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Chapter

Potential Role of Rice Plant Growth Promoting Phylloplane and Rhizospheric Bacteria in Controlling *Xanthomonas oryzae* pv. *oryzae*

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Abstract

Rice is an important cereal worldwide and it occupies the top position among the cereals in Bangladesh. Rice plant suffers from around 32 diseases of which ten are major in Bangladesh at present. Among the diseases, Bacterial Blight (BB) caused by *X. oryzae* pv. *oryzae* (*Xoo*) considered as a most destructive disease occurs in both rainfed and irrigated seasons of Bangladesh. BB causes considerable yield loss varies from 30 to 50% depending on the outbreak. It is also an important disease in most of the South and Southeast Asian countries. To develop environment-friendly sustainable management approach against BB of rice, in total sixty three plant growth promoting bacteria were identified from rice phylloplane and rhizosphere that are antagonistic to *X. oryzae* pv. *oryzae* during boro and aman seasons 2018 and 2019. These bacterial species inhibited the growth of *X. oryzae* pv. *oryzae* *in vitro* by 20.83 to 76.19%. These bacterial isolates were identified by sequencing of PCR products of 16SrDNA belonging to the genera mostly *Pseudomonas*, *Bacillus* and *Serratia*. Out of these bacterial species, 48 bacterial species were found as positive for IAA production, all 63 bacterial species were found positive for siderophore production and 48 were found capable to solubilize insoluble phosphate. Based on growth inhibition of *X. oryzae* pv. *oryzae* *in vitro*, thirty two bacterial species were selected for plant growth promotion assessment and evaluation of net house and field efficacy in controlling BB of rice. These bacterial species were formulated using talcum powder which was viable for at least three months post formulation. Assessment of plant growth promoting determinants revealed that all 32 bacterial species identified in this study enhance the growth of rice plants as measured by root and shoot length and reduced the BB severity in susceptible rice cultivar significantly as compared with untreated control.

Keywords: Rice, Plant growth promoting phylloplane and rhizospheric bacteria, control, *X. oryzae* pv. *oryzae*

1. Introduction

Rice (*O. sativa* L.) suffers from 32 diseases of which in Bangladesh 10 has been known as dreadful diseases [1]. Among the diseases three bacterial diseases are frequently occurred in Bangladesh. Among these three diseases, Bacterial Blight (BB) caused by *X. oryzae* pv. *oryzae* (Xoo) considered as a most destructive disease occurs in all Agro Ecological Zones (AEZs) of Bangladesh and mostly in two rice growing seasons namely viz. raifed and irrigated [2–4] and cause severe yield loss. In Japan, India and Bangladesh due to this devastating disease around 50%, 60% and 30% yield loss was observed [5], respectively in the highly infected rice fields. It is also a crucial disease in most of the South and Southeast Asian countries [6]. Bacterial blight (BB) is disease associated with several growth phases of rice plant showing either “Kressek” (acute wilting of young plants) symptoms and “leaf blight” (straw color blighted area with weavy margin) symtoms [7]. Excess amount of nitrogenous fertilizer in rice varieties (HYV) facilitates the emergence of this disease and its severity in the field [8–12]. In Bangladesh different pathogenic [13, 14] and genetic variability [15] have been detected and those were excessively perilous for rice [16].

Chemical fungicides (copper compounds, other chemicals and antibiotics) are not effective in controlling this disease [17]. However, control measures are including chemical, cultural, host resistance, genetic modification methods, among them cultural practices are not also effective in all circumstances as well as no fruitful chemical control and commercial product was found in this tropical climatic area which can be suppressed this disease nicely [18, 19]. Moreover, using antibiotics, toxic residues and chemicals have several limitations against BB of rice [20]. Apart from that, the uses of host resistance genes are used, in case of breeding single gene (Xa4) are manifested ineffective BLB management due to sub-populations [21].

Thus, biological control alleviates costs and it also serves as an environment friendly approach to mitigate this devastating threat [22], besides, the application of biological strains of PGPB would be the fullest alternative way of minimizing chemical pesticides, fertilizer and environmental pollution [23]. PGPB plays a crucial role in developing immunization in plants body, ISR is triggered by PGPB which is a signaling pathway while SAR mainly dependent on salicylic acid triggering a induced resistance by a particular infection, However, it is observed that ISR requires salicylic acid (SA) and ISR demands ethylene (ET) and jasmonic acid (JA) signal pathways [24] and both of these are triggered latent resistance mechanism subsequently after inoculation [25]. In recent years, application of PGPB in the field has been evaluated as an inducer showing systematic resistance [26, 27, 38]. Due to fruitful leaf colonization, quick growth, normal application procedure of *L. antibioticus* have been utilized as a bio control agents against Xoo [28]. *Bacillus* spp. also found effective in quelling BLB of rice under greenhouse condition [29]. According to [30], *S. globisporus* have been effective against rice blast. Sheath blight disease was alleviated by using a few biofilm and surfactant delivering strains of *Bacillus subtilis* [31]. Amalgamation of *B. subtilis* and *Streptomyces philanthi* were biologically effective against rice sheath blight adding with chemical fungicides [32]. HCN (Hydrogen cyanide) played an effective role inhibiting the surges of *M. oryzae* as well as developing its bio control agents against blast of rice [33]. These antagonistic bacteria have the ability to subvert plant pathogens by releasing chemicals such as glucanases, proteases and chitinases, siderophores [34]. Rice disease can be controlled by the antagonistic strains of *Bacillus* and *Pseudomonas* spp. up to 90% based on what kind of strains are used [35]. When systemic resistance is exposed is called as ISR, and conversely, by other phenomenon is called SAR [36]. No necrosis manifested while ISR developed by PGPB [36]. Last few decades, PGPB have been

showing as a systematic resistance in the field [26, 27, 37, 38]. ISR demands three systematic pathway which are jasmonic acid (JA), ethylene (ET), salicylic acid (SA) signaling pathways [24]. PGPB can induce priming by the release of volatiles. For instance, *Bacillus subtilis* GBO3 induces a signaling pathway that is independent of salicylic acid (SA), jasmonic acid (JA) and the Npr1 gene (SA insensitive or nonexpresser of PR genes), yet it requires ethylene [39]. Priming offers an energy cost efficient strategy, enabling the plant to react more effectively to any invader encountered by boosting infection induced cellular defense responses [40, 41]. The increased levels of defense related enzymes during ISR are known to play a crucial role in host resistance [42, 43], reported that *Pseudomonas fluorescens* have been used as a bacterial antagonists against BLB of rice. A plentiful of bacterial strains *B. cereus*, *B. pasteurii*, *pumilus*, *Bacillusmycoides*, *B. amyloliquefaciens*, *B. sphaericus*, *B. pumilus*, *B. cereus* have been effective in reducing disease resistance upon using ASM (acibenzolar-S-methyl) [39, 43].

Species such as *Bacillus* spp. which showed ISR are radically linked to plant growth modification promotion [39] and this strains have been manifested resistance activity against a number of plant diseases studied by several researcher [44–50]. In rice, limited number of studies found discussing on induced resistance, the main theme of PGPB also includes production of growth hormones such as IAA and IA (inorganic phosphate) (Khan et al., 1997 and [51]), and zinc solubilization [52], atmospheric nitrogen [53]. Plant health also maintained by PGPB by producing ISR, siderophores and competition [54] as well as mitigate plant pathogens by developing enzymes such as antibiotics, proteases, glucanases and chitinases [34]. In both lab and field conditions PGPB bacteria are significantly reduced plant disease incidence, among them *Bacillus* and *Pseudomonas* spp. suppressed diseases up to 90% based on rice variety and types of pathogens [35]. ISR (Induced systemic resistance) is an environment friendly option for plant disease control because it initiates defense related genes and enzymes in host plant through inoculated bacteria to reduce disease incidence [29]. Bacterial Blight pathogen, however, radical information on rice PGPB which can be used as both biopesticide and biofertilizer is not disclosed in Bangladesh. Besides, more investigation needs to be executed from other dimension to completely minimize this deadly disease.

2. Materials and methods

2.1 Isolation and identification of bacteria from rice phylloplane and rhizosphere

2.1.1 Plant sample collection

To isolate the bacteria from rice phylloplane and rhizosphere, the healthy rice plants with root system and soils of different rice cultivars were collected from 40 districts representing 30 Agroecological Zones (AEZs) of Bangladesh from the vicinity of BB infected rice plants during boro and aman season, 2018 and 2019 at maximum tillering stage to pre-ripening stage. Then the rice plant samples were brought into the laboratory in labeled polybags.

2.1.2 Isolation and purification of bacteria

The phylloplane bacteria were isolated using washing method. Freshly harvested 2nd, 3rd, 4th leaves were vortexed in sterile saline solution for 12 minutes with two or three brief intervals. Then 100 µl solution was placed at the center of Luria

Bartani (LB) or King's B agar plate and the solution was spread with glass spreader. The inoculated plates were incubated for 3–5 days at room temperature. After incubation of the inoculated plates, bacterial colonies appeared with various types of colors. Then the bacterial colonies were selected and isolated depending on their color and were streaked on LB media separately. Again the streaked LB plates were incubated at room temperature for 2 days. For isolation of antagonistic bacteria from rhizosphere, 1 g roots with rhizospheric soils were taken and then it was shaken with 100 ml sterile water for about 10–15 min to obtain soil suspension. Isolation of bacteria were carried out from rhizospheric soil by serial dilution technique up to 10^{-5} to 10^{-6} using LB (Luria Bertani) medium. Then the solution was placed at the center of Luria Bertani (LB) or King's B agar plate and the solution was spread with glass spreader. The inoculated plates were incubated for 3–5 days at room temperature. After incubation of the inoculated plates, bacterial colonies appeared with various types of colors. Then the bacterial colonies were selected and isolated depending on their color and were streaked on LB media separately. Again the streaked LB plates were incubated at room temperature for 2 days.

2.2 Assay of antagonism of bacterial spp. to *X. oryzae* pv. *oryzae* by dual culture method

Antimicrobial activity of antagonistic strains of *Pseudomonas* spp./*Bacillus* spp. were determined by agar diffusion technique method [55] with some modifications. Antagonistic bacterial suspension was spot inoculated (5 μ l of 10^8 CFU/ml) at three places on the NBY plates that were prior inoculated with *X. oryzae* pv. *oryzae* cell suspension (10^8 CFU/ml \sim optical density: 0.3). The plates were incubated for 7 days post inoculation at 28°C. Then *X. oryzae* pv. *oryzae* growth inhibition by the antagonistic bacterial isolates indicated by clear halo zones were measured with a ruler in mm. The percent growth inhibition of *X. oryzae* pv. *oryzae* by bacterial isolates was calculated as follows:

$$\text{Growth inhibition (\%)} = \left[\frac{\text{Total diameter (Colony diameter + clear halo zones)} - \text{Colony diameter}}{\text{Total diameter}} \right] \times 100 \quad (1)$$

2.3 Assessment of plant growth promoting determinants of bacteria antagonistic to *X. oryzae* pv. *oryzae*

Active isolates with antagonistic potential against *X. oryzae* pv. *oryzae* were further evaluated for their ability to produce plant growth promoting determinants viz. siderophore production, Indole acetic acid (IAA) production and phosphate solubilization capability as follows:

2.3.1 Assay for siderophore production

Siderophore productions by antagonistic bacterial isolates were tested qualitatively as described by Alexander and Zuberer [56]. 5 μ l of antagonistic bacterial cell suspension (5×10^8 CFU/mL) 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 FeCl₃.6H₂O

(in 10 mmol L⁻¹ HCl) with 50 mL of an aqueous solution of CAS (1.21 g L⁻¹). The resulting dark purple mixture was added slowly with constant stirring to 40 mL of aqueous solution of hexadecyl trimethyl ammonium bromide (1.821 g 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 30.24 g of piperazine-N, N-bis (2-ethane sufonic acid) (PIPES) in 750 mL of salt solution containing 0.3 g K₂PO₄, 0.5 g NaCl and 1.0 g NH₄Cl. 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 15 g of agar then cooled to 50°C. Solution 3 contained 2 g glucose, 2 g mannitol, 493 mg MgSO₄.7H₂O, 11 mg CaCl₂, 1.17 mg MnSO₄.2H₂O, 1.4 mg H₃BO₃, 0.04 mg CuSO₄.5H₂O, 1.2 mg ZnSO₄.7H₂O, 1.0 mg NaMoO₄.2H₂O 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.

