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Characterization of PGPR from rhizospheric soil of some vegetable crops cultivated at Sylhet district of Bangladesh

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Abstract

Plant Growth Promoting Rhizobacteria (PGPR) are rhizosphere-dwelling microorganisms which hold a great deal of potential for both plant growth stimulation and disease prevention. The characterization of PGPR will aid in the advancement and deployment of biocontrol agents. In this present work, rhizospheric soils were collected from several locations of Sylhet Agricultural University in order to obtain plant growth promoting rhizobacteria. Nineteen bacterial samples were extracted from a variety of fifteen distinct vegetable crops, viz. tomato, brinjal, beans, okra, cabbage, cauliflower, pumpkin, amaranth, malabar spinach, bitter gourd, ridge gourd, spiny gourd, sponge gourd, wax gourd, and snake gourd. These isolates were examined morphologically, biochemically, and screened for plant growth stimulating capability as well as their efficacy in combating the plant pathogen *Fusarium oxysporum* through antifungal activity. Among the isolates, only *Lysinibacillus macroides* (RB2), *Lysinibacillus fusiformis* (RB6) and *Acinetobacter baumannii* (RB15 and RB17) showed antifungal and growth promotion potentials. Therefore, the present study indicates that the vegetable rhizosphere contains potential rhizobacteria which could be utilized to enhance plant development and reduce disease incidence on vegetable crops.

Keywords: PGPR, vegetable crops, *Lysinibacillus macroides*, *Lysinibacillus fusiformis* and *Acinetobacter baumannii.*

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INTRODUCTION

Bangladesh, as a developing nation, relies heavily on its agro-economy, where vegetable production is an important agricultural activity that contributes to the country's food and nutritional needs. In this country, the market demand for vegetables comes in second place to that of rice (Mim et al., 2019). However, the average yield of vegetable crops is influenced by plant pathogenic microorganisms. Many diseases that occur throughout the crop growing season cause a significant loss (Azad et al., 2014). Therefore, people use a variety of tactics, including the application of a variety of chemicals, in order to fight against plant diseases while increasing agricultural production. Yet, the extended usage of these agrochemicals has significant adverse consequences for the ecosystem and people's health (Li et al., 2020). Contemporary public apprehensions regarding the detrimental impacts of agrochemicals have led to a growing curiosity about thoroughly understanding the cooperative relationships between plants and soil microorganisms. This has led to a pressing need for widely known biological agents (Mahanty et al., 2017). Utilizing plant growth promoting rhizobacteria appears to be a promising way to address this challenge (Gupta et al., 2015; Chauhan et al., 2021).

Plant growth promoting rhizobacteria (PGPR) are a collective of soil microorganisms thriving within the rhizosphere along with the plant root surface and benefit plant's overall health. Bacterial species, notably *Acinetobacter*, *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Gluconacetobacter*, *Lysinibacillus*, *Pseudomonas*, *Rhizobium*, and *Serratia* are widely acknowledged as

PGPR (Vessey 2003; Rokhbakhsh-Zamin et al., 2011; Bhattacharyya and Jha 2012; Huang et al., 2017; Naureen et al., 2017).

PGPR stimulates plant development by several mechanisms, categorized as direct and indirect. Direct mechanisms refer to processes which provide nutrients or generate growth stimulants in order to directly encourage plant development. In contrast, those processes that protect plants against infection or allow plants to grow properly under stressful conditions are termed indirect mechanics (Goswami et al., 2016). Direct mechanisms comprise the potential to nitrogen-fix, solubilize insoluble phosphate, sequester iron, and generate phytohormones (including auxins, cytokinins, and gibberellins), while the capacity to synthesize antibiotics, enzymes, or to generate systemic resistance within plants are examples of indirect mechanisms (Gouda et al., 2018; Meena et al., 2020).

