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# Identification of plant growth promoting antagonistic bacteria against *Xanthomonas oryzae* pv. *oryzae* in Bangladesh

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ABSTRACT

The purpose of this work was to isolate and identify the plant growth promoting bacteria from rice phylloplane and rhizosphere that antagonistic to *X. oryzae* pv. *oryzae*. Rice phylloplane and rhizosphere bacteria were isolated from the surface of rice leaves and stem as well as from the soil attached to the roots of rice plants, respectively. The antagonistic activity of these isolated bacteria was determined by dual culture method. The antagonistic bacterial isolates were identified by sequencing of 16SrDNA. The activities related to plant growth promotion were determined by Indole Acetic Acid (IAA) production, siderophore and phosphate solubilization assay.The promotion of plant growth was assessed by the determination of root length, shoot length and vigor index. Sixteen bacterial isolates were identified as antagonist to *X. oryzae* pv. *oryzae* out of 300 bacterial isolates by dual culture method. The maximum growth inhibition (33.5 mm) of *X. oryzae* pv. *oryzae* was recorded in plate inoculated with BDISOB05P while the minimum growth inhibition (5 mm) was recorded by BDISOB98P and BDISOB272R. The moderate growth inhibition was recorded in BDISOB241P, BDISOB16P, BDISOB306R, BDISOB242P, BDISOB220R, BDISOB04P, BDISOB258R, BDISOB219R, BDISOB221R, BDISOB275R, BDISOB283R and BDISOB61R. 16S rDNA sequencing was used to identify the bacterial isolates which were antagonistic. The bacterial isolates were identified BDISOB04P as *Pseudomonas putida*, BDISOB05P as *Pseudomonas putida*, BDISOB16P as *Bacillus* sp., BDISOB98P as *Stenotrophomonas maltophilia*, BDISOB241P as *Burkholderia* sp., BDISOB242P as *Burkholderia gladioli*, BDISOB219R as *Pseudomonas taiwanensis*, BDISOB220R as *Serratia* sp., BDISOB221R as *Pseudomonas* sp., BDISOB222R as *Pseudomonas plecoglossicida*, BDISOB258R as *Pseudomonas putida*, BDISOB272R as *Stenotrophomonas maltophilia*, BDISOB275R as *Pseudomonas putida*, BDISOB283R as *Pseudomonas fluorescens* and BDISOB306R as *P. putida*. Eight antagonistic bacterial isolates produce IAA, sixteen bacterial isolates were able to produce siderophore and nine bacterial isolates were found to show phosphate solubilizing capability. The results of plant growth promotion activities indicating that these bacterial isolates can increase the root growth, shoot growth and vigor index.

**Keywords:** Plant growth, antagonistic bacteria, *X. oryzae* pv. *oryzae*

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## 1 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). Among rice diseases, Bacterial Leaf Blight (BLB) caused by *X. oryzae* pv. *oryzae* considered as the most destructive disease occurs in all Agro Ecological Zones (AEZ) of Bangladesh and mostly in two rice growing seasons namely, Aman (June-July to November-December) and Boro (November-December to April-May) (Latif et al., 2011; Miah, 1973; Miah et al., 1985). Extensive cultivation of nitrogen-responsive modern rice cultivars is the reason for BLB to become one of the most devastating diseases of rice (Mew, 1993). It is also an important disease in most of the South and Southeast Asian countries (Sharma et al., 1991). The disease incurred over 50%, 60% and 30 % rice yield reduction in Japan (Soga, 1918), India (Srivastava et al., 1966) and Bangladesh (Shahjahan, 1993), respectively, in the severely diseased rice fields. The favorable conditions such as heavy rain, high humidity and temperature are considered to be responsible for creating higher incidence and severity of the disease (OCTA, 1970).

BLB appears in rice plant at different growth stages. The disease symptom mainly exhibits on leaf as leaf blight or either induces wilting of young plants, known as Kresek. Wounds or water pores are mainly the medium for *X. oryzae* pv. *oryzae* to easily invade the plant. Lesions with wavy edges commence from the leaf tip and margins. These water-soaked lesions coalesce and enlarge in size, turn yellow, and then ultimately dying of the plant (Ninoliu et al., 2006). To reduce the yield losses and avoid disease epidemics, various disease management strategies have taken in the past. Among those strategies, application chemicals has not been successful due to variation in sensitivity of pathogenic races towards applied chemicals. Moreover, planting resistant cultivars, is the most economical strategy as disease management, but these tactis have been partially successful because of the pathogen diversity. It has been reported the pathogenic variability of *X. oryzae* pv. *oryzae* in Bangladesh (Noda, 1996; Jalaluddin and Kashem, 1999). Twelve races of the *X. oryzae* pv. *oryzae* have been identified until 1995 in Bangladesh and the study indicated that some aggressive strains of Xoo occur in Bangladesh (Noda, 1996). Severe outbreak of BLB occurred in Bangladesh in Boro 2007-2008 and both hybrid and inbred varieties were affected. In current T. Aman 2017 Season, BLB outbreak is occurring in different regions of Bangladesh like each year which are documented in most of the daily newspapers.Cultural practices, chemical and biological control, disease forecasting, and, most importantly, host genetic resistance, typically major gene resistance, are commonly used control measures for BLB. But cultural practices are not found effective in all locations and its efficacy mainly depends on disease incidence records.