2.3.2 Assay for indole acetic acid (IAA) production

IAA production of antagonistic bacterial isolates were carried out as per the procedure described by Patten and Glick [57]. 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 FeCl₃.6H₂O 0.5 M solution in 80 mL 60% H₂SO₄) and the mixture was incubated at room temperature for 30 min, presence of pink color indicate qualitatively that isolate produced IAA. Formation of pink color indicated the presence of indoles [58].

2.3.3 Phosphate solubilization assay by antagonistic bacterial isolates

Phosphate solubilization was determined according to the method of Azman et al. [59]. Sterile filter papers (5.0 mm) were soaked in antagonistic bacterial cell suspension (5 × 10⁸ CFU/mL) 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 (10 g/L), Ca₃ (PO₄)₂ (5 g/L), MgCl₂.6H₂O (5 g/L), MgSO₄.H₂O (0.25 g/L), KCl (0.2 g/L), (NH₄)₂SO₄ (0.1 g/L), Bacteriological Agar (15 g/L) [60]. 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 (CRD) with 3 replications.

2.4 Identification of selected plant growth promoting antagonistic bacterial isolates by sequence analyses of 16SrDNA

2.4.1 Extraction of genomic DNA

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.

2.4.2 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'-AAG GAG GTG ATC CAN CCR CA-3') specific to 16SrDNA were used for amplification of 16SrDNA from the prepared genomic DNA template [61]. The PCR conditions were 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 using a Gel documentation System after separating the PCR products in the agarose gel for 50 min at 80 volt.

2.4.3 Sequencing of PCR products

A partial nucleotide sequencing of 16SrDNA was performed from amplified PCR products using primers 27F (5'-AGA GTT TGATCM TGG CTC AG-3') in the Macrogen Lab, South Korea via Biotech Concern Bangladesh. The sequencing was done directly from PCR products according to the standard protocols for the ABI 3730xl DNA genetic analyzer (Applied Biosystems, Foster City, CA, USA) with BigDye® Terminator v1.1 and 3.1 Cycle Sequencing Kits.

2.4.4 Processing of sequence data

The sequencing data were processed and nucleotide sequence data was exported using Chromas software version 2.6.4. The quality of nucleic acid sequences was evaluated using Chromas (Version 2.6) software to avoid the use of low quality bases.

2.4.5 Analyses of nucleotide sequences

The nucleotide sequences were analyzed using online bioinformatics tools. The DNA sequences of 16Sr DNA of the bacterial isolates were compared with 16Sr DNA of the bacterial spp. and the sequences of ITS region of the fungal isolates were compared with ITS region of the fungal spp. that were 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>).

2.5 Formulation of plant growth promoting antagonistic bacterial species

The pure cultures of thirty two selected potential bacterial antagonists were grown on LB agar medium for 24 hrs. Then the bacterial isolates were transferred in LB broth for about six hours by taking a loopful of bacteria from the LB agar plate. After that the liquid culture was then centrifuged and resuspended the pellet in previously prepared 200 ml peptone broth aimed to fortify the carrier materials. This culture broth was then grown for another two hours with shaking. After that 5 ml of sterile 100% glycerol was added to this 200 ml culture. Then the cultures of the bacterial antagonists (200 ml fortified with 1% peptone and 1% glycerol) were added to the mixture of 500 g talcum powder amended with 5 g carboxy methyl cellulose (CMC) and 7.5 g Calcium carbonate which were autoclaved for two consecutive days at 121°C under 15PSI pressure for 30 min each. The formulations were then being dried overnight in the tray. After that the formulations were powdered with hand, the formulated bacterial antagonists were packed in plastic bags. The formulated bacterial antagonists were then kept at both room and 4-8°C temperature in the refrigerator.

2.6 Assessment of viability of the formulated fungal and bacterial antagonists

The viability of the bacterial and fungal antagonists were checked by drawing 1 g of the formulated products in sterile water in every 30 days after formulation and diluted serially up to 10^{-4} or 10^{-5} . The numbers of viable cells (colony forming unit) were counted per gram formulations kept at both room temperature and 4-8°C temperature in the refrigerator.

2.7 Assessment of plant growth promotion induced by antagonistic bacterial and fungal isolates

Rice seeds (cv.IR24) were surface sterilized and dried. Then the sterilized rice seeds were treated with formulated bacterial and fungal antagonists (10 g/kg seeds) and the treated seeds were left for 1 h under shade. The rice seeds were then sown in the plastic pots previously filled with sterile soils. Fifty seeds were sown in each pot and three replications were maintained. Then the germination of seeds were recorded at 7DAS. The seedlings were uprooted at 7 DAS, 14 DAS and 28 DAS to measure the root length, shoot length and to calculate the vigor index [= (root length + shoot length) \times germination percentage] were measured.

2.7.1 Seed priming, raising of seedlings and transplanting

Seeds of IR24 were treated with 32 selected formulated PGP antagonistic bacterial isolates. The treated seeds were left for 1 hr. for adherence of the bacterial and fungal isolates with the treated seed surface. The treated seeds were then sown in the plastic pots filled with sterilized soils. One month old seedlings were then transplanted in the plastic pots filled with puddle soils.

2.7.2 Foliar spray of formulated PGP bacterial and fungal isolates

Formulated PGP antagonistic bacterial isolates were sprayed two times (at 50 and 55 DAS) before inoculation and two times after inoculation i.e. 65 and 70 DAS.

2.7.3 Inoculation of the rice plant with *X. oryzae* pv. *oryzae*

Rice plants were inoculated with a strain of *X. oryzae* pv. *oryzae* by Scissor clip method at 60 DAS.

3. Results

3.1 Isolation and identification of antagonistic bacteria against *X. oryzae* pv. *oryzae*

Rice plant samples were collected from 40 districts of Bangladesh representing 30 AEZs during boro seasons 2018–2019 and aman seasons 2018–2019. In total 300 bacterial isolates and 100 fungal isolates were isolated and purified from rice plant samples during boro season, 2018. Some selected representative bacterial species were shown in **Figure 1**. Out of 300 bacterial isolates, eighteen were identified as antagonist against *X. oryzae* pv. *oryzae* and inhibited the growth of *X. oryzae* pv. *oryzae* *in vitro* which was ranged by 28.39–76.19% (**Table 1** and **Figure 2**). The maximum (76.14%) growth inhibition of *X. oryzae* pv. *oryzae* *in vitro* was recorded by BDISOB05P while the minimum (28.59) growth inhibition was exhibited by

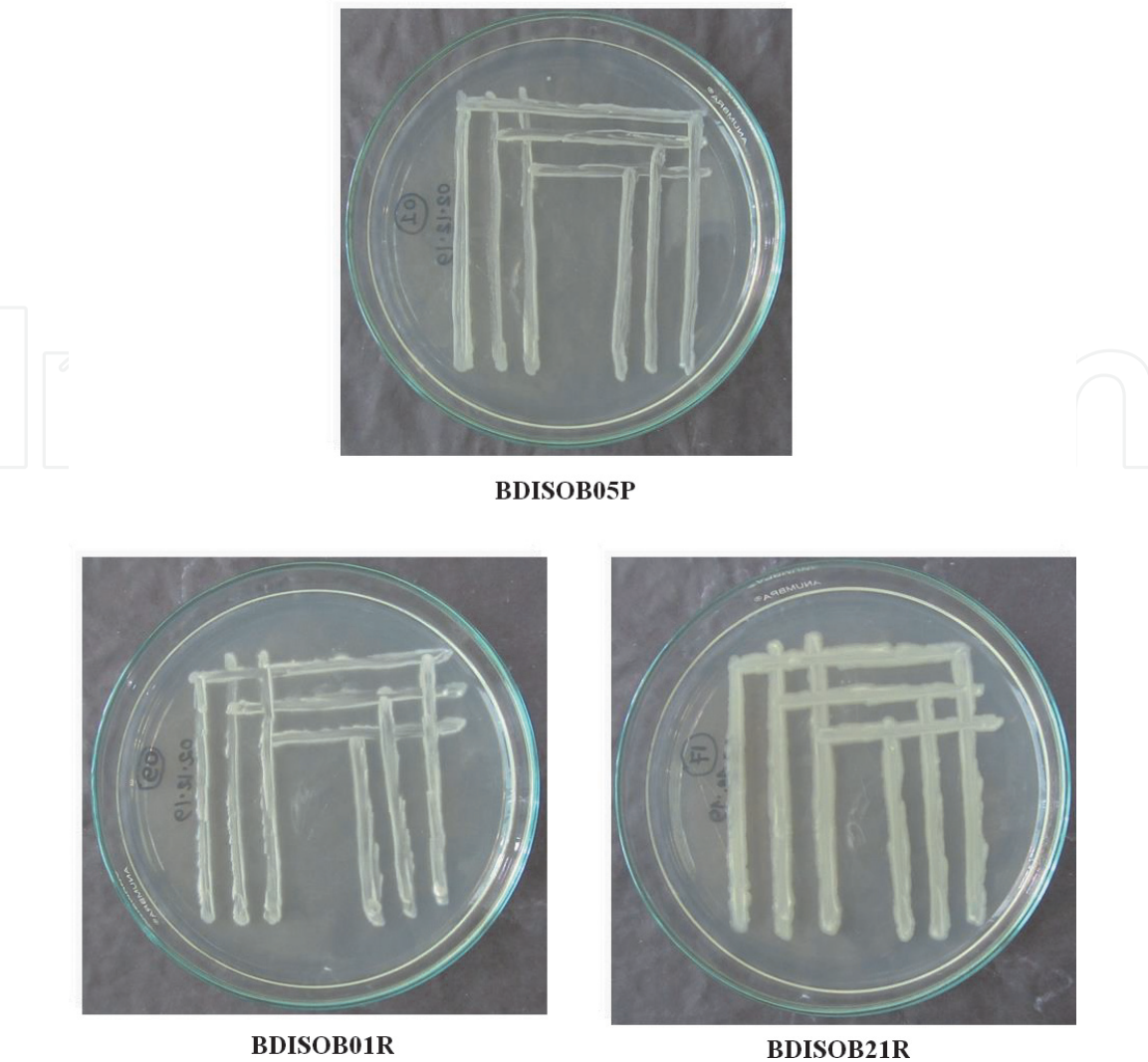


Figure 1.
Representative photographs of purified bacterial isolates obtained from rice phylloplane and rhizosphere. BDISOB05P: an isolate from Mymensingh, BDISOB01R: an isolate from Mymensingh and BDISOB21R: an isolate from Chattagram.

