

To identify the antagonistic bacterial isolates, the primer sets 27F (5'-AGA GTT TGATCM TGG CTC AG-3') and 1518R (5'-AAGGAGGTGATCCANCCR CA-3') were used for 16S rDNA amplification from the prepared genomic DNA template (Gio-vannoni SJ. 1991). The PCR condition was as follows: initial denaturation at 95°C for 5 min, 35 cycles denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min and finally a 7 min extension at 72°C. PCR products were visualized by electrophoresis on 1.0% agarose gel containing 0.5% of ethidium bromide.

Sequencing of PCR products

A partial nucleotide sequencing of 16S rDNA was performed from amplified PCR products using primers 27F (5'-AGA GTT TGATCM TGG CTC AG-3') and 1518R (5'-AAG GAG GTG ATC CAN CCR CA-3') in the Macrogen Lab, South Korea via Biotech Concern Bangladesh. The sequencing was done directly from PCR products in both orientations according to the standard protocols for the ABI 3730 x l DNA genetic analyzer (Applied Biosystems, Foster City, CA, USA) with BigDye® Terminator v1.1 and 3.1 Cycle Sequencing Kits. The quality of nucleic acid sequences was evaluated using Chromas (Version 2.6) software to avoid the use of low-quality bases.

Analyses of nucleotide sequences

The nucleotide sequences were analyzed using online bioinformatics tools. The DNA sequences were compared with other *Pseudomonas*, *Bacillus* spp. and other bacterial spp. available in the NCBI database using Basic Local Alignment Search Tool (BLAST) algorithm to identify closely related sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

Analysis of data

The collected data on radial mycelial growth and ANOVA were analyzed statistically by using MSTATC package program. The means for all the treatments were compared by DMRT (Duncan Multiple Range Test). The significance of the difference among the means was calculated by LSD (Least Significant Difference) test.

Assessment of plant growth promoting determinants

Active isolates with antagonistic potential against *Magnaporthe oryzae* were further evaluated for their ability to produce plant growth promoting determinants viz. siderophore production, IAA production and phosphate solubilization.

Assessment for production of IAA

IAA production of antagonistic bacterial isolates was carried out as per the procedure described by Patten and Glick (1996). Every isolate was grown in LB media supplemented with (0.005%) L-tryptophan and incubated in shaker at 30 °C with 160 rpm for 48 h. Then

bacterial culture was centrifuged at 8000 rpm for 15 min and 1 mL culture filtrate was mixed with 4 mL salkowski's reagent (1.5 mL $FeCl_3.6H_2O$ 0.5M solution in 80 mL 60% H_2SO_4) and the mixture was incubated at room temperature for 30 min, presence of pink color indicates qualitatively that isolate produced IAA. Formation of pink colour indicated the presence of indoles (Gordon and Weber, 1951).

Assessment of siderophore production

Siderophore production by antagonistic bacterial isolates was tested qualitatively as described by Alexander and Zuberer (1991). Five microliter of antagonistic bacterial cell suspension (5×108 CFU mL^{-1}) was spot inoculated on Chrome azurol S (CAS) agar plate. The plates were then incubated at 30 °C for 5 days. Development of yellow-orange halo zone around the bacterial growth was considered as positive for siderophore production. Experiment was performed with a completely randomized design with 3 replications. CAS agar was prepared from 4 solutions. Solution 1 (Fe-CAS indicator solution) was prepared by mixing 10 mL of 1 mmol L^{-1} $FeCl_3.6H_2O$ (in 10 mmol L^{-1} HCl) with 50 mL of an aqueous solution of CAS (1.21g L^{-1}). The resulting dark purple mixture was added slowly with constant stirring to 40 mL of aqueous solution of hexadecyl trimethyl ammonium bromide (1.821g L^{-1}). The yielded of dark blue solution which was autoclaved, then cooled to 50°C. The entire reagent was freshly prepared for each batch CAS agar. Solution 2 (buffer solution) was prepared by dissolving 30.24g of piperazine-N, N-bis (2-ethane sufonic acid) (PIPES) in 750 mL of salt solution containing 0.3g K_2HPO_4, 0.5g NaCl and 1.0g NH_4Cl. The pH was adjusted to 6.8 with 50% (w/v) KOH, and water was added to bring the volume 800 mL. The solution was autoclaved after adding 15g of agar then cooled to 50°C. Solution 3 contained 2g glucose, 2g mannitol, 493 mg $MgSO_4.7H_2O$, 11 mg $CaCl_2$, 1.17mg $MnSO_4.2H_2O$, 1.4 mg H_3BO_3, 0.04 mg $CuSO4 .5H_2O$, 1.2 mg $ZnSO_4 .7H_2O$, 1.0 mg $NaMoO_4 .2H_2O$ in 70 mL water, autoclaved, cooled to 50°C. Solution 4 was 30 mL filter sterilized 10% (w/v) casamino acid. Finally, solution 3 added to solution 2 along with solution 4, solution 1 was added last, with sufficient stirring.