Additional research has recognized the plant growth promoting (PGP) and antagonistic potential of PGPR. *Bacillus subtilis* obtained from *Solanum lycopersicum* (tomato) plants showed antifungal activity against *Alternaria alternata*, *Penicilium digitatum*, and *Fusarium oxysporum*, as well as improved both qualitative and quantitative growth parameters in *Solanum lycopersicum* and *Solanum melongena* plants (Sarbadhikary and Mandal, 2017). *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Acenetobacter pitti* exhibited antagonistic activity against *Rhizoctonia solani*, the pathogen responsible for foot rot disease in vegetable crops (Kumari et al., 2018). *Enterobacter cloacae*, *Chryseobacterium jejuense*, and *Klebsiella pneumonia*, all isolated from tomato rhizosphere were found to have antagonistic and PGP activities (Abdeljalil et al., 2016). *Pseudomonas aeruginosa* isolated from tomato rhizosphere inhibited the mycelial development of *Fusarium oxysporum* and *Alternaria solani* (Paramanandham et al., 2017).

Biofertilizers containing PGPR offer a more economical, sustainable, and productive alternative compared to chemical fertilizers. Moreover, they are highly effective and accessible to marginal farmers (Basu et al., 2021). Therefore, PGPR inoculants may be utilized to ensure the consistency of long-term agricultural production, presenting a more widely recognized substitute for intensive agriculture in numerous regions worldwide (Kenneth et al., 2019). Consequently, there is an immediate need to find viable PGPR strains for our farmers.

In line with this objective, the study aimed to isolate, characterize, and identify PGPR from vegetable crops rhizosphere, focusing on their PGP attributes and antagonistic activity. In this research work, nineteen bacterial isolates were collected, and their morphological and biochemical characteristics were examined. In addition, the isolates underwent screening for PGP properties, and selected representatives were molecularly characterized.

MATERIALS AND METHODS

The study site and cultivars

The research was conducted in the central laboratory of the Microbiology and Immunology Department, Faculty of Veterinary, Animal, and Biomedical Sciences, Sylhet Agricultural University, located in Sylhet, Bangladesh, from January 2021 to June 2021. Fifteen vegetable crops, e.g., tomato, brinjal, bean, okra, cabbage, cauliflower, pumpkin, amaranth, malabar spinach, bitter gourd, ridge gourd, spiny gourd, sponge gourd, wax gourd, and snake gourd were collected from different locations of SAU (24°54' to 36°36" N and 91°54' to 02°36" E). The climate in the region is characterized by subtropical monsoons. The majority of agricultural soils in the Sylhet region belong to the loamy sand textural class, acidic with pH values of 4.9-6.1 containing the organic matter of 0.5- 2.45% and EC of 0.26-1.17 dsm-1 (Hossain and Sattar, 2002).

Collection and preservation of rhizospheric soil

Nineteen samples were taken from two-month-old healthy vegetable crop plants. The rhizosphere around the vegetable crops was dug out to a 0–15 cm depth and then uprooted with sufficient rhizosphere soils without hampering the secondary and tertiary roots. The plant roots and rhizospheric soil were kept in plastic bags at 4°C.

Isolation of PGPR from rhizospheric soil

To isolate rhizobacteria, fresh roots weighing 2–5 g were washed and then surface sterilized with 5% NaOCl. The root samples underwent pulverization after being washed three times with sterile distilled water. Then, using both soil suspension and root samples, a serial dilution was prepared and it was carried out up to ten times. A portion of this solution (0.1 ml) was spread on nutritional agar (NA) medium and incubated for 24 hours at 37°C for bacterial culture. Then a single colony was isolated from the culture and re-streaked onto new plates, where it was cultured in the same way. Isolated colonies were selected for single isolation based on several features, such as shape, color, and margin. This was then cultured in nutrient broth slants to generate pure culture.

Morphological Characterization

Isolates cultured on NA plates were observed for morphological analysis. Several properties of colonies were recorded, including size, shape, elevation, margin, surface, pigmentation, etc., as indicated by Somasegaran and Hoben (2012). Light microscopy was used to observe cell size. Gram reaction and motility tests were performed. In vitro assessments were conducted for the abiotic stress tolerance activities of the isolates in different temperature ranges (10, 28, 37, and 45° C) and salt concentrations (5% and 10% NaCl).

Biochemical characterization

Bergey's Manual of Systematic Bacteriology was followed in the biochemical characterization process (Bergey et al., 1994). Routine biochemical tests such as citrate utilization activity, catalase activity, KOH solubilization,

indole test, Methyl Red (MR) test, Voges Proskauer (VP) test, and carbohydrate fermentation test (glucose, sucrose, maltose, and mannitol) were carried out for the PGPR isolates.