BDISOB272R. These antagonistic bacterial isolates were identified by sequencing of PCR products of 16SrDNA gene (**Figure 3A**). The identified bacterial species were BDISOB04P (*P. putida*), BDISOB05P (*P. putida*), BDISOB98P (*Stenotrophomonas maltophilia*), BDISOB241P (*Burkholderia* sp.), BDISOB242P (*B. gladioli*), BDISOB219R (*P. taiwanensis*), BDISOB220R (*Serratia* sp.), BDISOB221R (*Pseudomonas* sp.), BDISOB222R (*P. plecoglossicida*), BDISOB258R (*P. putida*), BDISOB272R (*Stenotrophomonas maltophilia*), BDISOB275R (*P. putida*), BDISOB186R (*Pseudomonas* sp.), BDISOB283R (*Pseudomonas fluorescens*), BDISOB306R (*P. putida*), BDISOB53R (*P. putida*), BDISOB61R (*Delftia tsuruhatensis*) (**Table 1**). In total 400 bacterial isolates and 40 fungal isolates were isolated and purified from rice plant samples collected in aman season, 2018. Seventeen bacterial isolates were identified as antagonist against *X. oryzae* pv. *oryzae* and inhibited the growth of *X. oryzae* pv. *oryzae* *in vitro* which was ranged by 38.33–60.66% (**Table 2**). The highest (60.66%) growth inhibition of *X. oryzae* pv. *oryzae* was exhibited by BDISO147P and the lowest (38.33%) growth inhibition was shown by BDISO135P. These antagonistic bacterial isolates were identified by sequencing of PCR products of 16SrDNA gene (**Figure 3B**). The bacterial species were BDISO04P (*P. putida*), BDISO45P (*Bacillus paramycoides*), BDISO356P (*P. hibiscicola*), BDISO198P (*Serratia plymuthica*), BDISO135P (*Bacillus* sp.), BDISO148P (*Serratia marcescens*), BDISO92P (*Serratia marcescens*), BDISO237P (*Alcaligenes faecalis*), BDISO12P (*Alcaligenes faecalis*),

Isolates	Closest relatives	Accession no.	Alignment	Homology	Growth inhibition of <i>X. oryzae</i> pv. <i>oryzae</i> (%)
BDISOB04P	<i>P. putida</i> strain	MF838698.1	968/1086	89	61.67
BDISOB05P	<i>P. putida</i> strain	MH085459.1	931/1140	82	76.14
BDISOB16P	<i>Bacillus</i> sp.	MH819972.1	702/738	95	59.94
BDISOB98P	<i>Stenotrophomonas maltophilia</i>	AY486381.1	1224/1271	96	33.04
BDISOB241P	<i>Burkholderia</i> sp.	GU979224.1	1154/1222	94	63.64
BDISOB242P	<i>B. gladioli</i>	MH748602.1	1186/1239	96	51.18
BDISOB219R	<i>P. taiwanensis</i>	KC293831.1	913/969	94	63.12
BDISOB220R	<i>Serratia</i> sp.	FM875872.1	150/186	81	61.77
BDISOB221R	<i>Pseudomonas</i> sp.	MG021242.1	303/341	89	68.33
BDISOB222R	<i>P. plecoglossicida</i>	KC864769.1	614/751	82	64.79
BDISOB258R	<i>P. putida</i>	MF417798.1	917/1050	87	64.40
BDISOB272R	<i>Stenotrophomonas maltophilia</i>	KJ534495.1	794/923	86	28.59
BDISOB275R	<i>P. putida</i>	KT984874.1	1201/1229	98	71.86
BDISOB186R	<i>Pseudomonas</i> sp.	JQ977022.1	29/29	100	64.43
BDISOB283R	<i>Pseudomonas fluorescens</i>	KF010368.1	969/1006	96	66.04
BDISOB306R	<i>P. putida</i>	KF030905.1	1298/1374	94	44.97
BDISOB53R	<i>P. putida</i>	JQ833720.1	53/60	88	48.19
BDISOB61R	<i>Delftia tsuruhatensis</i>	MF353931.1	976/1168	84	38.54

Table 1.
List of antagonistic bacterial isolates identified by homology search of sequences of 16SrDNA by BLAST program obtained from plant samples collected in boro season 2018.

BDISO196P (*Alcaligenes faecalis*), BDISO145P (*Serratia marcescens*), BDISO09P (*Serratia marcescens*), BDISO21R (*Serratia marcescens*), BDISO154P (*P. taiwanensis*), BDISO154P (*P. taiwanensis*), BDISO147P (*Serratia marcescens*), BDISO158R (*Serratia marcescens*), BDISO0R (*B. amyloliquefaciens*). In boro season 2019, 300 bacterial isolates were isolated and purified. In boro season 2019, out of 400 bacterial isolates fourteen were identified as antagonist against *X. oryzae* pv. *oryzae* and inhibited the growth of *X. oryzae* pv. *oryzae* *in vitro* which was ranged by 20.83–60.87% (**Table 3** and **Figure 3C**). The maximum (60.87%) growth inhibition of *X. oryzae* pv. *oryzae* *in vitro* was recorded by BDISOB37R while the minimum (20.83%) growth inhibition was exhibited by BDISOB14R. The bacterial species identified were BDISOB37R [*Pseudochrobactrum asaccharolyticum*], BDISOB16R [*Pseudochrobactrum asaccharolyticum*], BDISOB91R [*Pseudochrobactrum asaccharolyticum*], BDISOB17R [*Limnolyngbyacircumcreta*], BDISOB15R [*Pseudochrobactrum asaccharolyticum*], BDISOB86R [*Enterobacteraerogenes*], BDISOB30R [*Pseudochrobactrum asaccharolyticum*], BDISOB92R [*Pseudomonas fluorescens*], BDISOB178R [*Serratia marcescens*], BDISOB11R [*Pseudochrobactrum asaccharolyticum*], BDISOB21R [*Stenotrophomonas maltophilia*], BDISOB24R [*P. asaccharolyticum*], BDISOB23R [*Pseudochrobactrum asaccharolyticum*] and BDISOB14R [*Pseudochrobactrum asaccharolyticum*] by sequencing of bacterial 16SrDNA. In aman season 2019, 400 bacterial isolates were isolated and purified. In aman season 2019, out of 400 bacterial isolates fourteen were identified as antagonist against *X. oryzae* pv. *oryzae* and inhibited the growth of *X. oryzae* pv. *oryzae* *in vitro* which was ranged

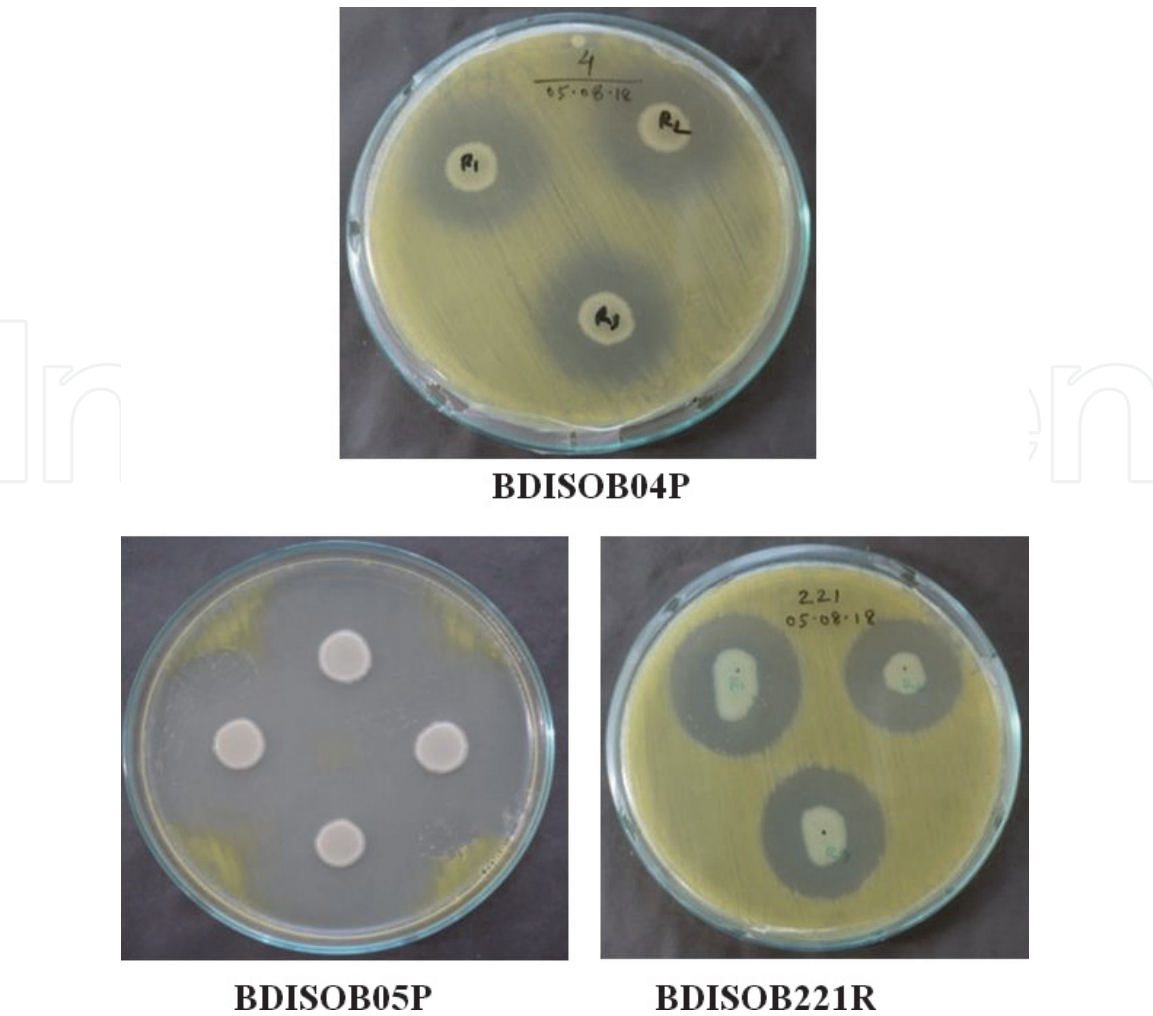


Figure 2. Representative photographs of in vitro growth inhibition of *X. oryzae* pv. *oryzae* by different potential bacterial isolates. BDISOB04P: an isolate from Cox’s Bazar, BDISOB05P: an isolate from Mymensingh and BDISOB221R: an isolate from Chattagram.

by 50.83–61.545% (**Table 4**). The maximum (61.54%%) growth inhibition of *X. oryzae* pv. *oryzae* in vitro was recorded by BDISOB54R while the minimum (50.93%) growth inhibition was exhibited by BDISOB12R. These antagonistic bacterial isolates were identified by sequencing of 16SrDNA gene (**Figure 3D**). The bacterial species were BDISOB70R [*Serratia marcescens*], BDISOB54R [*B. gladioli*], BDISOB08R [*Serratia marcescens*], BDISOB31R [*Serratia marcescens*], BDISOB06R [*Serratia marcescens*], BDISOB171R [*Alcaligenes faecalis*], BDISOB46R [*Serratia marcescens*], BDISOB09R [*Serratia marcescens*], BDISOB33R [*Serratia marcescens*], BDISOB11R [*Serratia marcescens*], BDISOB36R [*Serratia marcescens*], BDISOB07R [*Serratia nematodiphila*], BDISOB172R [*B. aerophilus*] and BDISOB12R [*Serratia marcescens*] by sequencing of bacterial 16SrDNA.

