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PLANT GROWTH-PROMOTING RHIZOBACTERIA FROM TOMATO FOR ANTAGONISTIC ACTIVITY AGAINST *Fusarium oxysporum* f. sp. *Lycopersici*

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Abstract

Plant-growth-promoting rhizobacteria (PGPR) are beneficial microorganisms that dwell in the rhizospheric region and positively affect plants. In the present study, bacteria were isolated from the rhizosphere of healthy tomato plants, and their effects on plant growth and *Fusarium oxysporum* f. sp. *lycopersici* were assayed. Sixteen isolates were obtained, among which five isolates, ERB4, ERB8, ERB12, ERB14, and ERB16, were capable of producing ammonia, IAA and phosphate solubilization. Meanwhile, they also showed *in vitro* antagonistic activities against *F. oxysporum.* The isolates were identified via morphology observation on selective and semi-selective media and 16S rRNA sequencing, which were classified as *Azotobacter* sp., *Bacillus* sp., *Pseudomonas stutzeri* and *Enterobacter cloacae*. The present study demonstrates that these bacteria can act as PGPRs to stimulate plant growth and suppress plant pathogenic fungi. It is important to develop new biocontrol agents for Fusarium wilt of tomato.

Keywords: PGPR, IAA production, Phosphate solubilization, *Pseudomonas strutzeri*, *Enterobacter cloacae.*

Introduction

Globally, the tomato (*Lycopersicon esculentum* L.) is the second most important edible vegetable after potato, belonging to the family Solanaceae (WPTC, 2022). In Bangladesh, tomato is grown on 69 thousand acres of land and produces 387 thousand MT per year (BBS, 2019). The tomato plant is prone to a massive attack by different fungal, bacterial, and viral diseases throughout the growing season. Fusarium wilt is one of the most important fungal diseases of tomatoes in Bangladesh (Tipu *et al.*, 2021) caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and loss of around 50% yield in susceptible hosts each year in India (Basco *et al.,* 2017). Although several cultural and physical practices are recommended to control this disease, chemical fungicides are the primary basis for disease management (Ons *et al.,* 2020). However, indiscriminate use of a wide range of fungicides is detrimental to the surrounding environment and the viability and survival of beneficial rhizospheric microbes (Heckel, 2012; *Mondal et al., 2017*). Furthermore, the growing cost of pesticides and the consumer demand for pesticide-free food has led to a search for substitutes for these products. Thus, there has been a need to find cost-effective and environment-friendly alternative pesticides to control the disease.

PGPR is rapidly gaining recognition in sustainable agricultural production for its naturally occurring plant-growth-promoting and antagonistic action against phytopathogens. Rhizobacteria are a heterogeneous group of bacteria that colonize the rhizospheric region at root surfaces and, in association with roots, exert beneficial effects on plant growth and development (Singh *et al*., 2017; Lugtenberg and Kamilova, 2009) through direct and indirect mechanisms. The Bacterial colonies, for example - *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus,* and *Serratia* have been reported to enhance plant growth (Ngalimat *et al.*, 2021; Zuluaga *et al.,* 2021). In general, PGPR promotes plant growth directly by enhancing nutrient availability (Singh *et al.,* 2017), stimulating root system development via the production of phytohormones, diazotrophic fixation of nitrogen, and solubilization of phosphate (Riaz *et al.,* 2021). Indirect effects on plants may involve the production of an antimicrobial compound that contains cell wall degrading enzymes and host defense enzymes to inhibit fungal growth (Dukare and Paul, 2021).

Several PGPRs isolated from the rhizosphere are effective against Fusarium on various plants. The strain of *P. fluorescens* isolated from the rhizosphere region of *Agrostic capillaries* was found to boost plant growth and inhabit the pathogenic fungi e.g., *Alternaria alternata*, *Fusarium oxysporum,* and *F. culmorum* (Grobelak *et al*., 2015). According to Hammami *et al*. (2013) fluorescent *Pseudomonas* sp. isolated from the rhizosphere soil of pepper and tomato plants

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observed an antagonist effect against the pathogens *S. sclerotiorum*, *F. solani*, and *A. alternate,* which were responsible for causing various infections in tomato plants (Hammami *et al*., 2013). In another report, Fatima *et al,* (2022) found that four different strains were isolated from the rhizosphere and used for controlling the chickpea wilt disease caused by *Fusarium oxysporum* f.sp. *ciceris* (Foc). Dong *et al.* (2022) demonstrated the bacteria isolated from Sanqi ginseng that suppressed the Fusarium root rot caused by *Fusarium oxysporum*. However, little is known about PGPRs that can potentially suppress Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* on tomato.