Assessment of HCN production

Nutrient sucrose agar (NSA) medium was used to detect the production of HCN by the antagonistic bacteria as described by Lorck (1948). The production of HCN was determined by the change in color of the picric acid saturated filter paper from yellow to red-brown. Relative quantification of HCN was done following the spectrophotometric method described by Nagarajkumar *et al.* (2004).

Screening for phosphate solubilization capability

Phosphate solubilization was determined according to the method of Azman *et al.* (2017). Sterile filter papers (5.0 mm) were soaked in antagonistic bacterial cell suspension (5×108 CFU mL^{-1}) was dispensed using pipette onto sterile filter paper (6.0 mm) that was placed on National Botanical Research Institute's phosphate (NBRIP) agar plate (Glucose

(10g L^{-1}), Ca$_3$(PO$_4$)$_2$ (5g L^{-1}), MgCl$_2$.6H$_2$O (5g L^{-1}), MgSO$_4$.H$_2$O (0.25g L^{-1}), KCl (0.2g L^{-1}), (NH$_4$)$_2$O$_4$ (0.1g L^{-1}), Bacteriological Agar (15g L^{-1}) (Nautiyal, 1999). The plates were then incubated at 28°C for 7 days. Phosphate solubilization was assessed by observing the clear halo zone. The experiment was performed with a completely randomized design with 3 replications.

Results

Antagonistic activity of the isolates

A total of 10 bacterial isolates obtained from rice rhizosphere (soils adhered to the roots). These bacterial isolates were screened to identify antagonist ability to *Magnaporthe oryzae*. Out of 10, only 4 bacterial antagonists found in vitro antagonistic activity against *Magnaporthe oryzae* strain tested. (Fig. 1 & Table 1).
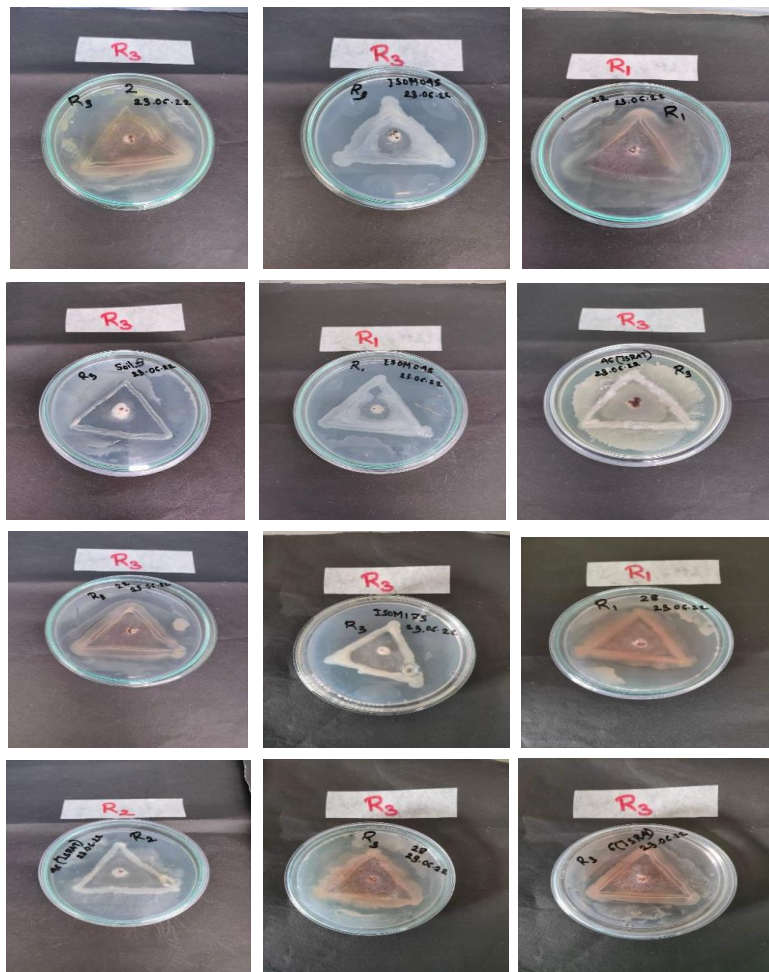