3.2 Assessment of plant growth promoting determinants

Three plant growth promoting determinants viz. siderophore and IAA production as well as phosphate solubilization capability were assessed. The results revealed that the development of yellow-orange halo zone around the bacterial growth on chrome azurol S agar plates was considered as positive (+) for siderophore production, formation of pink color by the culture supernatant of the bacterial isolates in presence of Salkowski’s reagent confirmed IAA production which was indicated by ‘+’ sign and observation of clear halo zone in National Botanical Research Institute’s phosphate (NBRIP) agar plates indicated the bacterial

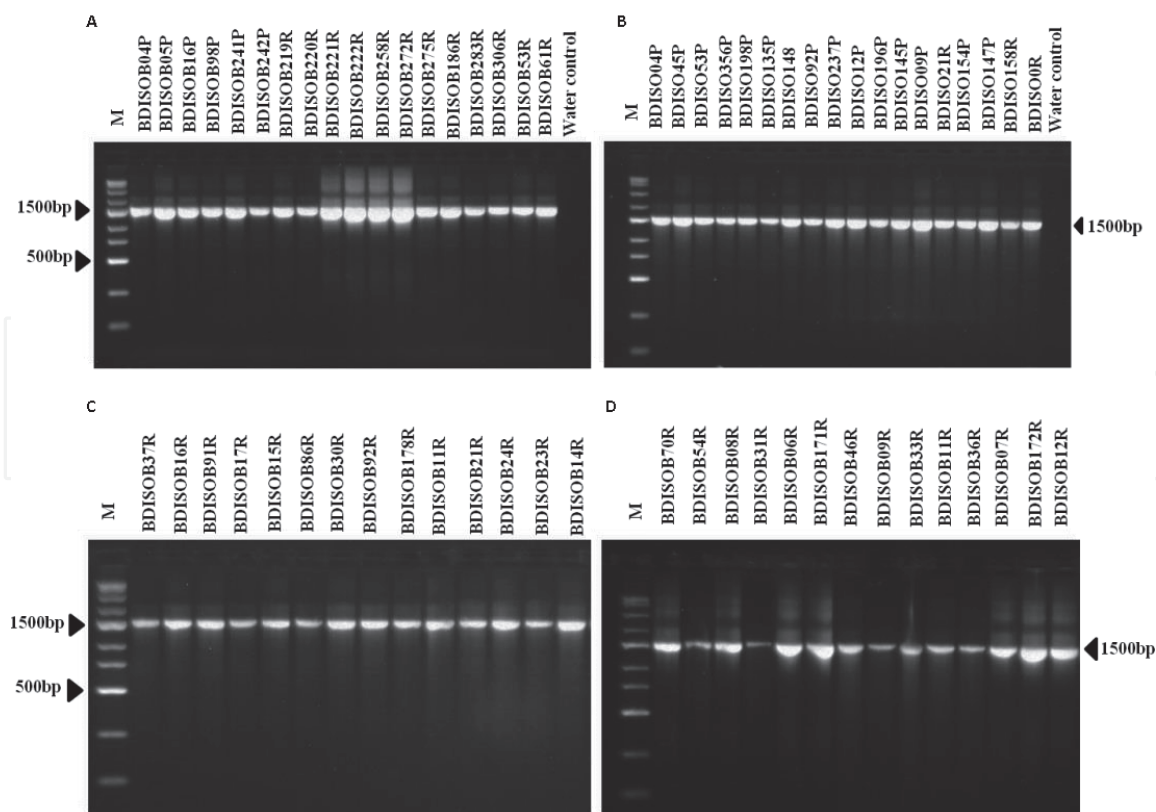


Figure 3.

PCR confirmation of the antagonistic bacterial isolates by amplification of 16S rDNA using primers 27F and 1518R obtained from plant samples collected in irrigated and rainfed seasons. These PCR products were then used for sequencing. Bacterial isolates obtained from (A) irrigated: BDISOB04P, BDISOB05P, BDISOB16P, BDISOB98P, BDISOB241P, BDISOB242P, BDISOB219R, BDISOB220R, BDISOB221R, BDISOB222R, BDISOB258R, BDISOB272R, BDISOB275R, BDISOB186R, BDISOB283R, BDISOB306R, BDISOB53R and BDISOB61R. (B) Rainfed: BDISO04P, BDISO45P, BDISO53P, BDISO356P, BDISO198P, BDISO135P, BDISO148P, BDISO92P, BDISO237P, BDISO12P, BDISO196P, BDISO145P, BDISO09P, BDISO21R, BDISO154P, BDISO147P, BDISO158R, BDISO0R, (C) irrigated: BDISOB37R, BDISOB16R, BDISOB91R, BDISOB17R, BDISOB15R, BDISOB86R, BDISOB30R, BDISOB92R, BDISOB178R, BDISOB11R, BDISOB21R, BDISOB24R, BDISOB23R and BDISOB14R: a rhizosphere isolate, Cumilla and (D) rainfed: BDISOB70R, BDISOB54R, BDISOB08R, BDISOB31R, BDISOB06R, BDISOB171R, BDISOB46R, BDISOB09R, BDISOB33R, BDISOB11R, BDISOB36R, BDISOB07R, BDISOB172R and BDISOB12R.

isolates are capable of phosphate solubilization which was denoted by “+” sign (Figure 3). Out of these bacterial species, 48 bacterial species were found as positive for IAA production, all 63 bacterial species were found positive for siderophore production and 48 were found capable to solubilize insoluble phosphate. In case of Indole Acetic Acid (IAA), BDISOB92FarR (*Pseudomonas fluorescens*), BDISOB172ThaR (*B. aerophilus*), BDISOB45PanP (*Bacillus paramycoides*), BDISOB01MymR (*Bacillus amyloliquefaciens*) showed highest IAA production. Whereas, BDISOB186KusR (*Bacillus paramycoides*) showed lowest IAA production. BDISOB54KhuR (*B. gladioli*) and BDISOB21ChaR (*S. maltophilia*) indicated moderate IAA production. BDISOB198HabP (*Seratiaplymuthica*), BDISOB148JoyP (*Seratia marcescens*), BDISOB145JoyP (*Seratia marcescens*), BDISOB07FarR (*Seratianematodiphilia*), BDISOB12FarR (*Seratia marcescens*), BDISOB31MagR (*Seratia marcescens*), BDISOB46GopR (*Seratia marcescens*) and BDISOB70KusR (*Seratia marcescens*) were statistically similar. The bacterial isolates BDISOB222GaiR (*P. plecoglossicida*), BDISOB45PanP (*Bacillus paramycoides*) BDISOB01MymR (*B. amyloliquefaciens*) BDISOB04KhaP (*P. putida*), BDISOB05MymP (*P. putida*), BDISOB221GaiR (*Pseudomonas sp.*) showed highest siderophore production. Whereas, BDISOB135SerP (*Bacillus sp.*), BDISOB145JoyP (*Seratia marcescens*) and BDISOB21ChaR (*Stenotrophomonas maltophilia*) showed

Isolate ID	Closest relatives	Accession no.	Alignment	Homology	Growth inhibition of <i>X. oryzae</i> pv. <i>oryzae</i> (%)
BDISO04P	<i>P. putida</i>	FR749878.1	827/1080	96	46.37
BDISO45P	<i>Bacillus paramycoides</i>	MK467557.1	1027/1133	91	50.00
BDISO356P	<i>P. hibiscicola</i>	KJ396817.1	1125/1148	98	46.83
BDISO198P	<i>Serratia plymuthica</i>	KU821695.1	472/530	89	50.00
BDISO135P	<i>Bacillus</i> sp.	KU146461.1	189/237	80	38.33
BDISO148P	<i>Serratia marcescens</i>	MN691926.1	929/990	94	54.26
BDISO92P	<i>Serratia marcescens</i>	MG996733.1	568/616	92	44.18
BDISO237P	<i>Alcaligenes faecalis</i>	KR827435.1	1048/1102	95	57.19
BDISO12P	<i>Alcaligenes faecalis</i>	MN513225.1	927/1094	85	57.44
BDISO196P	<i>Alcaligenes faecalis</i>	MN513225.1	901/1111	81	46.18
BDISO145P	<i>Serratia marcescens</i>	MF360051.1	545/630	87	40.00
BDISO09P	<i>Serratia marcescens</i>	MN252007.1	171/185	92	44.47
BDISO21R	<i>Serratia marcescens</i>	MG557818.1	194/200	97	54.60
BDISO154P	<i>P. taiwanensis</i>	MN416314.1	161/178	90	47.22
BDISO147P	<i>Serratia marcescens</i>	MF716688.1	1086/1130	96	60.66
BDISO158R	<i>Serratia marcescens</i>	MK346258.1	866/953	91	47.27
BDISO0R	<i>B. amyloliquefaciens</i>	KC888017.1	1151/1153	99	50.00

Table 2.
List of antagonistic bacterial isolates identified by homology search of sequences of 16SrDNA by BLAST program obtained from plant samples collected in aman season 2018.

lowest siderophore production. The Sierophore production found in BDISOB219GaiR (*P. taiwanensis*), BDISOB186KusR (*Pseudomonas* sp.), BDISOB283KisR (*Pseudomonas fluorescens*), BDISOB198HabP (*Seratiaplymuthic*), BDISOB54KhuR (*B. gladioli*) and BDISOB21ChaR (*S. maltophilia*) BDISOB198HabP (*Serratia plymuthica*), BDISOB148JoyP (*Serratia marcescens*), BDISOB158ChaR (*Serratia marcescens*) BDISOB148JoyP (*Serratia marcescens*), BDISOB145JoyP (*Serratia marcescens*), BDISOB07FarR (*Serratia nematodiphilia*), BDISOB12FarR (*Serratia marcescens*), BDISOB31MagR (*Serratia marcescens*), BDISOB46GopR (*Serratia marcescens*) and BDISOB70KusR (*Serratia marcescens*) were statistically similar. The bacterial isolates BDISOB05MymP (*P. putida*), BDISOB45PanP (*Bacillus paramycoides*) and BDISOB01MymR (*B. amyloliquefaciens*) showed highest siderophore production. Whereas, BDISOB186KusR (*Pseudomonas* sp.), BDISOB258GaiR (*P. putida*) and BDISOB70KusR (*Serratia marcescens*) showed lowest phosphate solubilization activity. The others bacteria in case of phosphate solubilization were statistically similar.