Tests for plant growth promoting (PGP) activities

Phosphate solubilizing ability

All 19 isolates were placed on the Pikovskaya agar plates and incubated at 28°C. After 24 hours of growth, the plates were inspected to see if the bacterial colonies had a halo zone surrounding them, an indication of their ability to dissolve tri-calcium phosphate (Pikovskaya, 1948).

Indole-3-Acetic Acid (IAA) production

IAA production was analyzed by following Salkowski's method (Ehmann, 1977). The isolates were cultured in yeast malt dextrose broth (YMD broth) for four days at 28°C, followed by centrifugation at 3000 rpm for 30 minutes. Subsequently, 1 ml of the resulting supernatant was incorporated with 2 ml of Salkowski's reagent, and the mixture was stored in the dark for 30 minutes. Controls included reagents mixed with distilled water. After that, the treated reagents were checked for turning red.

Production of ammonia

Peptone water was utilized to assess the ammonia production capacity of test isolates. Freshly cultured isolates were placed in every test tube containing 10 ml of peptone water, which were subsequently incubated at 37°C for 48 to 72 hours. Then each tube was filled with 0.5 ml of Nessler's reagent and checked for the formation of a brown to yellow color (Cappuccino and Sherman, 2013).

Production of Hydrogen Cyanide (HCN)

Isolates were grown on NA medium supplemented with 4.4 g per liter of glycine. A Whatman filter paper No. 1 was put on top of the plate after being soaked in a solution containing 2% sodium carbonate and 0.5% picric acid. Following the sealing of the plates with parafilm, they underwent incubation at 36±2°C for four days. Afterward, the filter paper was observed for a color transition from orange to brown (Lorck, 1948).

In Vitro Screening for Antagonism

The antagonistic behavior was assessed against *Fusarium oxysporum*, isolated from naturally infected tomato plants that showed typical Fusarium wilt symptoms, adopting the dual culture method (Skidmore and Dickinson, 1976). In this approach, isolates were cross-streaked on one side of PDA plates, while a five-day-old mycelial disc of the plant pathogen was placed on the other side. The plates were then incubated in the range of 28±2°C for a period of 5 days. The percentage of the fungal mycelial inhibition by the bacteria was determined using the following formula (Noumavo et al. 2015):

Percentage of growth inhibition (%)$= \frac{VI-V2}{V1} \times 100$

Where, V1= the diameter of the fungus growth (control),

V2= the diameter of the fungus growth in the dual culture plate.

Molecular characterization

Based on plant growthpromoting characteristics and antifungal properties, four bacterial isolates (RB2, RB6, RB15, and RB17) were selected for molecular characterization. This identification was accomplished by amplifying 16S rRNA using the PCR primers: 27F (AGA GTT TGA TCM TGG CTC AG) and 1492R (CGG TTA CCT TGT TAC GAC TT). The extracted bacterial DNA was utilized for PCR reaction. PCR reaction and sequencing were performed by the commercial service provider Invent Technologies Ltd., Dhaka, Bangladesh. The obtained sequences were compared with the GeneBank database using BLAST (Basic Local Alignment Search Tool), and the percentage similarity was subsequently determined. A phylogenetic tree was constructed by using the software package MEGA (Molecular Evolutionary Genetic Analysis) following the neighbor joining method (Saitou and Nei, 1987) with bootstrap values (Felsenstein, 1985) based on 1,000 replications.