Fig. 1. Antagonistic activity of isolated bacteria

Table 1. Closest relatives of the plant growth promoting antagonistic bacterial isolates identified in this study

Isolates	Closest relatives	Accession no.	Alignment	Homology
BDISO_01R	*Bacillus subtilis* strain HYM07	KT961120	251/271	93
	Bacillus sp. strain YL-200	OK147804.1	254/274	92.99
	Bacillus subtilis strain SC15	MT310692.1	249/268	100
	Bacillus subtilis strain AK159	ON156005.1	251/271	93

Identification of the isolates

Ten bacterial isolates (1. BDISO_01R, 2: BDISO_02R, 3: BDISO_03R, 4:BDISO_04R, 5: BDISO_05R, 6: BDISO_06R, 7: BDISO_07R, 8: BDISO_08R, 9: BDISO_09R, 10: BDISO_10R) were identified using primers specific to 16S rDNA gene of bacteria. The results of PCR as shown in the gel photograph confirmed the presence of amplicon size around 1500 bp which revealed that all the isolates obtained from rhizosphere of rice plant were bacteria (Fig. 2).
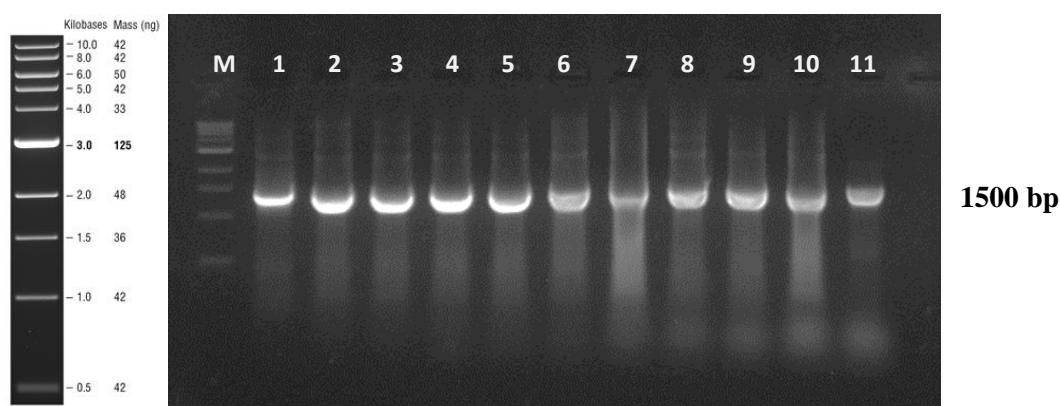

Fig. 2. PCR amplification of 16srDNA of the antagonistic bacterial isolates. 1kb+ DNA ladder. 1: BDISO_01R, 2: BDISO_02R, 3: BDISO_03R, 4: BDISO_04R, 5: BDISO_05R, 6: BDISO_06R, 7: BDISO_07R, 8: BDISO_08R, 9: BDISO_09R, 10: BDISO_10R