3.2.1 IAA production

In case of Indole Acetic Acid (IAA), four isolates those were BDISOB92FarR (*Pseudomonas fluorescens*), BDISOB172ThaR (*B. aerophilus*), BDISOB45PanP (*Bacillus paramycoides*), BDISOB01MymR (*Bacillus amyloliquefacience*) revealed highest IAA production. Conversely, only one BDISOB186KusR (*Bacillus paramycoides*) depicted lowest IAA production. Around, twelve isolates exhibited upper-moderate IAA production, besides, seven showed lower and lower-moderate IAA production. BDISOB198HabP (*Serratia plymuthica*), BDISOB148JoyP (*Serratia marcescens*),

Isolate ID	Closest relatives	Accession no.	Alignment	Homology	Growth inhibition of <i>X. oryzae</i> pv. <i>oryzae</i> (%)
BDISOB37R	<i>Pseudochrobactrum asaccharolyticum</i>	KC456599.1	275/298	92%	60.87
BDISOB16R	<i>Pseudochrobactrum asaccharolyticum</i>	KC456599.1	275/298	92	57.09
BDISOB91R	<i>Pseudochrobactrumsaccharolyticum</i>	KC456543.1	748/841	89	56.55
BDISOB17R	<i>Limnolyngbyacircumcreta</i>	KR697754.1	86/105	82	43.42
BDISOB15R	<i>Pseudochrobactrum asaccharolyticum</i>	KM921740.1	399/535	75	49.94
BDISOB86R	<i>Enterobacteraerogenes</i>	KM503142.1	444/483	92	45.75
BDISOB30R	<i>Pseudochrobactrum asaccharolyticum</i>	MK100767.1	166/177	94	47.73
BDISOB92R	<i>Pseudomonas fluorescens</i>	KJ027533.1	29/29	100	45.44
BDISOB178R	<i>Serratia marcescens</i>	MN691653.1	635/679	94	45.91
BDISOB11R	<i>Pseudochrobactrumsaccharolyticum</i>	MK377096.1	770/827	93	40.00
BDISOB21R	<i>Stenotrophomonas maltophilia</i>	MN173472.1	994/1084	92	38.42
BDISOB24R	<i>Pseudochrobactrumsaccharolyticum</i>	FJ950551.1	994/1084	92	36.55
BDISOB23R	<i>Pseudochrobactrum asaccharolyticum</i>	KC456600.1	1082/1122	96	32.46
BDISOB14R	<i>Pseudochrobactrum asaccharolyticum</i>	KC456600.1	535/541	99	20.83

Table 3.
List of antagonistic bacterial isolates identified by homology search of sequences of 16SrDNA by BLAST program obtained from plant samples collected in boro season 2019.

Isolate ID	Closest relatives	Accession no.	Alignment	Homology	Growth inhibition of <i>X. oryzae</i> pv. <i>oryzae</i> (%)
BDISOB70R	<i>Serratia marcescens</i>	MG571677.1	239/300	80	52.38
BDISOB54R	<i>B. gladioli</i>	MH748601.1	1050/1108	95	61.54
BDISOB08R	<i>Serratia marcescens</i>	KU963569.1	100/114	88	59.31
BDISOB31R	<i>Serratia marcescens</i>	MN691926.1	929/990	94	59.17
BDISOB06R	<i>Serratia marcescens</i>	MG571677.1	111/127	87	59.26
BDISOB171R	<i>Alcaligenes faecalis</i>	MN513225.1	927/1094	85	57.37
BDISOB46R	<i>Serratia marcescens</i>	MF360051.1	545/630	87	55.53
BDISOB09R	<i>Serratia marcescens</i>	MN252007.1	171/185	92	55.92
BDISOB33R	<i>Serratia marcescens</i>	KJ535346.1	127/143	89	52.27
BDISOB11R	<i>Serratia marcescens</i>	MK806681.1	88/98	90	53.57
BDISOB36R	<i>Serratia marcescens</i>	MK961214.1	787/910	86	58.33
BDISOB07R	<i>Serratia nematodiphila</i>	MN691930.1	572/639	90	52.00
BDISOB172R	<i>B. aerophilus</i>	KY307912.1	874/1043	84	51.19
BDISOB12R	<i>Serratia marcescens</i>	MH074778.1	780/841	93	50.93

Table 4. List of antagonistic bacterial isolates identified by homology search of sequences of 16SrDNA by BLAST program obtained from plant samples collected in aman season 2019.

BDISOB145JoyP (*Serratia marcescens*), BDISOB07FarR (*Serratia nematodiphilia*), BDISOB12FarR (*Serratia marcescens*), BDISOB31MagR (*Serratia marcescens*), BDISOB46GopR (*Serratia marcescens*) and BDISOB70KusR (*Serratia marcescens*) were statistically similar as well as BDISOB172ThaR, BDISO1MymR, BDISO45PanP and BDISOB92FarR were statistically similar, apart from these all were under the group of statistically dissimilar (**Table 5** and **Figure 3**).

3.2.2 Siderophore production

Six bacterial isolates BDISOB222GaiR (*P. plecoglossicida*), BDISOB45PanP (*Bacillus paramycoides*), BDISOB01MymR (*B. amyloliquefaciens*), BDISOB04KhaP (*P. putida*), BDISOB05MymP (*P. putida*), BDISOB221GaiR (*Pseudomonas sp.*) exposed highest siderophore production. On the opposite, three of them which were BDISOB135SerP (*Bacillus sp.*), BDISO04DinP (*P. putida*) and BDISOB21ChaR (*S. maltophilia*) in the list of lowest siderophore production. Nine of them produced upper-moderate level of siderophore as well as thirteen isolates were released lower-moderate level of siderophore. Sixteen isolates those who produced siderophore including BDISOB219GaiR (*P. taiwanensis*), BDISOB186KusR (*Pseudomonas sp.*), BDISOB283KisR (*Pseudomonas fluorescens*), BDISOB198HabP (*Serratia plymuthica*), BDISOB54KhuR (*B. gladioli*) and BDISOB21ChaR (*S. maltophilia*) BDISOB198HabP (*Serratia plymuthica*), BDISOB148JoyP (*Serratia marcescens*), BDISOB158ChaR (*Serratia marcescens*), BDISOB148JoyP (*Serratia marcescens*), BDISOB145JoyP (*Serratia marcescens*), BDISOB07FarR (*Serratia nematodiphilia*), BDISOB12FarR (*Serratia marcescens*), BDISOB31MagR (*Serratia marcescens*), BDISOB46GopR (*Serratia marcescens*) and BDISOB70KusR (*Serratia marcescens*) were statistically similar and rest of them were statistically dissimilar (**Table 5** and **Figure 3**).

Treatments/ bacterial isolates	Name of bacteria	Indole acetic acid (IAA) (ng/ml)	Siderophore production (orange color halo zone) (mm)	Phosphate solubilization (clear halo zone) (mm)
Control	—	0.00 o	0.00 h	0.00f
BDISOB04KhaP	<i>P. putida</i>	44.88kl	28.67a	8.17c-e
BDISOB05MymP	<i>P. putida</i>	44.54 l	29.00a	14.33a
BDISOB219GaiR	<i>P. taiwanensis</i>	70.98c-g	20.13b	7.83 c-e
BDISOB221GaiR	<i>Pseudomonas sp.</i>	42.93 lm	28.00a	8.67c
BDISOB222GaiR	<i>P. plecoglossicida</i>	41.46 m	29.83a	11.67b
BDISOB258GaiR	<i>P. putida</i>	49.27j	14.50d-f	6.83de
BDISOB186KusR	<i>Pseudomonas sp.</i>	36.83n	19.50bc	6.50e
BDISOB283KisR	<i>Pseudomonas fluorescens</i>	43.90 l	18.33bc	8.33 cd
BDISO04DinP	<i>P. putida</i>	46.59 k	13.00 fg	8.17 c-e
BDISO45PanP	<i>Bacillus paramycoides</i>	81.46a	28.17a	14.33a
BDISO198HabP	<i>S. plymuthica</i>	71.22c-f	20.00b	7.50 c-e
BDISO135SerP	<i>Bacillus sp.</i>	67.80 h	10.83 g	8.33 cd
BDISO148JoyP	<i>S. marcescens</i>	71.22c-f	20.00b	7.50 c-e
BDISO1MymR	<i>B. amyloliquefaciens</i>	81.46a	29.83a	14.17a
BDISO145JoyP	<i>S. marcescens</i>	71.71c-e	13.17 fg	6.83de
BDISO158ChaR	<i>S. marcescens</i>	69.60e-h	20.00b	7.50 c-e
BDISOB37KhaR	<i>Pseudochrobactrum asaccharolyticum</i>	69.93d-g	14.33d-f	8.33 cd
BDISOB16CumR	<i>Pseudochrobactrum asaccharolyticum</i>	61.46i	16.50c-e	8.17 c-e
BDISOB92FarR	<i>Pseudomonas fluorescens</i>	82.68a	0.00 h	7.50 c-e
BDISOB21ChaR	<i>S. maltophilia</i>	78.78b	11.17 g	7.00 c-e
BDISOB17CumR	<i>Limnolyngbya circumcreta</i>	68.93gh	18.33bc	7.67 c-e
BDISOB15CumR	<i>Pseudochrobactrum asaccharolyticum</i>	70.27c-g	18.06bc	8.17 c-e
BDISOB86FarR	<i>E. aerogenes</i>	68.93 h	18.33bc	7.33 c-e
BDISOB30ChaR	<i>Pseudochrobactrum asaccharolyticum</i>	69.27f-h	18.06bc	8.17 c-e
BDISOB07FarR	<i>S. nematodiphila</i>	71.22c-f	13.50e-g	7.50 c-e
BDISOB12FarR	<i>S. marcescens</i>	72.22c	20.17b	6.83de
BDISOB31MagR	<i>S. marcescens</i>	70.89c-g	17.50b-d	7.50 c-e
BDISOB36MagR	<i>S. marcescens</i>	71.55c-e	20.00b	7.33 c-e
BDISOB46GopR	<i>S. marcescens</i>	71.89 cd	20.00b	7.17 c-e
BDISOB54KhuR	<i>B. gladioli</i>	77.56b	18.33bc	7.33 c-e
BDISOB70KusR	<i>S. marcescens</i>	71.22c-f	20.00b	6.83de
BDISOB172ThaR	<i>B. aerophilus</i>	81.71a	20.17b	8.00 c-e
Level of significance		*	*	*
LSD	—	1.839	3.101	1.702
CV (%)	—	1.78	10.34	12.88

* indicated 5% level of significance.
Values in columns followed by the same letters indicate no significant differences.

Table 5.
Growth promoting determinants produced by different bacterial isolates antagonistic to *X. oryzae* pv. *oryzae*.

3.2.3 Phosphate solubilization

Among all bacterial isolates three of them those were BDISOB05MymP (*P. putida*), BDISOB45PanP (*Bacillus paramycoides*) and BDISOB01MymR (*B. amyloliquefaciens*) manifested supreme amount of phosphate solubilization activity. Whereas, another three of them which were BDISOB186KusR (*Pseudomonas sp.*), BDISOB258GaiR (*P. putida*) and BDISOB70KusR (*Serratia marcescens*) showed lowest phosphate solubilization activity. Except highest and lowest phosphate solubilization producing isolates rest of them were showed moderate type activity. In this case, a noticeable differences were observed that except two isolates BDISOB221GaiR and BDISOB222GaiR all other isolates are statistically similar. The others bacteria in case of phosphate solubilization were statistically similar (Table 5 and Figure 4).