PGPRs are highly efficient biocontrol agents that trigger plant growth by producing different hormones. Thus, the objectives of the study were to characterize the plant growth-promoting rhizobacteria (PGPR) from tomato cultivars of Sylhet and to evaluate the antagonistic activities of PGPR against *Fusarium oxysporum* f. sp. *Lycopersici*.

Materials and Methods

Sample collection site

Soil samples and fresh roots were collected from the rhizosphere (1-15 cm) of the tomato cultivar Raja F_1 (Hybrid tomato variety introduced by Advanta Seed International) from two sites located at Plant Pathology and Horticulture experimental field, Sylhet Agricultural University, Sylhet, Kandigao Bazar, and Mashuk ganj Bazar under Sylhet district, Bangladesh (24°53'57" N; 91° 52' 19" E). The climate is subtropical monsoon with an average annual temperature of 23.6 °C and rainfall of 5048 mm. Most of the agricultural soils of the Sylhet region are 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).

Isolation of PGPR strains

Fresh roots were sterilized using 5% NaOCl and ground with a sterilized mortar and pestle. Then serial dilutions were prepared from soil suspension and root samples following the serial dilution technique **(**Alexander 1965). On a nutrient agar (NA), a plate aliquot (0.1ml) of this suspended material was dispersed with a sterilized spatula. Plates were incubated for 24 h. at 37°C to observe the bacterial colonies. Bacterial colonies were transferred to other NA plates and were incubated at 37°C for 24 hrs. A well–isolated single colony was picked up, re-streaked to fresh NA plates, and incubated similarly to obtain a pure culture.

Morphological characterization

The morphology of the colony e.g., size, shape, elevation, margin, surface, opacity, pigmentation, growth pattern, etc. was recorded on the Nutrient Agar (NA) plate at 36 ± 2°C after 24 h the inoculation according to Somasegaran and Hoben (1994). Cell size was observed by light microscopy (Optima G-206). The Gram reaction and motility were performed as Vincent and Humphrey (1970) described, and growth in 5% NaCl was also observed.

Optimal growth temperature

The optimum growth temperature of the colony was investigated by growing the test strains at different temperatures (10, 28, 37, and 45°C) in a growth chamber following the protocol suggested by the manufacturer.

Biochemical characterization

A series of biochemical tests (production of catalase, indole, citrate utilization, fermentation of glucose, sucrose, maltase, and mannitol, Voges-Proskauer test, methyl red test, pectolytic test, growth on MacConkey, Cetrimide, Mannitol Egg Yolk Polymyxin agar (MYP) and Azotobacter agar) were performed to characterize the isolated rhizobacteria followed by Bergey's Systematic Bacteriology Manual (Bergey *et al.*, 1994**)**. Bacteria were aseptically removed from a Petri dish with an inoculating needle for the KOH solubility test, then mixed with 3% KOH solution on a clean slide for 1 minute and observed for the formation of a thread-like mass. According to Simmons (1926), the citrate test was performed using Simmons citrate agar medium to evaluate the adaptation of rhizobacterial isolates to aerobic or anaerobic conditions. Catalase tests were performed by adding drops of freshly prepared 3% H_2O_2 (Hydrogen peroxide) for 48 h. old colony. The methyl red (MR) test was done using three drops of 0.1% methyl red in 5ml MR-VP broth. The Voges Proskauer (VP) test was done using 10% KOH in 5 ml 10% KOH. The Indole test was done with 0.5 ml Kovac's reagent. The Carbohydrate fermentation test was done with four basic sugar e.g., glucose, sucrose, maltase, and mannitol.

Bioassays for plant growth-promoting traits

Phosphate solubilizing ability

Pikovskaya medium was used to test the isolated culture's capacity to solubilize phosphate according to Pikovskaya's technique (1948). After 24 h of incubation, the capacity of strains to solubilize phosphate was demonstrated by the development of a halo zone around the colony. A halo zone was developed when the bacterial isolates generated organic acid, which solubilizes tri-calcium phosphate in the medium.