The phylogenetic tree was constructed based on the sequence of 16S rDNA antagonistic bacterial isolates and compared with previously reported isolates of different places using MEGA 7 software (Saitou and Nei, 1987; Felsenstein, 1985; Tamura *et al.*, 2004; Kumar *et al.*, 2016). The length of the phylogenetic tree expressed the genetic distance of the isolates from the ancestors. The phylogenetic tree analysis of the isolates based on the nucleotide bases of 16S rDNA revealed that bacterial isolates were distributed in two main groups. Rhizobacterial isolates BDISO-01, accession no (KT961120, MT310692, OK147804) were in group- I and accession no KM573815.5 was present in the group -II (Fig. 3).
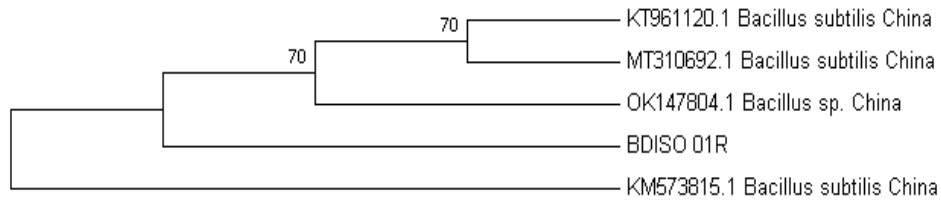
Fig. 3. Phylogenetic relationships of the *Bacillus subtilis* isolate using MEGA7 software

Plant growth promotiong determinants

Write some preleminary sentences on plant growth promoting determinants...

Indole acetic acid production (IAA)

Out of the 10 bacterial isolates, 7 isolates were found to produce IAA as indicated by the production of pink color in presence of Salkowski's reagent (Fig. 3 & Table 1).

Siderophore production

Out of the 10 bacterial isolates, we identified 6 bacterial isolates was found to produce siderophore as indicated by the production of orange yellow color on CAS agar (Fig. 4 & Table 1). Plant growth promoting rhizobacteria produce siderophores to compete and attain Fe^{3+} (ferric ions) from surrounding under iron scarcity (Whipps, 2001). Siderophores, derived from a Greek word meaning 'iron carrier' basically are the compounds with lower molecular weight with high iron affinity and these small iron chelating compounds are released by the beneficial microorganisms (Miller and Marvin, 2008).

Phosphate solubilization

Out of 10 bacterial isolates, 5 bacterial isolates showed the capability of phosphate solubilization. This capability was indicated by the production of clear halo zones on LB medium containing tri-calcium phosphate (Fig. 5 & Table 1).

Table 2. Assessment of plant growth promoting determinates of antagonistic bacterial strains

Isolate Name	Siderophore Production	HCN Production	Phosphate Solubilization	IAA Production
BDISO_01R	-	+	-	+
BDISO_02R	+++	-	++	+
BDISO_03R	-	+	++	+++
BDISO_04R	+	++	++	-
BDISO_05R	+++	+	++	+
BDISO_06R	+	-	-	+
BDISO_07R	+++	+++	-	+
BDISO_08R	-	+	-	+
BDISO_09R	++	-	++	-
BDISO_10R	-	-	-	-

Note: +++ Represents Very Aggressive; ++ Represents Medium Aggressive; + Represents Low Aggressive & - Represents No Results (Not Aggressive)

Hydrogen Cyanide (HCN) production

Out of 100 bacterial isolates, 5 bacterial isolates showed the capability of HCN production. The production of HCN was determined by the change in color of the picric acid saturated filter paper from yellow to red-brown.

Fig. 4. Indole acetic acid (IAA) production activity by different antagonistic bacterial isolates indicated by the presence of pink color when bacterial culture supernatant mixed with Salkowski reagent, Antagonistic bacterial isolates showed positive phosphate solubilizing activity by producing clear halo zone around the bacterial colony on National Botanical Research Institute's Phosphate (NBRIP) agar plates, Antagonistic bacterial isolates showed positive siderophore production activity as indicated by orange halo zone around bacterial colony on CAS agar plates and Antagonistic bacterial isolates showed HCN production activity as indicated by the change in color of the picric acid saturated filter paper from yellow to red-brown.