3.3 Plant growth promotion by bacterial isolates antagonistic to *X. oryzae* pv. *oryzae*

Based on the growth inhibition of *X. oryzae* pv. *oryzae* by these antagonistic bacterial species, 32 bacterial isolates were selected for plant growth promotion assay and for subsequent assessment of their net house and field performances. Different plant growth promoting bacterial antagonists enhanced the root length, shoot length and vigor index at 14, 21 and 28 DAS (Table 6). Among 32 bacterial

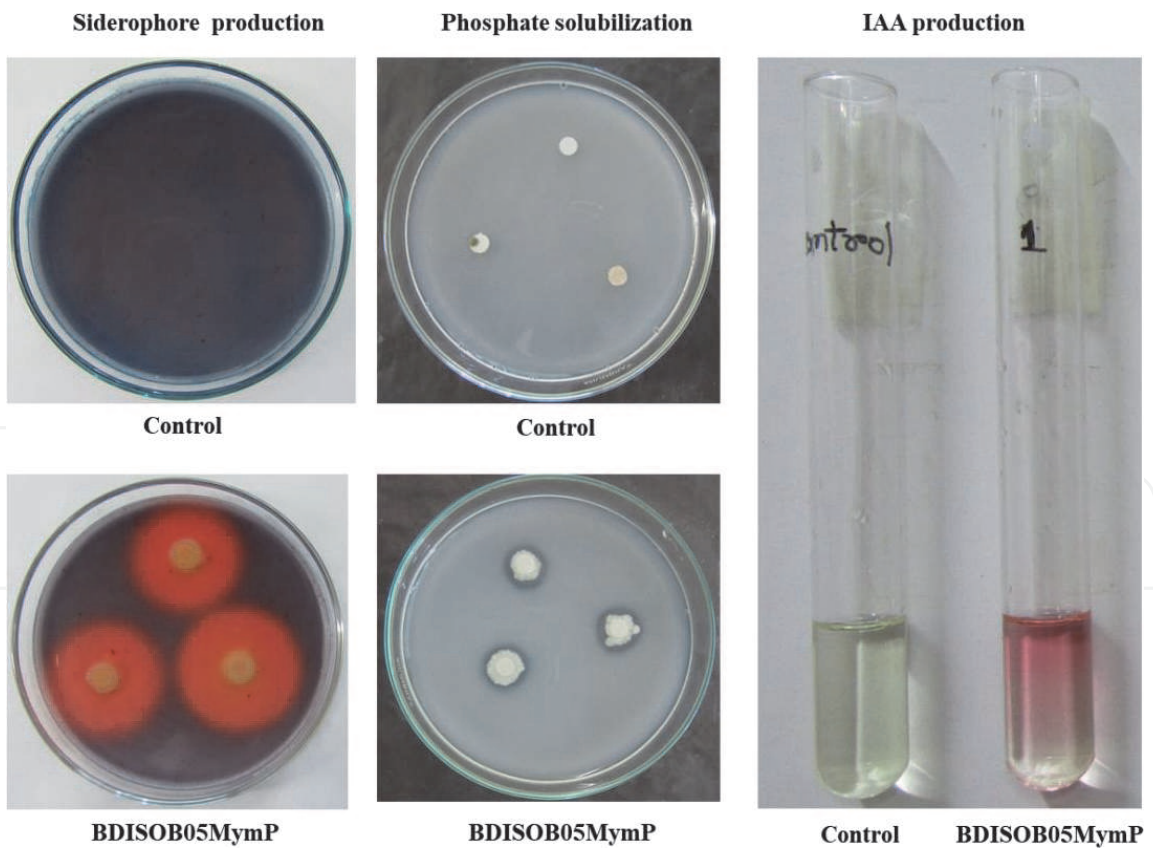


Figure 4. Representative photographs showing the assessment of different plant growth promoting determinants. Siderophore production: antagonistic bacterial isolates showed positive siderophore production activity as indicated by orange halo zone around bacterial colony on CAS agar plates, phosphate solubilization: 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 and indole acetic acid (IAA) production: IAA activity by different antagonistic bacterial isolates indicated by the presence of pink color when bacterial culture supernatant mixed with Salkowski's reagent. BDISOB05P: isolate from Mymensingh.

Treatments		Root length (cm)			% Increase of vigor index over control			Shoot length (cm)			% Increase of root length over control			Vigor index			% Increase of shoot length over control		
		Days after sowing (DAS)																	
		14	21	28	14	21	28	14	21	28	14	21	28	14	21	28	14.00	21.00	28.00
Control		6.76	9.20	11.28	0	0.00	0.00	10.72	11.97	17.23	0.00	0.00	0.00	1316.32	2046.56	2449.34	0.00	0.00	0.00
BDISOB04P	<i>P. putida</i>	9.12	12.31	13.20	34.93	33.77	17.05	12.37	16.77	23.07	15.40	40.11	33.89	1697.18	2306.48	2877.95	28.93	12.70	17.50
BDISOB05P	<i>P. putida</i>	8.23	12.22	12.84	21.85	32.83	13.80	12.37	16.53	18.32	15.40	38.16	6.29	1634.27	2549.46	2658.42	24.15	24.57	8.54
BDISOB219R	<i>P. taiwanensis</i>	8.69	12.22	12.58	28.56	32.83	11.55	12.40	16.53	18.88	15.71	38.16	9.57	1869.68	2549.46	2790.04	42.04	24.57	13.91
BDISOB221R	<i>Pseudomonas sp.</i>	8.43	11.13	11.30	24.81	21.01	0.18	11.90	15.65	19.53	11.04	30.78	13.35	1647.00	2169.45	2497.50	25.12	6.00	1.97
BDISOB222R	<i>P. plecoglossicida</i>	10.63	14.95	16.23	57.38	62.50	43.91	15.12	21.15	27.85	41.06	76.74	61.61	2360.42	3309.17	4040.97	79.32	61.69	64.98
BDISOB258R	<i>P. putida</i>	9.12	13.04	13.37	34.93	41.78	18.56	12.37	17.60	23.42	15.40	47.08	35.88	1697.18	2420.82	2906.41	28.93	18.29	18.66
BDISOB186R	<i>Pseudomonas sp.</i>	8.12	11.75	13.50	20.13	27.75	19.68	12.00	17.38	22.32	11.98	45.26	29.52	1595.92	2311.51	2841.72	21.24	12.95	16.02
BDISOB283R	<i>Pseudomonas fluorescens</i>	10.90	14.87	16.11	61.32	61.59	42.79	14.68	21.22	29.65	37.01	77.30	72.05	2285.44	3223.44	4087.60	73.62	57.51	66.89
BDISOB04P	<i>P. putida</i>	7.72	12.42	12.84	14.21	35.00	13.80	11.88	17.37	18.32	10.89	45.13	6.29	1672.53	2541.80	2658.42	27.06	24.20	8.54
BDISOB45P	<i>Bacillus paramycoides</i>	10.32	14.25	15.63	52.69	54.89	38.59	14.18	21.73	30.33	32.35	81.62	76.02	2237.67	3286.48	4198.29	69.99	60.59	71.41
BDISOB198P	<i>S. plymuthica</i>	8.65	11.38	12.33	28.02	23.73	9.34	11.43	13.35	20.07	6.69	11.56	16.44	1687.00	2127.07	2689.20	28.16	3.93	9.79
BDISOB135P	<i>Bacillus sp.</i>	7.82	11.45	12.05	15.69	24.46	6.83	12.90	15.53	20.05	20.37	29.81	16.34	1788.54	2329.56	2418.03	35.87	13.83	−1.28
BDISOB148P	<i>S. marcescens</i>	8.65	11.38	13.36	28.02	23.73	18.41	11.43	13.35	20.57	6.69	11.56	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82
BDISOB01R	<i>B. amyloliquefaciens</i>	8.33	11.38	13.42	23.33	23.73	18.94	12.72	13.35	23.65	18.66	11.56	37.23	1810.30	2127.07	3187.73	37.53	3.93	30.15
BDISOB145P	<i>S. marcescens</i>	8.65	11.38	13.36	28.02	23.73	18.41	11.43	13.35	20.57	6.69	11.56	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82
BDISOB158R	<i>S. marcescens</i>	8.65	11.38	13.36	28.02	23.73	18.41	11.43	13.35	20.57	6.69	11.56	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82
BDISOB37R	<i>P. asaccharolyticum</i>	8.13	12.66	12.33	20.37	37.61	9.34	12.18	16.52	20.07	13.69	38.02	16.44	1632.11	2324.41	2689.20	23.99	13.58	9.79

Treatments		Root length (cm)						% Increase of vigor index over control		Shoot length (cm)			% Increase of root length over control			Vigor index			% Increase of shoot length over control		
		Days after sowing (DAS)																			
BDISOB16R	<i>Pseudochractrum asaccharolyticum</i>	8.34	11.95	12.12	23.38	29.89	7.42	11.57	18.52	24.45	7.93	54.74	41.88	1585.63	2528.73	3071.60	20.46	23.56	25.41		
BDISOB92R	<i>Pseudomonas fluorescens</i>	7.10	13.06	12.38	5.08	41.92	9.78	12.02	15.87	20.28	12.19	32.59	17.70	1587.24	2429.56	2613.33	20.58	18.71	6.70		
BDISOB21R	<i>S. marcescens</i>	8.65	11.62	13.52	28.02	26.30	19.86	11.43	12.50	19.43	6.69	4.46	12.77	1687.00	1792.92	2449.53	28.16	−12.39	0.01		
BDISOB17R	<i>Limnolyngbya circumcreta</i>	7.10	11.45	13.36	5.08	24.46	18.41	12.02	15.53	20.57	12.19	29.81	19.38	1587.24	2329.56	2567.37	20.58	13.83	4.82		
BDISOB15R	<i>P. asaccharolyticum</i>	8.13	12.66	12.33	20.37	37.61	9.34	12.18	16.52	20.07	13.69	38.02	16.44	1632.11	2324.41	2689.20	23.99	13.58	9.79		
BDISOB86R	<i>E. aerogenes</i>	8.13	12.66	12.33	20.37	37.61	9.34	12.18	16.52	20.07	13.69	38.02	16.44	1632.11	2324.41	2689.20	23.99	13.58	9.79		
BDISOB30R	<i>P. asaccharolyticum</i>	8.13	12.66	12.33	20.37	37.61	9.34	12.18	16.52	20.07	13.69	38.02	16.44	1632.11	2324.41	2689.20	23.99	13.58	9.79		
BDISOB07R	<i>S. nematodiphila</i>	8.65	11.38	13.36	28.02	23.73	18.41	11.43	13.35	20.57	6.69	11.56	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82		
BDISOB12R	<i>S. marcescens</i>	8.65	11.38	13.36	28.02	23.73	18.41	11.43	13.35	20.57	6.69	11.56	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82		
BDISOB31R	<i>S. marcescens</i>	8.49	11.38	13.36	25.60	23.73	18.41	12.72	13.35	20.57	18.66	11.56	19.38	1604.39	2127.07	2567.37	21.88	3.93	4.82		
BDISOB36R	<i>S. marcescens</i>	8.65	11.38	13.36	28.02	23.73	18.41	11.43	13.35	20.57	6.69	11.56	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82		
BDISOB46R	<i>S. marcescens</i>	8.65	11.38	13.36	28.02	23.73	18.41	11.43	13.35	20.57	6.69	11.56	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82		
BDISOB54R	<i>B. gladioli</i>	7.87	11.62	13.52	16.43	26.30	19.86	11.77	12.50	19.43	9.80	4.46	12.77	1459.41	1792.92	2449.53	10.87	−12.39	0.01		
BDISOB70R	<i>S. marcescens</i>	8.65	11.38	13.36	28.02	23.73	18.41	11.43	13.35	20.57	6.69	11.56	19.38	1687.00	2127.07	2567.37	28.16	3.93	4.82		
BDISOB172R	<i>B. aerophilus</i>	8.40	12.35	12.84	24.32	34.24	13.80	13.00	16.92	22.92	21.31	41.36	32.98	1719.13	2351.09	2872.18	30.60	14.88	17.26		

Table 6.
Effect of different antagonistic bacteria on plant growth promotion of rice (cv. IR24).

isolates, the maximum vigor index (4198.29) was recorded in seedlings raised from seeds treated with BDISOB45PanR (*Bacillus paramycoides*) followed by BDISOB283R (*Pseudomonas fluorescens*) (4087.60), BDISOB222R (*P. plecoglossicida*) (4040.97) while the minimum (2418.03) vigor index was obtained in BDISOB135SheR (*Bacillus* sp.) followed by BDISOBP (*S. marcescens*) (2449.53) and BDISOB54R (*B. gladioli*) (2449.53) at 30 DAS. However, all the antagonistic bacterial isolates exhibited the increase of vigor index ranged by 0.01 to 71.41. This result implies that some of the selected antagonistic bacterial isolates have the potentiality in enhancing plant growth.