Statistical Analysis

The inhibitions of fungal growth by the bacterial isolates were observed, and the mean along with standard deviation of these inhibitions were estimated. The statistical analysis of the data was performed using the Agricole package within the R software. Significant differences among the means were determined utilizing Fisher's least significant (LSD) test, considering a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Strain isolation and morphological characterization

The bacterial strains isolated from vegetable crops rhizosphere were named as RB1, RB2, RB3, RB4, RB5, RB6, RB7, RB8, RB9, RB10, RB11, RB12, RB13, RB14, RB15, RB16, RB17, RB18, and RB19. Table 1 represents the result of the colony morphology and microscopic findings of PGPR isolates. From morphological analysis it was found that the bacterial isolates were fast-growing with a wide range of colony form. The isolates formed colonies that were typically circular, flat elevation, smooth surface, odorless, and varied in color. The colonies' diameter ranged from 0.7 to 1.6 mm. Under a microscope, ten rod-shaped isolates (RB2, RB3, RB5, RB6, RB7, RB11, RB12, RB14, RB18, and RB19), commonly known as bacilli, were discovered. Seven isolates (RB1, RB4, RB8, RB9, RB10, RB13 and RB16) were found to be cocci or spherical in form and two (RB15 and RB17)

were found to be short rod. There were 9 gram-positive isolates (RB2, RB3, RB6, RB7, RB11, RB12, RB13, RB14, and RB18) and 10 gram-negative isolates (RB1, RB4, RB5, RB8, RB9, RB10, RB15, RB16, RB17 and RB19) among the nineteen isolates. Except for RB15 and RB17, all isolates were motile. Morphological study revealed that the isolates varied greatly concerning their size, shape, elevation, color, margin, surface, odor and gram staining which were confirmed by other studies (Chen et al., 2009; Hardiansyah, 2020).

Table 1. Morphological features of colonies of PGPR isolates.

Isolates	Shape	Size (mm)	Elevation	Surface	Color	Odor	Cell Shape	Motility	Gram Staining
RB1	Round	0.8	Raised	Smooth	Off white	Odorless	Round	Motile	-
RB2	Round	1.2	Flat	Rough	Yellowish	Odorless	Rod	Motile	+
RB3	Oval	0.9	Flat	Rough	Yellowish	Odorless	Rod	Motile	+
RB4	Round	0.9	Raised	Smooth	Off white	Odorless	Round	Motile	-
RB5	Oval	0.7	Flat	Rough	Off white	Odorless	Rod	Motile	-
RB6	Oval	1.0	Raised	Smooth	Yellowish	Odorless	Rod	Motile	+
RB7	Oval	1.4	Flat	Rough	Off white	Odorless	Rod	Motile	+
RB8	Round	1.0	Raised	Smooth	Yellowish	Odorless	Round	Motile	-
RB9	Round	0.7	Flat	Smooth	Off white	Odorless	Round	Motile	-
RB10	Round	1.2	Raised	Smooth	Off white	Odorless	Round	Motile	-
RB11	Round	1.0	Raised	Rough	Off white	Odorless	Rod	Motile	+
RB12	Irregular	1.5	Flat	Rough	White	Odorless	Rod	Motile	+
RB13	Round	0.9	Flat	Smooth	Off white	Odorless	Round	Motile	+
RB14	Round	1.4	Flat	Rough	White	Odorless	Rod	Motile	+
RB15	Round	0.8	Raised	Smooth	White	Odorless	Short rod	Non-motile	-
RB16	Round	0.8	Flat	Rough	White	Odorless	Round	Motile	-
RB17	Round	1.4	Raised	Smooth	White	Odorless	Short rod	Non-motile	-
RB18	Round	1.6	Flat	Rough	Off white	Odorless	Rod	Motile	+
RB19	Round	1.2	Raised	Smooth	White	Odorless	Rod	Motile	-

NB: '+' indicates positive growth; and '-' indicates No growth

Growth of PGPR at abiotic stress tolerance activities (different temperature ranges and NaCl concentrations)

Temperature and salinity are two important limiting factors that have an impact on agricultural yield and plant development (Tsegaye et al., 2019). Under varied temperature conditions (i.e., 10, 28, 37, and 45° C), the test isolates grown in NA plates for 24 hours exhibited heavy to no growth, as shown in table 2. In 5% NaCl, 8 isolates (RB1, RB5, RB7, RB9, RB11, RB12, RB14, and RB17) were able to grow while the remaining 11 isolates didn't show any growth. No isolate developed growth at 10% salt concentration. The majority of isolates exhibited positive growth at 5% salt concentrations and temperatures of 28°C and 37°C. Table 2 shows the growth potential of isolates at various temperatures and NaCl concentrations.