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). Using an integrated plant nutrient management system helps not only to improve crop yield but it also plays a major role in developing sustainable agricultural system for crop production and ultimately it helps to restore the balance of an healthy environment. The use of biological agents has not widely popular for controlling BLB even though biological control is an environmentally friendly and cost-effective substitute to chemical. The use of chemical pesticides and fertilizers has great impact on environmental pollution. Therefore, application of antagonistic plant growth promoting bacterial isolates would be an attractive alternative to decrease the use of it (Ali et al., 2010). In recent days, the use of antagonistic bacteria as biological control consider as the best alternative way to reduce the application of chemicals in field (Yang et al., 2007; Misk and Franco, 2011).

The main aim of our research was to identify and characterize naturally occurring plant bacteria associated with rice plants which could effectively inhibit the growth of bacterial leaf blight pathogen,*X. oryzae* pv. *oryzae in vitro* and to assess the plant growth promoting effects of these isolates.

## 2 Materials and Methods

### 2.1 Plant sample collection

The healthy rice plants with root system of different rice cultivars were collected from twenty districts representing 30 agro-ecological zones (AEZs) of Bangladesh from the vicinity of BLB infected rice plants. The plant samples took to the Laboratory and used immediately after collection while in the refrigerator roots system with soils were preserved for further isolation of antagonistic bacterial isolates.

## 2.2 Isolation and purification of bacteria

For bacteria isolation from phylloplane, 1 g of leaf and shoot samples was washed in phosphate buffer saline with continuous stirring for 10 min. Then 100 $\mu$L of the washed solution was spread in either LB agar or King's B agar plate sand at 28 °C the plates were incubated until the bacterial colonies were grown. For further isolation from rhizosphere, 1 g of fresh roots with adhered soils were stirred in sterile distilled water for 10 min. Then 20 $\mu$L 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.

## 2.3 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. *X. oryzae* pv. *oryzae* strain was grown in NBY agar plates for 48 h and after that *X. oryzae* pv. *oryzae* strain was suspended in sterile distilled water up to cell density of $5\times10^8$ CFU mL$^{-1}$. Bacterial cell suspension was then spread in NBY agar plates using a cotton swab. The possible antagonistic bacterial cell suspension ($5\times10^8$ 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 *X. oryzae* pv. *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 *X. oryzae* pv. *oryzae* was estimated by using the formula of (Vincent, 1947) as given bellow:

$$I = \frac{T - C}{C} \times 100 \quad (1)$$

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

## 2.4 Molecular based identification

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

### 2.4.1 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 $\mu$L 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′) 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 was visualized by electrophoresis on 1.0% agarose gel containing 0.5% of ethidium bromide.

### 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′) 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 3730xl 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.

### 2.4.4 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).

### 2.4.5 Analysis of data

The collected data on radial mycelial growth and ANOVA were analyzed statistically by using MSTAT-C 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.

### 2.5 Assessment of plant growth promoting determinants

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, IAA production and phosphate solubilization.

### 2.6 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 indicate qualitatively that isolate produced IAA. Formation of pink colour indicated the presence of indoles (Gordon and Weber, 1951).

### 2.7 Assessment of siderophore production

Siderophore production by antagonistic bacterial isolates were tested qualitatively as described by Alexander and Zuberer (1991). Five microliter of antagonistic bacterial cell suspension ($5 \times 10^8$ 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 (1.21 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 $K_2HPO_4$, 0.5 g NaCl and 1.0 g $NH_4Cl$. 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 15 g of agar then cooled to 50 °C. Solution 3 contained 2 g glucose, 2 g mannitol, 493 mg $MgSO_4.7H_2O$, 11 mg $CaCl_2$, 1.17 mg $MnSO_4.2H_2O$, 1.4 mg $H_3BO_3$, 0.04 mg $CuSO_4.5H_2O$, 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.

### 2.8 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 ($5\times10^8$ 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 (10 g $L^{-1}$), $Ca_3(PO_4)_2$ (5 g $L^{-1}$), $MgCl_2.6H_2O$ (5 g $L^{-1}$), $MgSO_4.H_2O$ (0.25 g $L^{-1}$), KCl (0.2 g $L^{-1}$), $(NH_4)_2SO_4$ (0.1 g $L^{-1}$), 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.