Detection of Indole-3-Acetic Acid (IAA)

The Salkowski's technique was used to determine whether the isolates produced indole-3-acetic acid (IAA) or not (Mohite, 2013). The isolates were incubated in yeast malt dextrose broth (YMD broth) at 28°C for four days (Himedia, India). The broth culture was centrifuged at 3000rpm for 30 minutes, and the supernatant was saved. Two milliliters of Salkowski's reagent (2% 0.5 FeCl3 in 35 % HCLO4 solution) were mixed with 1 ml of the supernatant and stored in the dark. Reagent mixed with sterile distilled water was used as a control. The inoculated reagents that turned red after 30 min of incubation suggest that the tested organism is an IAA-producing agent.

Production of ammonia

The ammonia production of the test isolates was accessed using Nessler's reagent (0.5ml) in a test tube containing freshly grown cultures mixed with 10ml peptone water. After 48-72 h of incubation at 37°C, each test tube was observed to form a brown to yellow color that was a positive indication of ammonia production (Cappuccino and Sherman, 1992).

Screening for antagonism

The antagonism was tested against FOL on 9-cm PDA plates using the dual culture technique (Riungu *et al*., 2008). FOL was collected from naturally infected tomato plants exhibiting a typical symptom of Fusarium wilt. A bacterial streak of each rhizobacterial strain suspension containing 10^8 CFU/ml was cross-streaked on the four edges of the petri-dish. A five mm actively growing mycelial disc was placed at the center of the PDA plate; incubated for five days at 28±2°C, and inhibition was calculated as described in Hossain *et al*. (2018).

$$\%\ \text{Inhibition of mycelial growth} = \frac{R1 - R2}{R1} \times 100$$

Where R1 = Radial mycelial growth of the pathogen in the absence of antagonists
R2 =Radial mycelial growth of FOL antagonistic bacteria treated plate. The experiment was repeated three times independently for each treatment.

Molecular characterization

Considering the plant growth-promoting traits and maximum antagonism against FOL, two isolates, ERB8 and ERB12, were selected for molecular characterization. Genomic DNA was extracted by Promega DNA extraction kit (Model: Maxwell 16, Origin: Promega, USA) following the manufacturer's instructions. The universal primers of 16SF-(AGAGTTTGATCCTGGCTCAG) and 16SR-(GGTTACCTTGTTACGACTT) were used for PCR amplification (Stackebrandt and Goodfellow, 1991). The PCR amplification was performed as Hasan et al. (2022) described. The Purified double-stranded PCR fragments were directly sequenced through a 3730xl DNA Analyzer using Big Dye Terminator Cycle sequencing kits (Applied Biosystems, Forster City, CA, USA) following the manufacturer's instructions. Sequences for each region were edited using Chromas Lite 2.01. The 16S rRNA gene was manually aligned with reference sequences retrieved from Gen Bank database1 and EzBio Cloud (Yoon *et al*., 2017) following BLAST searches for fast identification. The phylogenetic tree was constructed by the software package MEGA 10.2.1 (Tamura *et al.*, 2013) after multiple alignments of sequences data by CLUSTAL_X (Thompson *et al.*, 1997). The corrected evolutionary distance was evaluated according to Kimura's two-parameter model (Kimura, 1980), and the clustering was based on the neighbour-joining method (NJ tree, Saitou and Nei, 1987). Bootstrap analysis was applied to the tree topology by performing 1000 resampling (Felsenstein, 1985).

Statistical analysis

The mean and standard deviations of the inhibitions of radial growth were calculated. All the data were subjected to analysis of variance (ANOVA) using R software agricolae package v4.1.2 (R Core Team, 2021). Significant differences (P≤0.05) among the means were determined by Fisher's least significant differences test (LSD).

Results & Discussion

Strain isolation and morphological characterization

The isolated sixteen strains named ERB1, ERB2, ERB3, ERB4, ERB5, ERB6, ERB7, ERB8, ERB9, ERB10, ERB11, ERB12, ERB13, ERB14, ERB15, and ERB16, were naturally established in the acidic conditions located in Sylhet, Bangladesh. The morphological features of colonies of the isolates are shown in Table-1. The characteristics of the colony varied greatly. The isolates were fast-growing, round in shape, odorless, raised elevation, smooth shinning surface, smooth margin, different colors, and diameter. Under the microscope (ERB1, ERB2, ERB5, ERB6, ERB7, ERB8, ERB9, ERB10, ERB11, ERB12, ERB13, ERB14, ERB15, and ERB16) were observed rod-shaped which is also called bacilli. Bacterial isolates ERB3 and ERB4 were cocci or round-shaped, and all the isolates were motile. In gram reaction, seven isolates (ERB2, ERB3, ERB6, ERB7, ERB9, ERB14, and ERB16) were gram-positive, and nine isolates (ERB1, ERB4, ERB5, ERB8, ERB10, ERB11, ERB12, ERB13, and ERB15) were gram-negative.