Antagonistic activity of the rhizobacterial isolate (BDISO-01R) against rice blast pathogen, *M. oryzae*

10 bacterial isolates were subjected to a triangular dual culture assay to ensure antifungal activity and to determine the best isolate against *M. oryzae*. Out of the 10 bacterial isolates, 5 isolates showed antagonistic activity against the rice blast pathogen, *M. oryzae*. The data recorded 14 days after incubation were analyzed. Most of the selected bacteria inhibited *M. oryzae* growth to varying degrees, and the inhibition was significant when compared to relative controls. The quantitative inhibitory activity of identified antagonist bacteria against *M. oryzae* is shown as a percentage of growth inhibition for each bacterial isolate. The percent of mycelial growth inhibition of *M. oryzae* (over control) by different rhizospheric bacterial isolates was recorded and ranged from 66.52% to 81.00%. The maximum inhibition of *M. oryzae* (81.00%) was obtained by bacterial isolate BDISO-01R (Fig. 5).
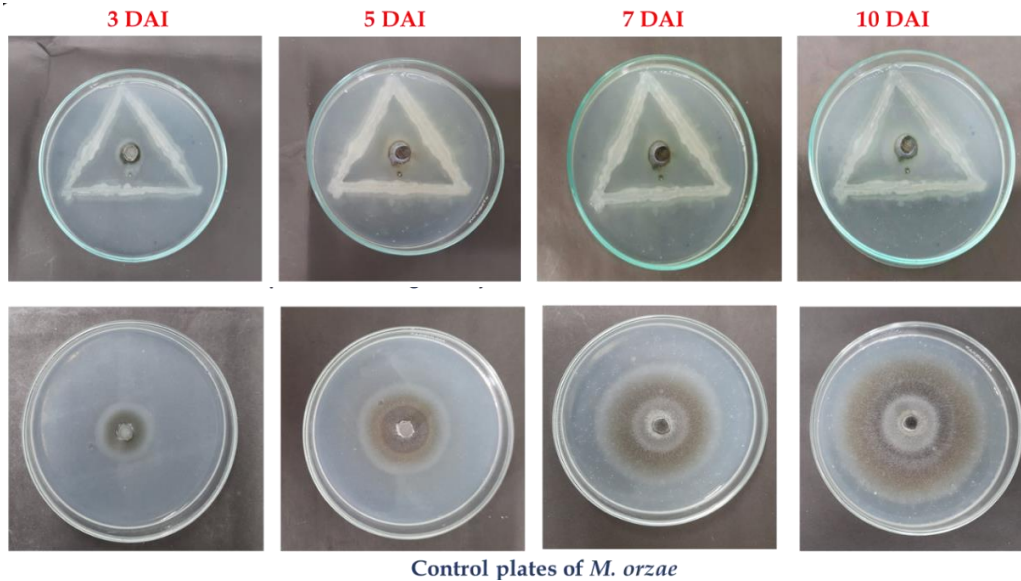
Fig. 5. Dual culture plate containing *M. oryzae* isolate

Discussion

Rice is our stable crop and rice production in Bangladesh is hampered every year due to the attack of different fungal, bacterial and viral diseases. Till to date, the use of chemical pesticides is the only effective measure to control those diseases at the field level. But the unjudicial use of chemical pesticides is harmful to both human health and environment. Due to that, the current study was conducted to isolate and identify plant growth-promoting rhizobacteria (PGPR) from rice rhizosphere and to check their antagonistic ability against some rice pathogens such as *M. oryzae* causing blast diseases of rice respectively as part of bio-control of plant rice diseases. In the present study, a total of 105 rhizobacterial isolates were obtained from the rice rhizosphere (soils adhered to the roots). It has been stated that bacteria isolated from soil can control diseases (Rahaman 2010). In the current study, 10 bacterial isolates out of 5 exhibited mycelial growth inhibition against *Magnaporthe oryzae* in various percentages ranging from 66.52% to 81.00% growth inhibition in a dual culture experiment. In this work, 10 rhizobacterial isolates (1: BDISO_01R, 2: BDISO_02R, 3: BDISO_03R, 4: BDISO_04R, 5: BDISO_05R, 6: BDISO_06R, 7: BDISO_07R, 8: BDISO_08R, 9: BDISO_09R, 10: BDISO_10R) showed strong antagonistic activity against *M. oryzae* ranging from 88.01% to 91.00%. Out of 10 bacterial isolates, only 4 isolates of different species were showed antagonistic activity against *Magnaporthe oryzae*. In this study, it was observed that 66.52% to 81.00% radial growth inhibition of *Magnaporthe oryzae* exhibited by all of those, acterial isolates. 66.52% to 81.00%. Similar findings were reported by Jha, Y *et al.* (2011). They showed defense related enzymes such as chitinase, polyperoxidase (PO) and polyphenol oxidase (PPO) in the presence of *Magnaporthe grisea*,