3.4 Plant growth promotion by different bacterial isolates antagonistic to *Xanthomonas oryzae* pv. *oryzae*

3.4.1 Fresh shoot weight

At 28 days after sowing the highest shoot weight (2260 mg) was recorded in plants raised from the seed treated with the bacterial isolate BDISOB01MymR followed by the bacterial isolates BDISOB05MymP (2250 mg), BDISOB45PanP (2173 mg), BDISOB04DinP (2033 mg), BDISOB86FarR (2033 mg), BDISOB07FarR, (2033 mg) BDISOB283KisR (1950 mg). But the lowest shoot weight was observed in control (untreated seed) (933 mg) Rest of the isolates were showed moderate fresh shoot weight. Among all bacterial isolates seventeen were statistically similar and others denoted statistically dissimilar (**Table 7**).

3.4.2 Dry shoot weight

At 28 days after sowing the highest shoot weight (546 mg) was recorded in plants raised from the seed treated with the bacterial isolate BDISOB01Mym) followed by the bacterial isolates BDISOB04DinP (473mgmg), BDISOB04KhaP (470 mg), BDISOB92Far (466 mg), BDISOB222GaiR (443 mg) were statistically similar Whereas, the lowest (260 mg) was reorded in the plants raised from untreated seed followed by the plants sprayed with [Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)] (313 mg), BDISOB172ThaR (266 mg), BDISOB07FarR (270 mg), BDISOB86FarR (273 mg), BDISOB70KusR (276 mg), BDISOB54KhuRwere statistically similar. On the otherhand, the plants raised from the seed treated with the bacterial isolatesBDISOB21ChaR (376 mg), BDISOB186KusR (330 mg), BDISOB219GaiR (373 mg), BDISOB21ChaR (376 mg) were statistically similar (**Table 7**).

3.4.3 Fresh root weight

At 28 days after sowing the highest rootweight (1350 mg) was recorded in plants raised from the seed treated with the bacterial isolate BDISOB45PanPfollowed by the bacterial isolates BDISOB05MymP (1316 mg), BDISOB21ChaR (1306 mg) BDISOB15CumR (1256 mg), BDISOB01MymR (1253 mg), BDISOB92Far (1246 mg), BDISOB16CumR (1213 mg) were statistically similar Whereas, the lowest (830 mg) was recorded in the plants raised from untreated seed followed by thebacterial isolate BDISOB219GaiR (983 mg), plants sprayed with [Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)] (1016 mg), BDISOB30ChaR (1080 mg). Other bacterial isolates were statistically similar (**Table 7**).

Treatment	Isolate ID		Fresh shoot weight (mg)	Dry shoot weight (mg)	Fresh root weight (mg)	Dry root weight (mg)
To	Control	—	933.33 k	333.33d-g	830.00 g	170.00 l
T1	Positive control	—	1300.00j	360.00 cd	1016.67ef	220.00jk
T2	BDISOB04KhaP	<i>P. putida</i>	1693.33f-i	470.00a	1166.67a-f	403.33b
T3	BDISOB05MymP	<i>P. putida</i>	2250.00ab	450.00a	1316.67ab	416.67b
T4	BDISOB219GaiR	<i>P. taiwanensis</i>	1816.67d-i	410.00b	983.33 fg	246.67 hi
T5	BDISOB221GaiR	<i>Pseudomonas sp.</i>	1533.33ij	293.33 h	1113.33c-f	240.00ij
T6	BDISOB222GaiR	<i>P. plecoglossicida</i>	1883.33c-h	443.33a	1116.67c-f	440.00a
T7	BDISOB258GaiR	<i>P. putida</i>	1666.67f-i	323.33e-h	1166.67a-f	220.00jk
T8	BDISOB186KusR	<i>Pseudomonas sp.</i>	1633.33f-i	330.00d-g	1133.33b-f	233.33i-k
T9	BDISOB283KisR	<i>Pseudomonas fluorescens</i>	1950.00a-f	320.00e-h	1116.67c-f	266.67 h
T10	BDISO04DinP	<i>P. putida</i>	2033.33a-e	473.33a	1120.00b-f	246.67 hi
T11	BDISO45PanP	<i>Bacillus paramycoides</i>	2173.33a-c	326.67e-g	1350.00a	343.33d-f
T12	BDISO198HabP	<i>S. plymuthica</i>	1660.00f-i	350.00c-f	1093.33d-f	326.67 fg
T13	BDISO135SerP	<i>Bacillus sp.</i>	1766.67d-i	336.67d-g	1133.33b-f	323.33 fg
T14	BDISO148JoyP	<i>S. marcescens</i>	1693.33f-i	320.00e-h	1100.00b-f	313.33 g
T15	BDISO1MymR	<i>B. amyloliquefaciens</i>	2260.00a	346.67c-g	1253.33a-d	450.00a
T16	BDISO145JoyP	<i>S. marcescens</i>	1950.00a-f	313.33 gh	1136.67b-f	240.00ij
T17	BDISO158ChaR	<i>S. marcescens</i>	1763.33d-i	293.33 h	1180.00a-f	246.67 hi
T18	BDISOB37KhaR	<i>P. asaccharolyticum</i>	1686.67f-i	363.33 cd	1190.00a-e	226.67i-k
T19	BDISOB16CumR	<i>P. asaccharolyticum</i>	1730.00e-i	406.67b	1213.33a-e	230.00i-k
T20	BDISOB92FarR	<i>Pseudomonas fluorescens</i>	1933.33b-g	466.67a	1246.67a-d	326.67 fg
T21	BDISOB21ChaR	<i>S. maltophilia</i>	1800.00d-i	376.67c	1306.67a-c	336.67ef

Treatment	Isolate ID		Fresh shoot weight (mg)	Dry shoot weight (mg)	Fresh root weight (mg)	Dry root weight (mg)
T22	BDISOB17CumR	<i>Limnolyngbya circumcreta</i>	2066.67a-d	363.33 cd	1220.00a-d	310.00 g
T23	BDISOB15CumR	<i>P. asaccharolyticum</i>	1866.67c-h	346.67c-g	1256.67a-d	363.33 cd
T24	BDISOB86FarR	<i>E. aerogenes</i>	2033.33a-e	326.67e-g	1170.00a-f	353.33c-e
T25	BDISOB30ChaR	<i>P. asaccharolyticum</i>	1733.33e-i	363.33 cd	1080.00d-f	266.67 h
T26	BDISOB07FarR	<i>S. nematodiphila</i>	2033.33a-e	316.67f-h	1146.67b-f	373.33c
T27	BDISOB12FarR	<i>S. marcescens</i>	1816.67d-i	320.00e-h	1113.33c-f	310.00 g
T28	BDISOB31MagR	<i>S. marcescens</i>	1580.00 h-j	323.33e-h	1116.67c-f	236.67i-k
T29	BDISOB36MagR	<i>S. marcescens</i>	1613.33 g-i	376.67c	1120.00b-f	230.00i-k
T30	BDISOB46GopR	<i>S. marcescens</i>	1700.00f-i	353.33c-e	1123.33b-f	246.67 hi
T31	BDISOB54KhuR	<i>B. gladioli</i>	1513.33ij	353.33c-e	1126.67b-f	213.33 k
T32	BDISOB70KusR	<i>S. marcescens</i>	1566.67 h-j	363.33 cd	1113.33c-f	233.33i-k
T33	BDISOB172ThaR	<i>B. aerophilus</i>	1510.00ij	360.00 cd	1160.00a-f	246.67 hi
Level of significance	—	—	*	*	*	*
LSD	—		270.7	27.85	161.9	20.58
CV	—		9.39	4.73	8.65	4.36

* indicated 5% level of significance.
Values in columns followed by the same letters indicate no significant differences.

Table 7.
Plant growth promotion by different bacterial isolates antagonistic to *X. oryzae* pv. *oryzae*.

Isolate ID	Name of bacteria	Lesion length* (mm)	Reduction of lesion length (%)
Control	—	23.67a	0
Positive control	—	6.33b-d	73.31
BDISOB04P	<i>P. putida</i>	1.50ij	92.61
BDISOB05P	<i>P. putida</i>	1.00j	95.71
BDISOB219R	<i>P. taiwanensis</i>	5.67c-f	76.04
BDISOB221R	<i>Pseudomonas</i> sp.	5.00d-g	78.85
BDISOB222R	<i>P. plecoglossicida</i>	0.83j	96.56
BDISOB258R	<i>P. putida</i>	1.50ij	93.61
BDISOB186R	<i>Pseudomonas</i> sp.	5.33c-g	77.38
BDISOB283R	<i>Pseudomonas fluorescens</i>	1.33ij	94.38
BDISOB04P	<i>P. putida</i>	5.83c-e	75.25
BDISOB45R	<i>Bacillus paramycoides</i>	2.00ij	91.55
BDISOB198P	<i>S. plymuthica</i>	5.83c-e	52.36
BDISOB135R	<i>Bacillus</i> sp.	2.83hi	88.08
BDISOB148P	<i>Serratia marcescens</i>	5.83c-e	75.69
BDISOB1R	<i>B. amyloliquefaciens</i>	2.33ij	90.16
BDISOB145P	<i>S. marcescens</i>	6.83bc	71.12
BDISOB158R	<i>S. marcescens</i>	6.83bc	50.14
BDISOB37R	<i>P. asaccharolyticum</i>	5.33c-g	77.44
BDISOB16R	<i>P. asaccharolyticum</i>	5.17d-g	78.01
BDISOB92R	<i>Pseudomonas fluorescens</i>	4.50e-g	80.85
BDISOB21R	<i>S. marcescens</i>	2.17ij	93.80
BDISOB17R	<i>Limnolyngbyacir cumcreta</i>	4.00 gh	83.33
BDISOB15R	<i>P. asaccharolyticum</i>	5.33c-g	54.03
BDISOB86R	<i>E. aerogenes</i>	4.00 gh	83.33
BDISOB30R	<i>P. asaccharolyticum</i>	4.33e-h	81.64
BDISOB07R	<i>S. nematodiphila</i>	4.00 gh	83.33
BDISOB12R	<i>S. marcescens</i>	4.00 gh	83.06
BDISOB31R	<i>S. marcescens</i>	5.00d-g	78.97
BDISOB36R	<i>S. marcescens</i>	5.83c-e	75.49
BDISOB46R	<i>S. marcescens</i>	4.17f-h	82.28
BDISOB54R	<i>B. gladioli</i>	4.17f-h	82.41
BDISOB70R	<i>S. marcescens</i>	2.83hi	87.96
BDISOB172R	<i>B. aerophilus</i>	7.50b	68.21
Level of significance		*	
CV (%)		16.80	

* indicated 5% level of significance.
Values in columns followed by the same letters indicate no significant differences.