Table 2. Growth Performance of PGPRs at abiotic stress tolerance activities

Isolates	Temperature (°C)				Salt concentration (%)	
	10	28	37	45	5	10
RB1	-	++	+	+	+	-
RB2	+	++	++	++	-	-
RB3	-	+	++	-	-	-
RB4	+	+	+	+	-	-
RB5	-	++	++	+	+	-
RB6	+	++	++	++	-	-
RB7	+	++	++	+	+	-
RB8	-	++	+	+	-	-
RB9	-	++	+	+	+	-
RB10	-	++	++	+	-	-
RB11	+	++	++	-	+	-
RB12	+	++	++	++	+	-
RB13	+	+	++	++	-	-
RB14	-	+	++	++	+	-
RB15	+	+	+	+	-	-
RB16	-	++	+	-	-	-
RB17	-	+	+	+	+	-
RB18	+	++	++	+	-	-
RB19	+	+	++	+	-	-

NB: '++' indicates heavy growth; '+' indicates positive growth; and '-'indicates No growth

Biochemical Characterization

As for biochemical analysis, the isolates demonstrated a high level of diversity where most of them gave positive results for the Citrate utilization test, Catalase test, KOH solubility test, MR test, VP test, and Carbohydrate fermentation tests. Only one isolate (RB16) was positive for the Indole test. Similar biochemical tests were performed by Asrafujamman et al. (2009) and Tsegaye et al., (2019). The outcomes of all these biochemical tests are presented in Table 3.

Table 3. Biochemical characterization of PGPR isolates.

Isolates	Citrate	Catalase	KOH	Indole	MR	VP	Glucose	Sucrose	Maltose	Mannitol
RB1	-	+	-	-	+	-	+	+	+	+
RB2	+	+	+	-	-	-	+	+	+	+
RB3	-	+	+	-	-	+	+	+	-	+
RB4	+	+	-	-	+	-	+	-	+	-
RB5	-	+	-	-	-	+	+	+	+	+
RB6	+	+	+	-	-	-	+	+	-	+
RB7	-	+	+	-	-	+	-	+	+	+
RB8	+	+	-	-	+	-	+	+	+	-
RB9	-	+	-	-	-	+	+	+	+	-
RB10	+	+	-	-	-	-	+	-	-	+
RB11	-	+	+	-	-	-	+	+	+	+
RB12	+	+	+	-	+	+	+	+	-	+
RB13	-	+	+	-	+	+	+	+	+	+
RB14	-	+	+	-	+	-	+	+	+	+
RB15	+	-	-	-	+	+	+	-	+	-
RB16	-	+	-	+	+	-	-	+	+	+
RB17	+	-	-	-	+	+	+	+	+	-
RB18	+	+	+	-	+	-	+	+	-	+
RB19	+	+	-	-	+	-	+	+	+	+

NB: '+' corresponds to positive response; '-' corresponds to negative response, MR= Methyl Red, and VP= Voges Proskauer

Characterization for plant growth promoting properties

All 19 isolates were examined for their PGP properties (ammonia synthesis, IAA generation, HCN production, and phosphate solubilization). Phosphorus is a vital element for optimal plant development. However, a significant amount of the phosphorus in soil exists in an insoluble form, preventing direct uptake by plants. Some PGPR help to convert them into soluble form by secreting organic acids and phosphatases, thereby enhancing its availability to plants (Souchie et al., 2005; Dash et al., 2017). In the current study, Out of the nineteen rhizobacterial isolates, only six formed a halo zone surrounding the colonies. As a result, these six isolates (RB1, RB2, RB6, RB15, RB16, and RB17) were reported as positive in the phosphate solubilization test, whereas the other thirteen were found to be negative. Other studies have also documented the phosphate solubilization ability of PGPR strainsderived from different vegetable crops (Bechtaoui et al., 2019; Liu et al., 2015; Baliah et al., 2016; Paiter et al., 2019; Mei et al., 2021).