### 2.9 Plant growth promotion assessment

Rice seeds (cv. BRRI dhan49) were surface sterilized and dried. Then the sterilized rice seeds were dipped in antagonistic bacterial suspension for 2 h with shaking. After shaking, the rice seeds were shed dried and sown in the plastic pots. Fifty seeds were sown and three replications were maintained. After germination, seedlings were uprooted at 7 DAS, 14 DAS and 30 DAS. Then the root length, shoot length and vigor index [= (root length + shoot length) × germination percentage] were measured.

## 3 Results

### 3.1 Antagonistic activity of the isolates

A total of 300 bacterial isolates obtained from rice phylloplane and rhizosphere (soils adhered to the roots). These bacterial isolates were screened to identify antagonist ability to *X. oryzae* pv. *oryzae*. Out of 300, only 16 bacterial antagonists showed in vitro antagonistic activity against *X. oryzae* pv. *oryzae* strain tested. The results revealed that all bacterial isolates inhibited the growth of *X. oryzae* pv. *oryzae* by 95%. (Fig. 1). All 16 identified bacteria were tested for potential antagonistic activities against *X. oryzae* pv. *oryzae* strains visually by dual culture assay. The growth of *X. oryzae* pv. *oryzae* was inhibited by all selected bacteria by different extents and inhibition was significant in comparison with relative controls. Comparative results of the quantitative assay of the inhibitory activity of identified antagonist bacteria

against *X. oryzae* pv. *oryzae* are shown as both mean and percent growth inhibition for each bacterial isolates. The average inhibition zone of the antagonistic bacteria against *X. oryzae* pv. *oryzae* in varied from 5 to 33.5 mm. Among all the identified isolates, maximum inhibition was 33.5 mm which was showed by BDISOB05P isolate while BDISOB98P and BDISOB272R isolates exhibited minimum inhibition (5 mm). The moderate level of inhibition varied from 10 to 20.5 mm was recorded in other bacterial isolates. The percent growth inhibition of 16 isolates were recorded and ranged from 28.56 to 76.14% (Fig. 2a & Fig. 2b).

## 3.2 Identification of the isolates

Sixteen bacterial isolates 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 phylloplane and rhizosphere of rice plant were bacteria (Fig. 3). 16S rDNA gene sequence analysis confirmed that all identified antagonistic bacteria belonged to the six different genera *viz. Pseudomonas*, *Bacillus*, *Stenotrophomonas*, *Burkholderia*, *Serratia* and *Delftia*. Among these 16 isolates, 9 isolates (BDISOB04P, BDISOB05P, BDISOB219R, BDISOB221R, BDISOB222R, BDISO258BR, BDISOB275R, BDISOB283R and BDISOB306R) were determined and aligned to *Pseudomonas* sequences deposited in GenBank. On the other hand, bacterial isolate BDISOB16P showed 95% identity with *Bacillus* sp. while rest of the 5 isolates BDISOB98P, BDISOB241P, BDISOB242P, BDISOB220R and BDISOB61R showed 96%, 94%, 96%,81% and 84% identity with *Stenotrophomonas*, *Burkholderia*, *Serratia*, respectively. The bacterial isolates were identified BDISOB04P as *Pseudomonas putida*, BDISOB05P as *Pseudomonas putida*, BDISOB16P as *Bacillus* sp., BDISOB98P as *Stenotrophomonas maltophilia*, BDISOB241P as *Burkholderia* sp., BDISOB242P as *Burkholderia gladioli*, BDISOB219R as *Pseudomonas taiwanensis*, BDISOB220R as *Serratia* sp., BDISOB221R as *Pseudomonas* sp., BDISOB222R as *Pseudomonas plecoglossicida*, BDISOB258R as *Pseudomonas putida*, BDISOB272R as *Stenotrophomonas maltophilia*, BDISOB275R as *Pseudomonas putida*, BDISOB283R as *Pseudomonas fluorescens* and BDISOB306R as *Pseudomonas putida* (Table 1)

## 3.3 Plant growth promotion

### 3.3.1 Indole acetic acid production (IAA)

Out of the 16 bacterial isolates, 8 isolates were found to produce IAA as indicated by the production of pink color in presence of Salkowski's reagent (Fig. 4a). The results also showed that out of 8 isolates, 6 isolates [BDISOB04P (*Pseudomonas putida*), BDISOB05P (*Pseudomonas putida*) , BDISOB219R (*Pseudomonas taiwanensis*) , BDISOB221R (*Pseudomonas* sp), BDISOB222R (*Pseudomonas plecoglossicida*), BDISOB258R (*Pseudomonas putida*)] producing IAA belonged to *Pseudomonas* spp. (Table 2).