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the causative agent of rice blast. The results indicate that the endophytic bacteria showed a better response to the fungal infection than the rhizospheric one. The findings of the present study also underpinning by the findings of Suprapta *et al.* (2016), who showed Ninetyfive isolates of rhizobacteria were isolated from diverse plant rhizospheres grown in Bali and tested for their ability to promote the rice growth and induce resistance against blast disease. Nine isolates have been proven serve as PGPR and among them four isolates namely KDDA, O38, OR3 and Al7Kla are capable of inducing resistance against blast disease on Padi Merah Medang Putih. Based on 16S rDNA sequence analysis, these PGPR are respectively identified as *Bacillus methylotrophicus* KDDA, *Bacillus amyloliquefaciens* O38, *Pseudomonas aeruginosa* OR3 and *Achromobacter xylosoxidans* Al7Kla. All of these PGPR can be further utilized to induced resistance against blast disease on Bali local rice variety. Antagonistic bacteria can suppress plant pathogens either by directly or indirectly. Antibiotics, enzymes like chitinases, glucanases, proteases, and siderophores produce direct or indirect mechanisms in which the antagonistic bacteria compete with the pathogen for a niche or nutrient sites (Bardin *et al.*, 2015). Out of 30 bacterial isolates, 10 isolates were aligned and identified as *Bacillus* spp. Isolates of *Bacillus* spp, have widely studied and exploited bacterial species as biocontrol agents (Kloepper *et al.*, 1989; Okon and Labandera-Gonzalez, 1994). It has been reported that *B. velezensis* strain has the potential to be developed into a biopesticide for the biocontrol of rice blast (Chen, *et al.*, 2021). Molecular identification of antagonistic bacteria such as *B. subtilis, B. amyloliquefaciens, B. valismortis, Streptomyces sp., Pseudomonas chlororaphis* and *Acinetobacter baumannii* based on 16S rRNA sequence analysis were reported (Ranjbariyan *et al.*, 2011). Molecular techniques are implied to carry out the distinct classification and identification of bacteria by isolating the genomic DNA, polymerase chain reaction generates copies of DNA sequence and then 16S ribosomal DNA (rDNA)-based identification of bacteria. 16S rDNA gene sequencing provides explicit data even for rare isolates, which are reproducible in and between laboratories. The increase in accurate new 16S rDNA sequences and the development of alternative genes for molecular identification of certain taxa should further improve the usefulness of molecular identification of bacteria. The use of 16S rDNA gene sequences to study bacterial phylogeny and taxonomy has been by far the most common housekeeping genetic marker used for many reasons (Patel, 2001). However, 16S rRNA gene sequencing provides genus identification in most cases (>90%) but less so with regard to species (65 to 83%), with from 1 to 14% of the isolates remaining unidentified after testing (Janda and Abbott, 2007). Out of the 30 bacterial isolates, 8 isolates were found to produce IAA. The results also showed that out of 30 isolates, 8 isolates [BDPMIL-03, BDPMIL-06, BDPMIL-15, BDPMIL-28, BDPMIL-34, BDPMIL-38, BDPMIL-46, BDPMIL-47] producing IAA belonged to *Bacillus spp*. IAA also has been speculated to improve the fitness of plant-microbe interactions (Patten and Glick, 2002). It was proved that many plant-associated bacteria have the ability to produce IAA take part in the most important role in plant growth promotion by stimulating plant roots development and improving absorption of water and nutrients from soil (Aslanta¸s *et al.*, 2007; Wu *et al.*, 2005). The IAA producing bacteria encouraged adventitious root formation, produced the