Another crucial PGP characteristic of rhizobacteria is the generation of phytohormones (Patten and Glick, 2002). IAA, an important phytohormone, has a beneficial impact on plant root system elongation and development, contributing in water and nutrient intake as well as the activation of cambial cell division (Grobelak et al., 2015; Kusumawati et al., 2017). Eight isolates (RB1, RB2, RB6, RB10, RB12, RB15, RB17, and RB18) were capable of producing IAA which was evident by their distinctive reddish to pinkish appearance in the solution. Similar results were found by other researchers (Dias et al. 2013; Meliani et al., 2017; Yousef, 2018; Mike-Anosike et al., 2018; Kalimuthu et al., 2019; Cavalcante et al., 2020).

The generation of ammonia was evidenced when the broth culture became brown with the addition of Nesler's reagent. All nineteen bacterial isolates produced ammonia under test conditions, a phenomenon known to have an indirect influence on plant health. This study's findings on the ammonia generating ability of rhizobacteria are aligned with other prior studies (Goswami et al., 2015; Moustaine et al., 2017; Mazumdar et al., 2018; Singh et al., 2019).

Six bacterial isolates (RB2, RB6, RB10, RB15, RB16, and RB17) generated HCN. It was demonstrated by an alteration in filter paper's color from yellow or orange to brown. HCN is a well-known secondary metabolite that has been associated with pathogen control in soil (Kesaulya et al. 2015). Other researchers also reported HCN producing PGPR strains (Vaikuntapu et al., 2014; Kesaulya et al., 2015; Marakana et al., 2018; Abd El-Moaty et al., 2018; Agustiyani et al., 2022). Table 4 represents the performance of PGPR in growth promotion activities.

Effects of antagonistic activity of rhizobacteria

To assess antagonistic activity, dual cultures were performed using the test isolates against *Fusarium oxysporum*. Nine of the nineteen isolates showed some level of antagonism, while the maximum and minimum inhibitions were observed for RB2 (44.74%) and RB12 (23.68%), respectively. Rhizobacteria can limit the growth

of phytopathogens through different ways, such as competing for nutrients and space, generating bacteriocins, enzymes, antibiotics, and siderophores (Jing et al. 2007). Other research also found the antagonistic behavior of rhizobacteria (Manasa et al., 2017; Ali et al., 2020; Attia et al., 2020; Pellegrini et al., 2020).

Table 4. Performance of PGPR in growth promotion activities.

Isolates	Phosphate solubilization	IAA production	Ammonia production	HCN production
RB1	+	+	+	-
RB2	+	+	+	+
RB3	-	-	+	-
RB4	-	-	+	-
RB5	-	-	+	-
RB6	+	+	+	+
RB7	-	-	+	-
RB8	-	-	+	-
RB9	-	-	+	-
RB10	-	+	+	+
RB11	-	-	+	-
RB12	-	+	+	-
RB13	-	-	+	-
RB14	-	-	+	-
RB15	+	+	+	+
RB16	+	-	+	+
RB17	+	+	+	+
RB18	-	+	+	-
RB19	-	-	+	-