### 3.3.2 Siderophore production

In this study we identified 16 bacterial isolates and all of them was found to produce siderophore as indicated by the production of orange yellow color on CAS agar (Fig. 4b & Table 2). 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 meanings '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).

### 3.3.3 Phosphate solubilization

Out of 16 bacterial isolates, 9 bacterial isolates [BDISOB04P (*Pseudomonas putida*), BDISOB05P (*Pseudomonas putida*), BDISOB241P (*Burkholderia* sp.), BDISOB242P (*Burkholderia gladioli*), BDISOB219R (*Pseudomonas taiwanensis*), BDISOB220R (*Serratia* sp.), BDISOB222R (*Pseudomonas plecoglossicida*), BDISOB258R (*Pseudomonas putida*), BDISOB61R (*Delftia tsuruhatensis*)] 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. 4c & Table 2). The results also revealed that among 9 phosphate solubilizing bacterial isolates, 5 isolates [BDISOB04P (*Pseudomonas putida*), BDISOB05P (*Pseudomonas putida*), BDISOB219R (*Pseudomonas taiwanensis*), BDISOB222R (*Pseudomonas plecoglossicida*), BDISOB258R (*Pseudomonas putida*)] belonged to *Pseudomonas* spp. (Table 2).

### 3.3.4 Plant growth

Different plant growth promoting bacterial antagonists has impact in increasing root length, shoot length and vigour index. Among 16 bacterial isolates, 7 bacterial isolates [BDISOB04P (*Pseudomonas putida*), BDISOB05P (*Pseudomonas putida*), BDISOB219R (*Pseudomonas taiwanensis*), BDISOB221R (*Pseudomonas* sp.), BDISOB222R (*Pseudomonas plecoglossicida*), BDISOB258R (*Pseudomonas putida*), BDISOB283R (*Pseudomonas fluorescens*)] were selected based on their growth promoting activity compared to control. After 30 DAS, the maximum root length was 12.78 cm recorded in plants grown from seed treated with BDISOB283R (*Pseudomonas fluorescens*), whereas the minimum root length was 10.20 cm recorded in plants

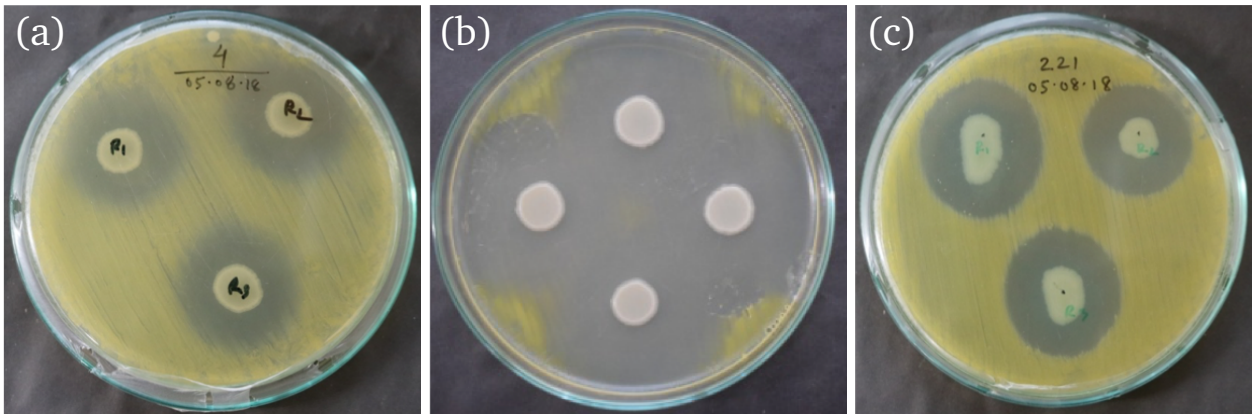


Figure 1. Representative photographs of *in vitro* growth inhibition of *X. oryzae* pv. *oryzae* by different potential bacterial isolates (a) BDISOB04P, (b) BDISOB05P, and (c) BDISOB221R