greatest roots and shoots weight (Cakmakci *et al.*, 2007). All 16 bacterial isolates were found to produce siderophore. It was known that microorganism that can produce siderophore provided Fe nutrition to enhance plant growth when iron element bioavailability was low (Crowley, 2006). It was also known for more than three decades that different bacterial species were capable to improve plant growth, contributed into plant Fe nutrition and promoted roots and shoots growth by producing siderophores (Verma *et al.*, 2011). Siderophore is particularly important when evaluating the potential of a strain for biocontrol (Manninen and Mattila-Sandholm 1994). Siderophores have been suggested to be an environmentally friendly alternative to hazardous pesticides (Schenk *et al.*, 2012). The biological control mechanism depended on the role of siderophore as competitors for Fe in order to reduce Fe availability for the phytopathogen (Beneduzi *et al.*, 2012). Siderophores produced by numerous bacteria had a significant role in the biocontrol and negatively affected the growth of several pathogens (Yu *et al.*, 2011; Beneduzi *et al.*, 2012).Out of 30 isolates, 5 bacterial isolates [BDPMIL-06, BDPMIL-17, BDPMIL-25, BDPMIL-28, BDPMIL-46] showed the capability of phosphate solubilization which were capable of phosphate solubilization related to *Bacillus spp*. It has been reported that phosphate solubilizing bacteria (PSB) induced plant growth promotion (c *et al.*, 2015). Plant roots-associated PSB have been considered as one of the possible alternatives for inorganic phosphate fertilizers for promoting plant growth and yield (Thakuria *et al.*, 2004). Plant growth and phosphate uptake have increased in many crop species due to the results of PSB inoculants (Fankem *et al.*, 2015; Gusain *et al.*, 2015). It has also been documented that the application rates of phosphate fertilizers reduced to 50% by inoculating phosphate solubilizing microbes (PSM) in crops without significantly reducing crop yield (Yazdani *et al.*, 2009). In sustainable agriculture, certain plant pathogens can be controlled by biological agents like plant growth promoting bacteria (PGPB) and PGPB can also be used as bio-fertilizer (Shanthi and Vittal, 2013). There are a lot of PGPB strains that reported to suppress numerous of plant pathogen, reduce the disease incidence, stimulate the plant growth factor and supplies the nutrition for the growth of the plant (Hariprasad *et al.*, 2009; Yasmin *et al.*, 2017). Therefore, it has been considerable research interest in the potential use of antagonistic bacteria as PGPB (Babalola, 2010; Kumar *et al.*, 2012). 23 bacterial isolates exhibit the capability to produce HCN. The production of HCN was a more common trait of *Pseudomonas* (88.89%) (Ahmad *et al.* 2008). Cyanide occurs in solution as free cyanide, which includes the cyanide anion (CN-) and the non-dissociated HCN. Cyanide is a phytotoxic agent capable of inhibiting enzymes involved in major metabolic processes and is considered one of the typical features of deleterious rhizobacterial isolates (Bakker and Schippers 1987). Nevertheless, at present its applications in areas of biocontrol methods are increasing (Devi *et al.* 2007). Some cyanogenic rhizobacteria are typically host specific and associated with the roots of their host plants. Therefore, HCN produced in the rhizosphere of seedlings by selected rhizobacteria is a potential and environmentally compatible mechanism (Kremer and Souissi, 2001). Studies concerning commercialization and field applications of integrated stable bio-formulations as effective biocontrol strategies would be needed in future.

Conclusion

This study identified plant growth-promoting bacteria from rice plants with antagonistic properties against blast disease. *Bacillus subtilis* showed antagonistic behavior, and several isolates displayed abilities like IAA production, siderophore secretion, and phosphate solubilization, revealing their potential for promoting rice growth and disease control. The findings of this study clearly showed that plant growth promoting bacterial isolate BDISO-01R which have significant antagonistic behaviour against *M. oryzae*, can be employed as bio-control agents against rice diseases.

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