NB: '+' indicates positive growth; and '-'indicates No growth

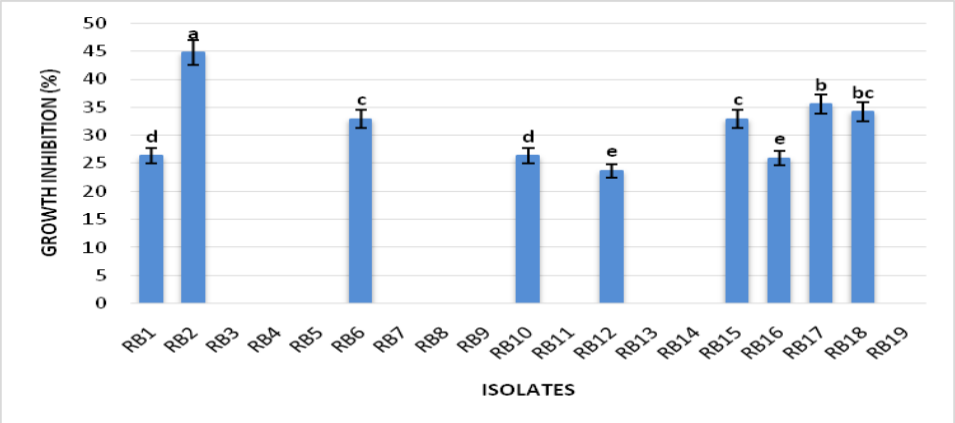

Figure 1. Antagonistic activity of PGPR against *Fusarium oxysporum*

Molecular Characterization and Identification

In accordance with morphological and biochemical findings, nine out of the nineteen isolates belonged to five different genera, namely *Acinetobacter sp., Bacillus sp., Enterobacter sp., Lysinibacillus sp.,* and *Pseudomonas sp.* (Huang et al., 2017; Pramanik et al., 2018; Singh et al., 2020; Jyolsna et al., 2021). Of them, Isolates RB2, RB6, RB15, and RB17 were molecularly characterized (Figure 2). The outcome indicated that RB2 and RB6 had the highest similarity with *Lysinibacillus macroides* and *Lysinibacillus fusiformis* respectively while RB15 and RB17 both showed similarity with *Acinetobacter baumannii*. Identification of different *Lysinibacillus spp.* as PGPR was also recorded by other studies (Vendan et al., 2010; Singh et al., 2013; Jyolsna et al., 2021; Ahsan and Shimizu, 2021; Passera et al., 2021; Jha and Mohamed 2023; Pantoja-Guerra et al., 2023). *Acinetobacter* has been reported as PGPR by previous studies such as Rokhbakhsh-Zamin et al. (2011), Padmavathi et al. (2015), kumari et al. (2018), Santosa et al. (2018), Leontidou et al. (2020), Singh et al. (2020) and Mujumdar et al. (2023). Phylogenetic analysis was done to evaluate the evolutionary relationships of RB2, RB6, RB15, and RB17 with other species, as illustrated in Figure 3. According to this tree, isolates RB15 and RB17 were discovered to be closely related to two distinct strains of *Acinetobacter baumannii*, with bootstrap values of 99% and 67%, respectively, while isolates RB2 and RB6 demonstrated a close relationship with *Lysinibacillus macroides* and *Lysinibacillus fusiformis*, with bootstrap values of 50% and 17%, respectively.

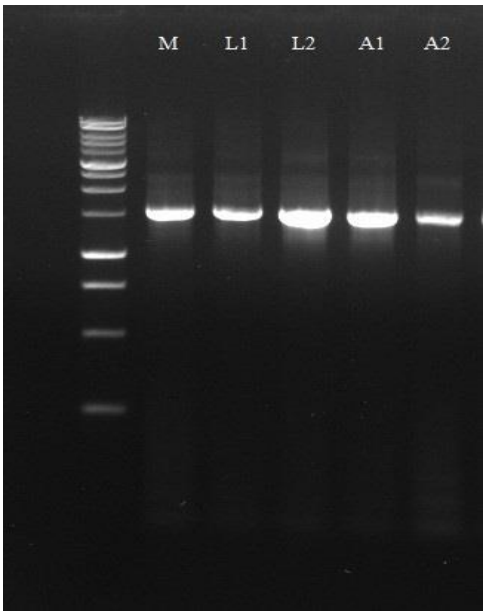

Figure 2. Agarose gel electrophoresis of the DNA amplified with primers 27F and 1492R from genomic DNA of bacterial strains.