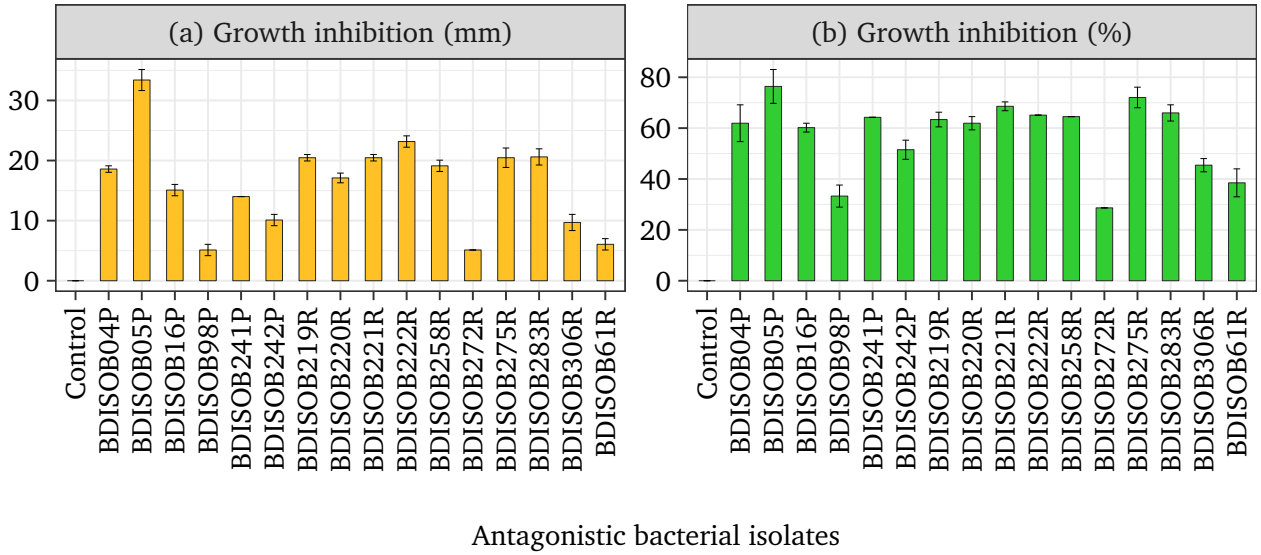


Figure 2. Growth inhibition of *X. oryzae* pv. *oryzae* by different bacterial antagonists

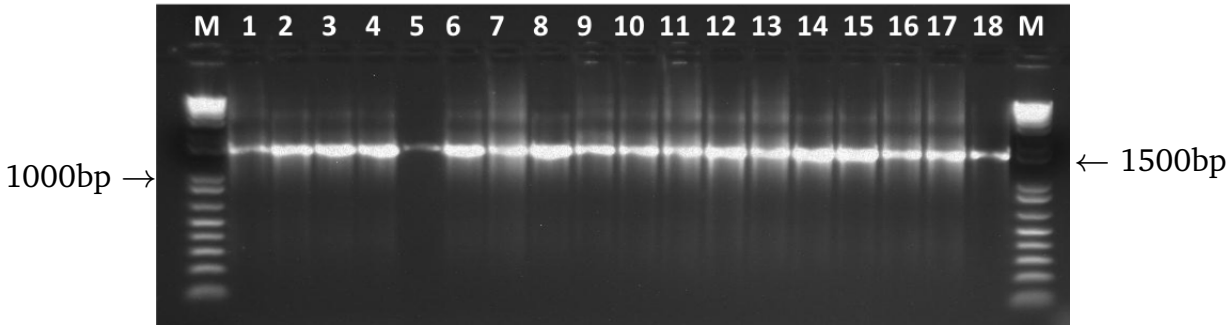


Figure 3. PCR amplification of 16srDNA of the antagonistic bacterial isolates. M: 1 kb plus DNA ladder. 1: BDISOB04P, 2: BDISOB05P, 3: BDISOB16P, 4: BDISOB98P, 5: BDISOB241P, 6: BDISOB242P, 7: BDISOB219R, 8: BDISOB220R, 9: BDISOB221R, 10: BDISOB222R, 11: BDISOB258R, 12: BDISOB272R, 13: BDISOB275R, 14: BDISOB186R, 15: BDISOB283R, 16: BDISOB306R, 17: BDISOB53R, and 18: BDISOB61R