Table 1. Morphological features of colonies of PGPR isolates.

Isolates	Shape	Size (mm)	Elevation	Surface	Margin	Color	Odor	Cell shape	Motility	Gram staining
ERB1	Circular	0.9	Umbonate	Smooth	Entire	Slightly yellow	Odorless	Rod	Motile	-
ERB2	Circular	0.8	Flat	Smooth	Filiform	Off white	Odorless	Rod	Motile	+
ERB3	Circular	0.9	Flat	Smooth	Endulate	Creamy	Odorless	Rod	Motile	+
ERB4	Circular	0.9	Flat	Smooth	Entire	Whitish	Odorless	Round	Motile	-
ERB5	Circular	0.8	Flat	Smooth	Entire	Watery	Odorless	Round	Motile	-
ERB6	Irregular	1.2	Flat	Rough	Erose	Off white	Odorless	Rod	Motile	+
ERB7	Circular	1.4	Umbonate	Smooth	Entire	Off white	Odorless	Rod	Motile	+
ERB8	Circular	0.9	Umbonate	Smooth	Entire	Yellowish	Odorless	Rod	Motile	-
ERB9	Circular	1.6	Flat	Smooth	Endulate	Off white	Odorless	Rod	Motile	+
ERB10	Circular	2.0	Flat	Smooth	Entire	Watery	Odorless	Rod	Motile	-
ERB11	Circular	0.8	Flat	Smooth	Filiform	Yellowish	Odorless	Rod	Motile	-
ERB12	Circular	1.2	Flat	Smooth	Endulate	Off white	Odorless	Rod	Motile	-
ERB13	Circular	0.7	Flat	Smooth	Entire	Off white	Odorless	Rod	Motile	-
ERB14	Circular	0.9	Flat	Smooth	Entire	Yellowish	Odorless	Rod	Motile	+
ERB15	Circular	1.0	Flat	Smooth	Filiform	Off white	Odorless	Rod	Motile	-
ERB16	Circular	0.9	Umbonate	Dry	Endulate	white	Odorless	Rod	Motile	+

NB: '+' indicates gram positive; and '-' indicates gram negative.

Growth of PGPR strains at different temperatures

The growth potential of the isolated rhizobacterial strain at different temperatures in Nutrient Agar media is shown in Table-2. Among sixteen PGPR isolates ERB6, ERB8, ERB12 and ERB14 were able to grow at 10°C and the rest 12 isolate (ERB1, ERB2, ERB3, ERB4, ERB5, ERB7, ERB9, ERB10, ERB11, ERB13, ERB15, ERB16) did not grow at 10°C. At 28°C temperatures, all the isolates were able to grow except ERB10 and ERB15. All 16 isolates showed heavy growth in Nutrient agar media at 37°C. Ten isolates ERB2, ERB3, ERB6, ERB7, ERB8, ERB9, ERB11, ERB13, ERB14, and ERB16 were able to grow at 45°C temperatures, but another six isolates (ERB1, ERB4, ERB5, ERB10, ERB 12 and ERB15) were unable to grow.

Table 2: Growth performance PGPRs at different temperatures.

Isolates	10°C	28°C	37°C	45°C
ERB1	-	++	++	-
ERB2	-	+	++	++
ERB3	-	+	++	++
ERB4	-	++	++	-
ERB5	-	+	++	-
ERB6	+	++	++	++
ERB7	-	++	++	++
ERB8	+	++	++	+
ERB9	-	++	++	++
ERB10	-	-	++	-
ERB11	-	+	++	+
ERB12	+	+	++	-
ERB13	-	++	++	+
ERB14	++	+	++	++
ERB15	-	-	++	-
ERB16	-	+	++	+