Table 8.
Effect of some selected antagonistic bacterial isolates on the reduction of lesion length in susceptible check cultivar (IR24) caused by *X. oryzae* pv. *oryzae*.



Figure 5.
Reduction of lesion length by some selected antagonistic bacterial in susceptible check cultivar (IR24). Photographs were taken at 14 days after inoculation.. BDISOB04P (P. putida), BDISOB05P (P. putida), BDISOB219R (P. taiwanensis), BDISOB221R (Pseudomonas sp.), BDISOB222R (P. plecoglossicida), BDISOB258R (P. putida), BDISOB186R (Pseudomonas sp.), BDISOB283R (Pseudomonas fluorescens), BDISO04P (P. putida), BDISO45R (Bacillus paramycoides), BDISO198P (S. plymuthica), BDISO135R (Bacillus sp.), BDISO148P (S. marcescens), BDISOB01R (B. amyloliquefaciens), BDISO145P (S. marcescens), BDISO158R (S. marcescens), BDISOB37R (P. asaccharolyticum), BDISOB16R (P. asaccharolyticum), BDISOB92R (Pseudomonas fluorescens), BDISOB21R (S. maltophilia), BDISOB17R (Limnolyngbya circumcreta), BDISOB15R (P. asaccharolyticum), BDISOB86R (E. aerogenes), BDISOB30R (P. asaccharolyticum), BDISOB07R (Serratia nematodiphila), BDISOB12R (Serratia marcescens), BDISOB31R (Serratia marcescens), BDISOB36R (Serratia marcescens), BDISOB46R (Serratia marcescens), BDISOB54R (B. gladioli), BDISOB70R (Serratia marcescens) and BDISOB172R (B. aerophilus).

3.4.4 Dry root weight

At 28 days after sowing the highest dry root weight (450 mg) was recorded in plants raised from the seed treated with the bacterial isolate BDISOB01MymR, BDISOB222GaiR (440 mg) followed by the bacterial isolates BDISOB05MymP (413 mg), BDISOB04KhaP (403 mg). Whereas, the lowest (170 mg) was recorded in the plants raised from untreated seed followed by the plants sprayed with [Bactroban (inducer) + SICOGREEN® (nutrient and hormonal solution) + Hemoxy (Copper fungicide)] (220 mg), BDISOB54KhuR (213 mg). Other bacterial isolates were statistically similar (Table 7).

3.5 Effect of some selected antagonistic bacterial isolates on the reduction of lesion length in susceptible check cultivar (IR24) caused by *X. oryzae* pv. *oryzae*

To evaluate the mechanisms of BB severity reduction by plant growth promoting antagonistic bacteria, susceptible check variety IR24 was used. The results of plant inoculation showed a significant reduction of lesion length in plants sprayed with formulated bacterial bioagents as compared with untreated control.

(Table 8). 96.56% reduction of lesion length was marked as highest spraying with BDISOB222R followed by BDISOB05P (95.71%), BDISOB283R (94.38%), BDISOB21R (93.80%), BDISOB258R (93.61%), BDISOB04P (92.61%), BDISO45P (91.55%) and BDISO1R (90.16%). The minimum (50.145%) reduction of lesion length were observed in plants sprayed with BDISO158R followed by BDISO198P (52.36%) and BDISOB15R (54.03%). Ten bacterial isolates were revealed upper-moderate level of lesion length reduction and eleven isolates were marked their place at lower-moderate level of lesion length reduction. However, all other bacterial isolates reduced lesion length significantly as compared with the untreated plants (Table 8 and Figure 5).

4. Discussion

Antagonistic bacterial isolates were identified mostly as different species of *Pseudomonas*, *Bacillus*, *Serratia* and *Delftia*. In a previous study, frequency of antagonistic bacteria on LB medium was low [62], but another study revealed that using different growth media such as King's B, and Gould's S1 and Nutrient Agar were effective for the isolation of higher number of antagonistic bacteria [63]. It was reported that some antagonistic bacteria such as *B. subtilis*, *B. amyloliquefaciens*, *B. valismortis*, *Streptomyces* sp., *P. chlororaphis* and *Acinetobacter baumannii* were identified based on 16S rRNA sequence analysis [64]. A number of bacteria from species *Alcaligenes*, *Arthobacter*, *Burkholderia*, *Alcaligenes*, *Arthobacter*, *Burkholderia*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter* and *Serratia* have been observed to develop plant growth. However, as bio control agents, isolates of *fluorescens*, *Pseudomonas*, and *Bacillus* have been the most exploited and studied [65–68]. Nowadays, antagonistic bacteria were also used for plant roots as a biological control infecting by numerous plant pathogens [26, 69]. Out of 300 bacterial isolates sixteen isolates of several species were evaluated *in vitro* and they exposed antagonistic activity to *X. oryzae* pv. *oryzae*. It was observed that 54.03% to 96.56% of lesion length was diminished when treating with antagonistic bacteria. These findings were identical to the reported by Monteiro *et al.* [63] because they also showed that BB pathogen was suppressed by antagonistic bacteria. According to Ranjbariyan *et al.* [70] who also experimented that three bacterial isolates

significantly acted higher growth inhibition of *X. oryzae* pv. *oryzae*. Antibiotics, enzymes like chitinases, glucanases, proteases, and siderophore produce directly or indirect mechanisms in which the antagonistic bacteria compete with the pathogen for a niche or nutrient sites [34].

Out of the 63 bacterial isolates, 48 bacterial species were found as positive for IAA (Indole Acetic Acid) production, all 63 bacterial species were found positive for siderophore production and 48 were found capable to solubilize insoluble phosphate. IAA also has been speculated to fasten the overall fitness of plant-microbe associations [57]. It was proved that numerous plant-associated bacteria have the ability to produce IAA by stimulating plant roots development and improving absorption of water and nutrients from soil [71, 72]. The IAA producing bacteria encouraged adventitious root formation, produced the greatest roots and shoots weight [73]. All 63 bacterial isolates were found to produce siderophore. When iron availability is in stress microorganism those who produce siderophore supplied Fe nutrition to enhance plant growth [74]. Siderophore also assists when it comes to the growth condition of shoots, roots as well as nutrition in plants [75]. Siderophore plays a crucial role in selecting a potential bioagent [76], besides, it has been considered as an alternative to ruinous pesticides effects [77]. The biological control mechanism depended on the role of siderophore as competitors for Fe in order to reduce Fe availability for the phytopathogen [78]. Siderophores produced by numerous bacteria had a significant role in the biocontrol and negatively affected the growth of several pathogens [78, 79]. Forty eight bacterial isolates showed the capability of phosphate solubilization. It has been also experimented that phosphate solubilizing bacteria (PSB) can also triggered plant growth promotion [80]. This PSB inoculants have been exploited as a possible alternative for phosphate fertilizers which is inorganic [81] and it also influences phosphate uptake and plant growth [82, 83]. It has also been documented that the application rates of phosphate fertilizers reduced to 50% by inoculating phosphate solubilizing microbes (PSM) added phosphate fertilizers reduced the disease incidence up to 50% [84].

Among the bacterial isolates, 32 were selected based on their antagonistic capability and growth promoting determinants. PGPB have significant impact in surging root length, vigor index and shoot length. Sakthivel *et al.* [85] and Mishra and Sinha [86] reported to enhance growth of rice seedling with bioagent application. Van Peer and Schippers [87] stated that shoot, root and fresh weight was raised for cucumber, lettuce, tomato and potato as a result of bacterization with *Pseudomonas* strains. The results of the present study depicts that the effect of plant growth promoting bacterial isolates on growth and vigor of rice plants was significantly higher than control. It has been reported that *P. fluorescens* and other plant growth promoting rhizobacteria can show antagonisms to potentially harmful bacterial pathogens and eventually those bacteria contribute to enhance plant growth [88]. Biological agents like plant growth promoting bacteria (PGPB) can be used as bio-fertilizer [89].

Forty eight bacterial species were found positive for phosphate solubilization out of 63 antagonistic bacterial species identified in this study. It has been reported that phosphate solubilizing bacteria (PSB) induced plant growth promotion [80]. Plant roots-associated PSB have been considered as one of the possible alternatives for inorganic phosphate fertilizers for promoting plant growth and yield [81]. Plant growth and phosphate uptake have increased in many crop species due to the results of PSB inoculants [82, 83]. 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 [84]. In sustainable agriculture, specific plant pathogens can be suppressed by biological agents such as plant growth promoting bacteria (PGPB) which can also be used as bio-fertilizer [89]. There are a plenty of PGPB strains that reported to suppress numerous of

plant pathogen, reduced disease incidence, triggered plant growth factor and provides nutrition for the growth of the plant [63, 90]. Thus, it has been considerable research interest in the potential use of antagonistic bacteria as PGPB [91, 92]. To evaluate plant-interaction with bacteria, such as endophytes, biocontrol agents, phytopathogens, and symbionts needs to be re-infection and development of those experimental strains in or on field grown plants [93]. Effective root colonization by fluorescent *Pseudomonas* spp. has been manifested to take an inevitable part in controlling plant pathogens as a biocontrol agent [94]. The significance of this study is that functionally characterized all antagonistic bacteria may be used for biocontrol of BB along with enhanced rice growth. Even though, *Pseudomonas* spp. are indigenous and involve in various rhizomicrobiomes but few of them have the ability to grow above 37°C and become opportunistic pathogens, thus predictable biosafety regulations are needed to implement this technology practically for field application [95]. In a nutshell, based on all results achieved from during this study, bacterial strains may be an effective bio-inoculant for controlling BB of rice by ensuring its biosafety aspects.

5. Conclusion

Thirty two potential bacterial isolates were identified belong to the genera mostly *Pseudomonas*, *Bacillus* and *Serratia* from rice phylloplane and rhizosphere among sixty three that inhibited the growth of *X. oryzae* pv. *oryzae* in *in vitro* significantly and were found positive for enhancing plant growth promotion by the production of plant growth promoting determinants viz. IAA, siderophore and phosphate solubilization. Formulated bacterial isolates can be viable in talcum powder for at least three months post formulation. Reduction of lesion length caused by *X. oryzae* pv. *oryzae* on susceptible cultivar IR24 by the formulated bacterial isolates primarily indicates their potentiality in controlling BB of rice. Patenting, registration, large scale formulation and commercialization of these PGP bacteria would be the next step of this work.

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Conflict of interest

There is no conflict of interest among the authors.

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