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

| Isolates | Closest relatives | Accession | Alignment | Homol. |
|---|---|---|---|---|
| BDISOB04P | *Pseudomonas putida* strain PF41 | MF838698.1 | 968/1086 | 89 |
| BDISOB05P | *Pseudomonas putida* strain TB3 | MH085459.1 | 931/1140 | 82 |
| BDISOB16P | *Bacillus* sp. (in: Bacteria) strain VPS44 | MH819972.1 | 702/738 | 95 |
| BDISOB98P | *Stenotrophomonas maltophilia* strain AUX077_Japan | AY486381.1 | 1224/1271 | 96 |
| BDISOB241P | *Burkholderia* sp. RBA1 | GU979224.1 | 1154/1222 | 94 |
| BDISOB242P | *Burkholderia gladioli* strain LMG 2121 | MH748602.1 | 1186/1239 | 96 |
| BDISOB219R | *Pseudomonas taiwanensis* strain GGRJ11 | KC293831.1 | 913/969 | 94 |
| BDISOB220R | *Serratia* sp. B2-254 | FM875872.1 | 150/186 | 81 |
| BDISOB221R | *Pseudomonas* sp. strain M2.2.1 | MG021242.1 | 303/341 | 89 |
| BDISOB222R | *Pseudomonas plecoglossicida* strain HFgGr | KC864769.1 | 614/751 | 82 |
| BDISOB258R | *Pseudomonas putida* strain B-18 | MF417798.1 | 917/1050 | 87 |
| BDISOB272R | *Stenotrophomonas maltophilia* strain JC178 | KJ534495.1 | 794/923 | 86 |
| BDISOB275R | *Pseudomonas putida* strain P6 | KT984874.1 | 1201/1229 | 98 |
| BDISOB283R | *Pseudomonas fluorescens* strain B8 | KF010368.1 | 969/1006 | 96 |
| BDISOB306R | *Pseudomonas putida* strain DNCA01 | KF030905.1 | 1298/1374 | 94 |
| BDISOB61R | *Delftia tsuruhatensis* strain As-23 | MF353931.1 | 976/1168 | 84 |

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

| Isolates | Plant growth promoting determinants | | |
|---|---|---|---|
| | IAA | Siderophore | Phosphate solubilizing |
| BDISOB04P | + | + | + |
| BDISOB05P | + | + | + |
| BDISOB16P | − | + | − |
| BDISOB98P | − | + | − |
| BDISOB241P | − | + | + |
| BDISOB242P | − | + | + |
| BDISOB219R | + | + | + |
| BDISOB220R | + | + | + |
| BDISOB221R | + | + | − |
| BDISOB222R | + | + | + |
| BDISOB258R | + | + | + |
| BDISOB272R | + | + | − |
| BDISOB275R | − | + | − |
| BDISOB283R | − | + | − |
| BDISOB306R | − | + | − |
| BDISOB61R | − | + | + |

Table 3. Effect of potential plant growth promoting antagonistic bacteria on the root and shoot length, and vigour index of rice

| Treatments | Germ. (%) | Root length (cm) | | | Shoot length (cm) | | | Vigor index | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 7 DAS | 14 DAS | 30 DAS | 7 DAS | 14 DAS | 30 DAS | 7 DAS | 14 DAS | 30 DAS |
| Control | 90 | 8.77 | 5.56 | 9.9 | 9.47 | 14.7 | 22.15 | 1641 | 1823.4 | 2884.5 |
| BDISOB04P | 90 | 9.7 | 8.18 | 12.18 | 9.96 | 18.1 | 24.84 | 1769.4 | 2364.75 | 3331.8 |
| BDISOB05P | 90 | 9.28 | 7.25 | 12.24 | 11.12 | 18.82 | 22.4 | 1836 | 2346.3 | 3117.6 |
| BDISOB219R | 90 | 9.28 | 7.25 | 12.24 | 11.12 | 18.82 | 22.4 | 1836 | 2346.3 | 3117.6 |
| BDISOB221R | 90 | 9.22 | 10.25 | 10.84 | 11.82 | 17.52 | 25.85 | 1893.6 | 2499.3 | 3302.1 |
| BDISOB222R | 90 | 9.4 | 10.14 | 10.56 | 12.88 | 19.16 | 24.5 | 2005.2 | 2637 | 3155.4 |
| BDISOB258R | 90 | 9.15 | 9.16 | 10.2 | 10.06 | 18.88 | 24.18 | 1728.9 | 2523.6 | 3094.2 |
| BDISOB283R | 90 | 9.27 | 7.14 | 12.78 | 11.82 | 17.78 | 25.5 | 1897.8 | 2242.8 | 3445.2 |