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

Biochemical characterization

The isolates ERB4, ERB6, ERB8, ERB9, ERB10, ERB12, ERB14, and ERB16 could use citrate as their sole carbon source, but the rest eight were negative in the citrate utilization. All sixteen isolates were catalase positive. Among sixteen isolates, ERB1, ERB4, ERB5, ERB8, ERB10, ERB11, ERB12, ERB13, and ERB15 were able to solubilize KOH and become a sticky substance. The rest seven isolates (ERB2, ERB3, ERB6, ERB7, ERB9, ERB14, and ERB16) showed negative results in the KOH solubility test. At 5% NaCl, seven isolates (ERB5, ERB7, ERB8, ERB10, ERB12, ERB14, and ERB15) were able to grow, but the remaining nine isolates (ERB1, ERB2, ERB3, ERB4, ERB6, ERB9, ERB11, ERB13, ERB16) failed to grow at the same concentration. Only a couple of strains, namely – ERB3, ERB4, ERB8, ERB12, ERB14, and ERB816 were able to breakdown the amino acid tryptophane to indole, whereas the remaining isolates (ERB1, ERB2, ERB5, ERB6, ERB7, ERB9, ERB10, ERB11, ERB13 and ERB15) exhibited a negative reaction in the indole test. The isolates ERB6, ERB7, and ERB9 were positive for the MR test, whereas ERB1, ERB2, ERB3, ERB4, ERB5, ERB8, ERB10, ERB11, ERB12, ERB13, ERB14, ERB15, and ERB16 tested negative for MR test. Five isolates (ERB6, ERB7, ERB9, ERB14, and ERB16)responded well to the VP test, but the remaining ERB1, ERB2, ERB3, ERB4, ERB5, ERB8, ERB10, ERB11, ERB12, ERB13, and ERB15 did not. All isolates fermented basic sugar glucose and produced acid in the glucose fermentation test except these three isolates ERB6, ERB8, and ERB13. In the case of sucrose, nine isolates (ERB2, ERB4, ERB5, ERB6, ERB7, ERB10, ERB12, ERB14, and ERB16) fermented it, whereas seven isolates (ERB1, ERB3, ERB8, ERB9, ERB11, ERB13, and ERB15) did not. Except for ERB5, ERB6, ERB8, ERB9, ERB11, and ERB15, all isolates showed the capacity to ferment maltose. Except for ERB9 and ER13, all isolates showed a positive response in the mannitol fermentation test. The response of different isolates to different biochemical tests is shown in Table-3.

Table 3: Biochemical characteristics of PGPR isolates.

Isolates	Citrate	Catalase	NaCl	KOH	Indole	MR	VP	Glucose	Sucrose	Moltase	Mannitol
ERB1	-	+	-	+	-	-	-	+	-	+	+
ERB2	-	+	-	-	-	-	-	+	+	+	+
ERB3	-	+	-	-	+	-	-	+	-	+	+
ERB4	+	+	-	+	+	-	-	+	+	+	+
ERB5	-	+	+	+	-	-	-	+	+	-	+
ERB6	+	+	-	-	-	+	+	-	+	-	+
ERB7	-	+	+	-	-	+	+	+	+	+	+
ERB8	+	+	+	+	+	-	-	-	-	-	+
ERB9	+	+	-	-	-	+	+	+	-	-	-
ERB10	+	+	+	+	-	-	-	+	+	+	+
ERB11	-	+	-	+	-	-	-	+	-	-	+
ERB12	+	+	+	+	+	+	-	+	+	+	+
ERB13	-	+	+	-	-	-	-	-	-	+	-
ERB14	+	+	-	-	+	-	+	+	+	+	+
ERB15	-	+	+	+	-	-	-	+	-	-	+
ERB16	+	+	-	-	+	-	+	+	+	+	+

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

Characterization for plant growth promoting traits

The bioassay for plant growth-promoting traits is presented in Table-4. Sixteen pure bacterial isolates were screened for plant growth-promoting traits such as phosphate solubilization, IAA production, and NH_3 production. Among 16 rhizobacterial isolates, only five isolates (ERB4, ERB8, ERB12, ERB14, and ERB16) were positive in Pikovskayas agar and formed a halo zone around the colonies which indicates phosphate solubilization. The ammonia production was confirmed by the formation of brown color in the presence of Nesler's reagent in broth culture. The production of ammonia was observed in all the isolates. The typical reddish to pinkish hue indicator in the solution suggested the IAA production in the cultured medium. Five bacterial isolates (ERB4, ERB8, ERB12, ERB14, and ERB16) were positive for Salkowski's reagent.