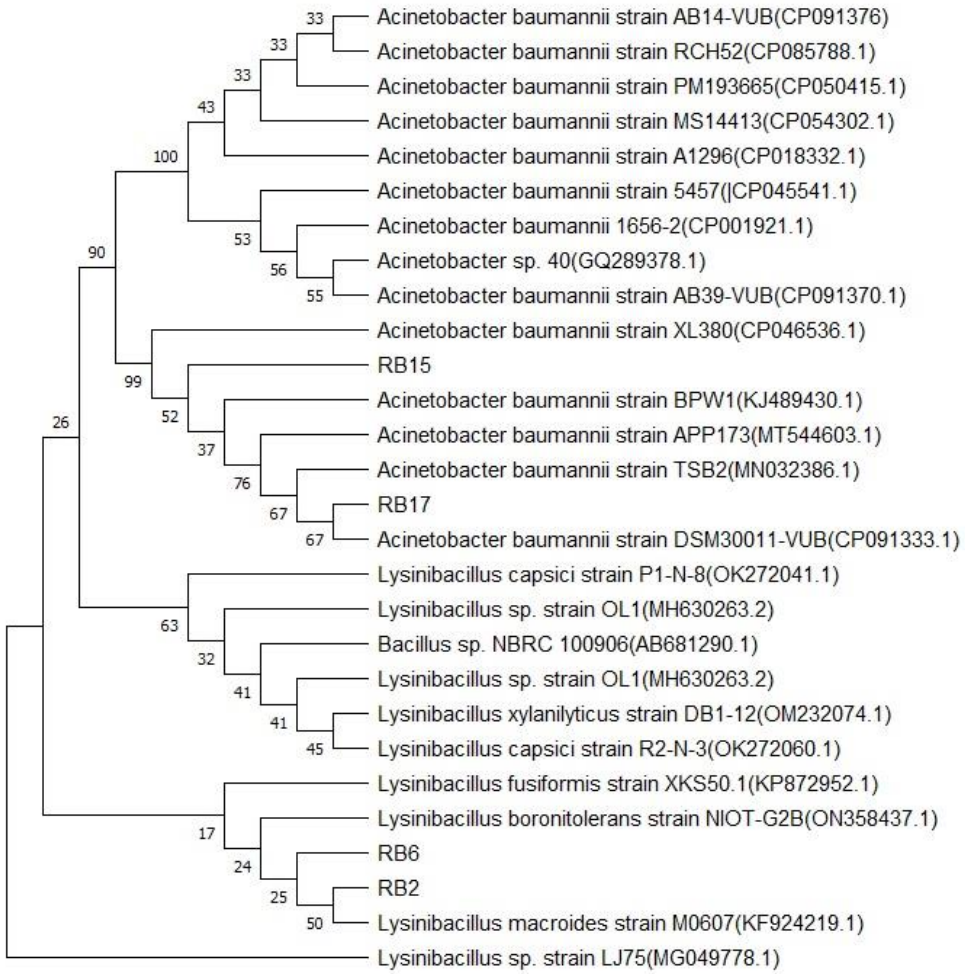

Figure 3: Phylogenetic tree formed by using the neighbor-joining method based on16S rRNA showing the relationship of isolates RB2, RB6, RB15 and RB17 to related isolates.

CONCLUSION

The application of PGPR has become an environmentally friendly strategy to enhance crop yields by promoting plant growth by means of diverse mechanisms, encompassing the maintenance of nutritional status, activation of disease resistance in plants, and nutrients dissolution for easier plant uptake (Santoyo et al., 2021). This research aimed to identify potential PGPR from vegetable rhizosphere and 19 bacterial isolates were obtained with this purpose. The collected isolates were cultured in NA agar media and observed for morphological, biochemical and molecular characteristics. PGP attributes and antagonistic activity of the isolates were also evaluated. Among the nineteen bacterial isolates studied, four isolates (RB2, RB6, RB15 and RB17) were found most potential considering PGP properties and antagonistic effect against *Fusarium oxysporum*. These isolates were identified as *Lysinibacillus macroides* (RB2), *Lysinibacillus fusiformis* (RB6), and *Acinetobacter baumannii* (RB15 and RB17) based on 16S rRNA gene sequence analysis. The study findings therefore support the hypothesis that these four isolates possess the potential to act as PGPR and can inhibit *Fusarium oxysporum* induced diseases in vegetable crops. The strains RB2, RB6, RB15, and RB17 collectively show promise as candidates for the production of biopesticide and biofertilizer for field application. Further research is needed to evaluate their effectiveness as biofertilizers. In addition, their antagonistic activity against various other pathogens should be examined to ensure a broader range of disease management for sustainable agriculture.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

No political conflict of interest was reported by the authors.

Author contribution

Saima Sadia Jui made a draft of the design of work, data collection, acquisition & analysis of the dataset, and wrote a draft of manuscript. Md. Monirul Islam made the plan of actions to execute and revised the design and analysis critically, and made the interpretation of the results. Rakibul Hasan carefully observed the design and analysis, also revised the manuscript. Israt Jahan Ema revised the manuscript and made valuable suggestions. Hasan Tareq Nasim helped in data collection. All authors read and approved the final manuscript.

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