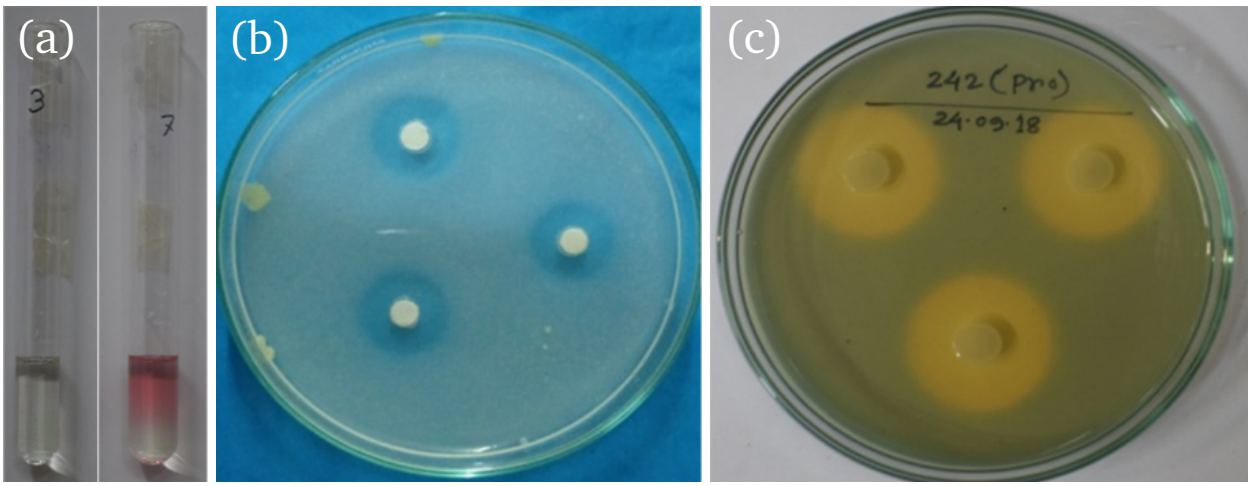


Figure 4. (a) Indole acetic acid (IAA) production activity by different antagonistic bacterial isolatesindicated by the presence of pink color when bacterial culture supernatant mixed with Salkowski reagent, (b) Antagonistic bacterial isolates showed positive phosphate solubilizing activity by producing clear halo zone around the bacterial colony on National Botanical Research Institute's Phosphate (NBRIP) agar plates, and (c) Antagonistic bacterial isolates showed positive siderophore production activity as indicated by orange halo zone around bacterial colony on CAS agar plates.

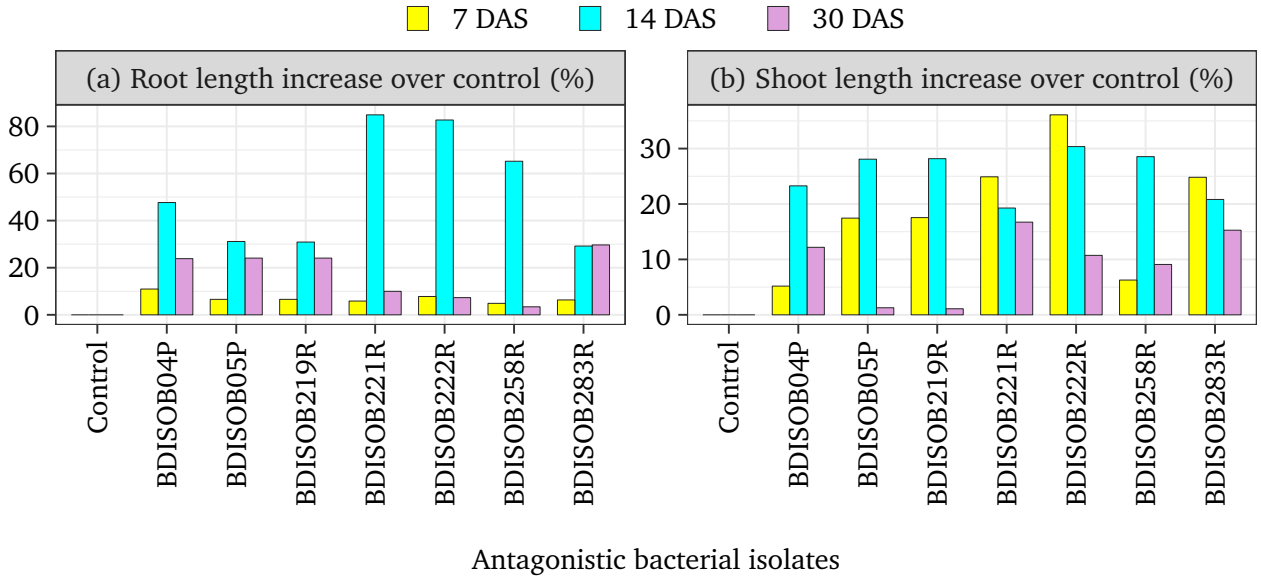


Figure 5. Effect of potential bacterial anatagonists on percent increase of (a) root length (cv. BRRI dhan49), and (b) shoot length over control. BDISOB04P: *Pseudomonas putida*, BDISOB05: *Pseudomonas putida*, BDISOB219R: *Pseudomonas taiwanensis*, BDISOB221R: *Pseudomonas* sp., BDISOB222R: *Pseudomonas plecoglossicida*, BDISOB258R: *Pseudomonas putida*, BDISOB186R: *Pseudomonas* sp., and BDISOB283R: *Pseudomonas fluorescens*

grown from seed treated with BDISOB258R. However, the other isolates [BDISOB04P (*Pseudomonas putida*), BDISOB05P (*Pseudomonas putida*), BDISOB219R (*Pseudomonas taiwanensis*), BDISOB221R (*Pseudomonas* sp.), BDISOB222R (*Pseudomonas plecoglossicida*)] showed moderate root length varied from 10.56 to 12.24 cm (Table 3).