Table 4: Plant growth-promoting traits of PGPR isolates.

Isolates	Phosphate solubilizing ability	IAA production	Production of ammonia
ERB1	-	-	+
ERB2	-	-	+
ERB3	-	-	+
ERB4	+	+	+
ERB5	-	-	+
ERB6	-	-	+
ERB7	-	-	+
ERB8	+	+	+
ERB9	-	-	+
ERB10	-	-	+
ERB11	-	-	+
ERB12	+	+	+
ERB13	-	-	+
ERB14	+	+	+
ERB15	-	-	+
ERB16	+	+	+

NB: '+' indicates positive growth; and '-'indicates no growth.

3.4. Effects of antagonistic activity of Rhizobacteria

In vitro, antagonistic activity tests showed that Rhizobacteria could inhibit the growth of *Fusarium oxysporum* f. sp. *Lycopersici* (Figure-1). Among sixteen rhizobacterial isolates, six (ERB2, ERB5, ERB8, ERB12, ERB14, and ERB16) exhibited significant antagonistic activity against FOL on PDA plates. The highest inhibition zone was observed in ERB8 (66.85%), followed by ERB12 (62.38%), and the lowest inhibition zone was observed in ERB2 (36.67%)

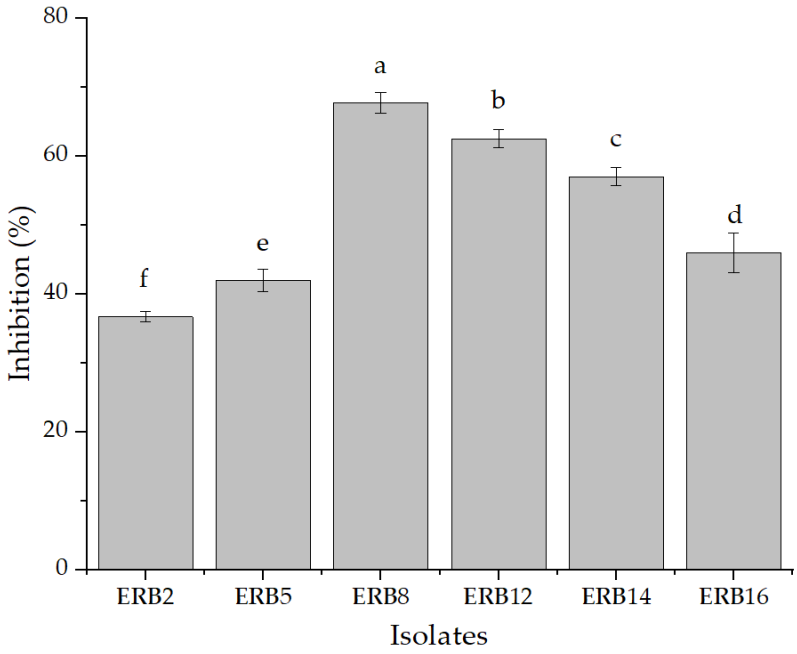

Figure 1: In vitro radial mycelial growth inhibition of *F. oxysporum* by sixteen different bacterial isolates. Different letters indicate significant differences between treatments at ($P<0.05$) using the LSD test.

3.5 Molecular characterization and identification

Among the sixteen isolates, ERB4, ERB8, ERB12, ERB14, and ERB16 were found to be promising for all PGP attributes and antagonism of phytopathogen (Fig.1). Based on the morphological and biochemical analysis (Table-1, Table-2, Table-3, Table-4), two *Bacillus* species (ERB12, ERB14), one *Pseudomonas* (ERB8), and one *Azotobacter* species (ERB4), and one *Enterobacter* species (ERB12) were identified. Based on the highest antagonistic attributes, rhizobacteria (ERB8 and ERB12) were molecularly characterized by 16S rRNA sequences. BLAST searches on the NCBI data libraries for similarities between ERB8 and ERB12 were found to be most similar (about 88%) *with Pseudomonas stutzeri* and *Enterobacter cloacae*, respectively. The sequence identified was further characterized by multiple alignment with the similar sequences found in the BLAST search. The phylogenetic tree was constructed from translated protein and showed a close relationship between ERB8 and ERB12 with *Pseudomonas stutzeri* and *Enterobacter cloacae*, respectively (Figure-2).

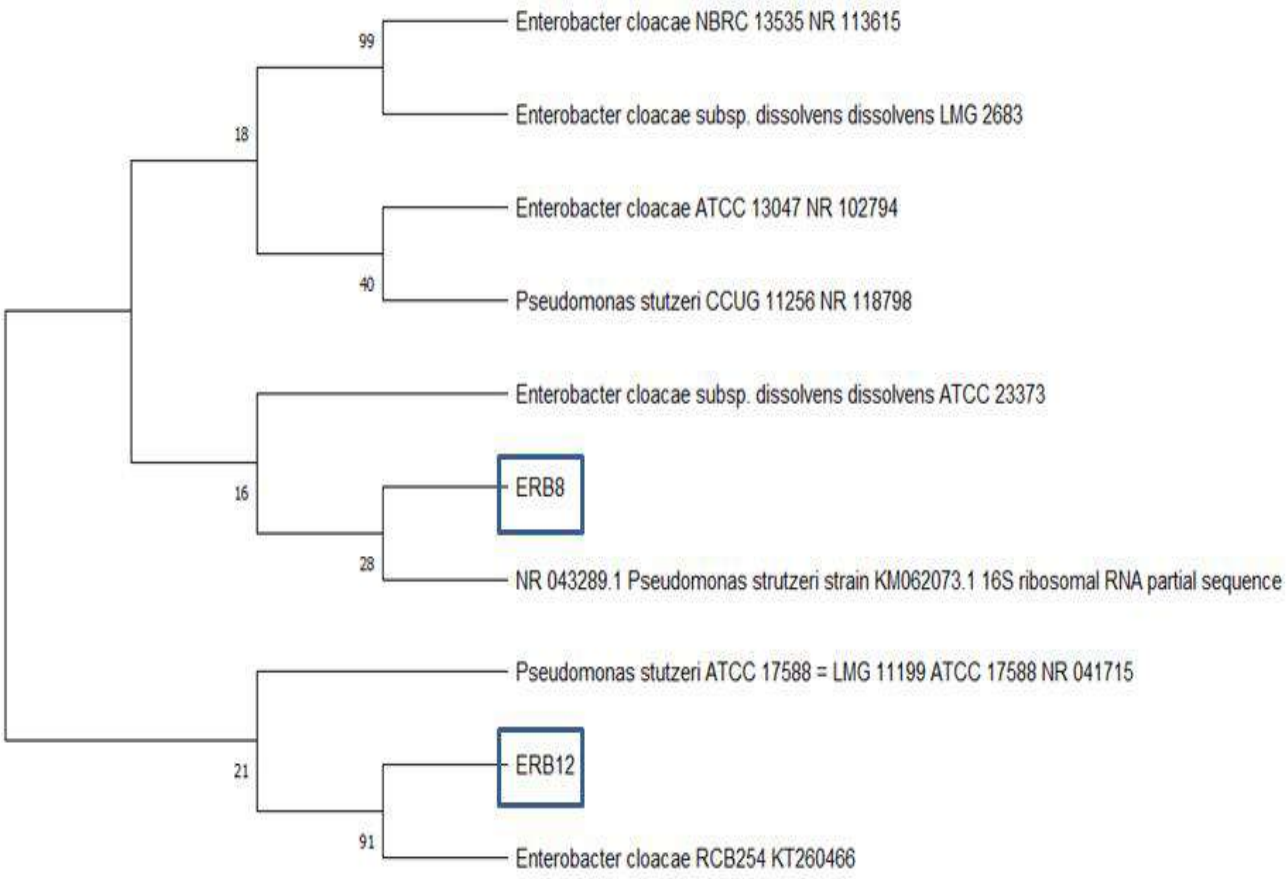

Figure 2: Phylogenetic relationship of PGPR isolates and the typical known PGPR isolates inferred by analysis of 16S rRNA sequences.