After 30 DAS the maximum shoot length was 25.85 cm measured in plants grown from seed treated with BDISOB221R (*Pseudomonas* sp.). On the contrary, the minimum root length was 22.40 cm recorded in plants grown from seed treated with both BDISOB05P (*Pseudomonas putida*) and BDISOB219R (*Pseudomonas taiwanensis*), respectively. The other isolates BDISOB04P (*Pseudomonas putida*), BDISOB222R (*Pseudomonas plecoglossicida*), BDISOB258R (*Pseudomonas putida*), BDISO283R (*Pseudomonas fluorescens*) showed moderate shoot length varied from 23 to 25 cm (Table 3 & Fig. 5).

In case of vigor index after 30 DAS, maximum vigor index (3445.20) was recorded in plants grown from seed treated with BDISOB283R (*Pseudomonas fluorescens*). On the other hand, the minium vigor index (3094.20) was observed in plants grown from seed treated withBDISOB258R (Pseudomonas sp.). However, the moderate vigor index ranged from 3094.20 to 3331.80 exhibited by BDISOB04P (*Pseudomonas putida*), BDISOB05P (*Pseudomonas putida*), BDISOB219R (*Pseudomonas taiwanensis*),BDISOB221R (*Pseudomonas* sp.), BDISOB222R (*Pseudomonas plecoglossicida*), BDISOB258R (*Pseudomonas putida*) isolates (Table 3).

## 4 Discussion

Sixteen bacterial isolates of different species were tested *in vitro* and found antagonistic to *X. oryzae* pv. *oryzae*. In this study, it was observed that 28.56 to 76.14% radial growth inhibition of *X. oryzae* pv. *oryzae* exhibited by all of this bacterial isolates. Similar findings were reported by Yasmin et al. (2017). They showed consistent suppression of BLB pathogen in rice by different bacterial isolates. The findings of the present study also underpinning by the findings of Rahman et al. (2007), who showed three bacterial isolates exhibited comparatively higher growth inhibition of *X. oryzae* pv. *oryzae*. Antagonistic bacteria can suppress plant pathogens either by directly or indirectly. Antibiotics, enzymes like chitinases, glucanases, proteases, and siderophores produce directly or indirect mechanisms in which the antagonistic bacteria compete with the pathogen for a niche or nutrient sites (Bardin et al., 2015). Out of 16 bacterial isolates, 9 isolates were aligned and identified as *Pseudomonas* spp. Isolates of *Pseudomonas* 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 *P. fluorescens* PDY7 can control BLB and enhance the growth of rice variety IR24 (Velusamy et al., 2013).

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 unambiguous 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 16 bacterial isolates, 8 isolates were found to produce IAA. The results also showed that out of 8 isolates, 6 isolates [BDISOB04P, BDISOB05P, BDISOB219R, BDISOB221R, BDISOB222R, BDISOB258R] producing IAA belonged to *Pseudomonas* 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 (Aslantaş 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). Nine bacterial isolates [BDISOB04P, BDISOB05P, BDISOB241P, BDISOB242P, BDISOB219R, BDISOB220R, BDISOB222R, BDISOB258R, BDISOB61R] showed the capability of phosphate solubilization and among them 5 isolates [BDISOB04P, BDISOB05P, BDISOB219R,BDISOB222, BDISOB258R] which were capable of phosphate solubilization related to *Pseudomonas* spp. It has been reported that phosphate solubilizing bacteria (PSB) induced plant growth promotion (Oteino 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). Different plant growth promoting bacterial antagonists has significant impact in increasing root length, shoot length and vigour index. Among 16 bacterial isolates, 7 bacterial isolates [BDISOB04P, BDISOB05P, BDISOB219R, BDISOB221R, BDISOB222R, BDISOB258R, BDISOB283] were selected based on their antagonistic capability and also in increasing plant growth compared to control. Similarly, Sakthivel et al. (1986) and Mishra and Sinha (1998) also reported enhanced growth of rice seedling with bioagent application. van Peer and Schippers (1989) stated that shoot, root and fresh weight was increased for tomato, cucumber, lettuce, 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 vigour of rice plants was significantly higher than control. Kloepper (1980) 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. Studies concerning commercialization and field applications of integrated stable bio-formulations as effective biocontrol strategies would be needed in future.

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## Conflict of Interest

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

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