Discussion

PGPR invades plant roots and encourages the growth and development of plants through several mechanisms. The screening of PGPR and benefits of PGPR are widely reported in crops, especially rice, wheat, corn, and sugar cane, but few from tomatoes. In this research, rhizobacteria isolated from the tomato rhizosphere were screened for different plant-growth-promoting activities. Five strains were selected based on plant growth promotion and antagonistic activities against *Fusarium oxysporum* (ERB4, ERB8, ERB12, ERB14, and ERB16). Colony morphology and results of the biochemical tests indicated that ERB4, ERB8, ERB12, ERB14, and ERB16 belonged to *Azotobacter*, *Pseudomonas*, *Enterobacter*, and *Bacillus* genera. For more accurate identification ERB8 and ERB12 were molecularly characterized. 16S rRNA sequence analysis of strain ERB8 showed 88% similarity with *Pseudomonas strutzeri* (Accession No. KM062073.1), and ERB12 had 88% homology with *Enterobacter cloacae* (Accession No. KT260466.1). Among 16 isolates, only five isolates (ERB4, ERB8, ERB12, ERB14, and ERB16) showed positive plant growth promotion activities such as phosphate solubilization, IAA and NH_3 production. Phosphorus is one of the most important essential plant nutrients that remain insoluble in soil. As a result, plants cannot utilize it (Song *et al.,* 2021). Phosphate solubilizing bacteria solubilize mineral phosphate by forming organic acids, thus making phosphorus available for plants and increasing plant growth by enhancing cell elongation attributed to phosphorous (Wagh *et al.*, 2014). IAA is the precursor of phytohormone auxin. These five isolates are reported to produce IAA that can improve plant growth (Hossain *et al.*, 2016). All the isolates in the study were positive in the NH3 production test with the Neslers reagent. PGPR increases the nitrogenase enzyme activity that converts molecular nitrogen into ammonia and directly improves plant growth by improving root architecture and indirectly affecting plant pathogens (Yadav *et al.*, 2016). Production of ammonia by PGPR in the presence of Nesler's reagent was also reported by Goswami et. al. 2014 and Moustaine et. al., 2017.

Control of plant disease through fungal antagonists such as *Trichoderma viride*, *T. harzianum*, *T. asperellum*, *T. longibrachiatum*, and *Beauveria bassiana* was reported by several workers (Ghosh *et al.*, 2018). Some bacteria also possess the potential to control plant diseases. Isolated PGPR was also tested to know their ability to inhibit the mycelial growth of pathogenic fungi *Fusarium oxysporum*. The highest antagonistic activity was observed by ERB8 (66.85%) followed by ERB12 (62.38%), ERB14 (56.73%), ERB16 (49.91%), ERB5 (42.32%), and the lowest was observed by ERB2 (36.67%). PGPR shows antagonistic activities by producing toxins such as HCN, antibiotics, and some defense-related enzymes, viz., proteases, amylases, glucanases, and chitinases (Bardin *et al.*, 2015). . Previous research works have supported the fungal antagonism by PGPR and bio-control activities of *Pseudomonas, Azotobacter, Bacillus,* and *Enterobacter* (Thanh *et al.*, 2009, Rawat *et al.*, 2020). *Bacillus subtilis* can protect against the Fusarium wilt of tomato by triggering the synthesis of phenolics, flavonoids, and plant defense enzymes (Akram *et al.,* 2021). Islam *et al.*, (2016) isolated potential PGPR from cucumber rhizosphere in Bangladesh that significantly suppressed Phytophthora crown rot caused by *Phytophthora capsici*. Bio-control of Phytopathogens viz. *Fusarium* sp., *Macrophomina* sp., and *Pythium* sp. can be obtained by *Pseudomonas aeruginosa*(Pandya & Shelat, 2017). Verma and Shahi (2015) also found *Enterobacter cloacae* as a potential plant-growth-promoting agent in the potato rhizosphere. Deshwal & Thapliyal (2019) reported a significant improvement in plant growth in rice by PGPR. PGPR is also gaining importance as a biofertilizer because these microbial populations can improve soil health by fixing atmospheric nitrogen and making it available for plant uptake (Islam *et al.*, 2016).

Conclusions

The study clearly demonstrated that tomato rhizospheric soils harbour diversified beneficial bacteria, which may be promising plant-growth-promoting and bio-control agents. *Azotobacter* sp., *Bacillus* sp., *Pseudomonas strutzeri,* and *Enterobacter cloacae* were highly effective in antifungal activity against *Fusarium oxysporum in vitro*. Their ability to produce plant growth-promoting compounds and antagonistic activity indicated that they might be used as bio-fertilizer and biocontrol agents.

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Conflicts of Interest

The authors declare no conflict of interest.

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