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RESEARCH ARTICLE

Plant growth promoting rhizospheric bacteria associated with *Oroxylum indicum* (L.) Benth. ex Kurz

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Abstract

Oroxylum indicum (L.) Benth. ex Kurz is a threatened plant species, largely due to overexploitation for its valuable medicinal properties. This study focused on isolating and screening rhizospheric bacteria associated with *O. indicum* for their plant growth-promoting (PGP) traits, including ammonia production, hydrogen cyanide (HCN) production, indole-3-acetic acid (IAA) production, and phosphate solubilization. Out of 60 bacterial isolates, 35 exhibited one or more PGP activities. Morpho-molecular characterization of these 35 isolates identified seven bacterial species, with relative abundances of 2.86% *Enterobacter roggenkampii*, 2.86% *Pseudomonas taiwanensis*, 2.86% *Staphylococcus arlettae*, 5.71% *Bacillus tropicus*, 5.71% *Bacillus velezensis*, 20% *Bacillus thuringiensis*, and 60% *Bacillus cereus*. All isolates tested positive for ammonia production, while 51.43% demonstrated phosphate solubilization with varying solubility indices. Additionally, 11.43% of the isolates tested positive for HCN production, and 8.57% for IAA production. The highest IAA production was recorded in *Enterobacter roggenkampii* (*Erog*-1) at 5.75 µg/ml, followed by *Bacillus tropicus* (*Btro*-7) and *Bacillus cereus* (*Bcer*-13) at 0.125 µg/ml. The most effective phosphate solubilizer was *Pseudomonas taiwanensis* (*Ptai*-40), with a solubility index of 3.916 ± 0.144 mm, followed by *Enterobacter roggenkampii* (*Erog*-1) with an index of 3.076 ± 0.292 mm. Significant variations in PGP activities were observed at the strain level within species. These findings highlight the potential of certain rhizospheric bacteria associated with *O. indicum* as promising bioinoculants for enhancing plant growth and agricultural productivity.

Keywords: PGPR; Ammonia production; HCN production; IAA production; Phosphate solubilization

1. Introduction

Oroxylum indicum (L.) Benth. ex Kurz is a plant of significant medicinal importance that belongs to the family Bignoniaceae. Commonly, it is known as the midnight horror, Indian trumpet flower, or broken bones tree in English. In India, it is recognized by various vernacular names, such as *Shoyanka*, *Sonpatha*, and *Bhatghila*, and has been used since time immemorial for its broad spectrum of medicinal properties (Debi and Parkash, 2020). The plant's distribution is primarily limited to the Eastern and Western Ghats, as well as the northeastern regions of India. Every part of this tree has medicinal value, and different parts are sometimes used to treat different ailments. Root of this plant is used as one of the key components of the well-known 'Dasamula' group in the Indian Ayurvedic system of medicine, where it is utilized for its astringent, anti-helminthic, anti-inflammatory, anti-leucodermatic, anti-bronchitic, anti-rheumatic, and anti-anorexic properties, as well as for treating leprosy and tuberculosis (Manonmani et al., 1994, 1995; Taru et al., 2022). Detailed information on its medicinal properties and uses in various diseases can be found in review articles (Raghu et al., 2013; Jagetia, 2021).

Due to its immense medicinal value, *O. indicum* has been subject to unscientific harvesting and exploitation from wild habitats (Debi and Parkash, 2020). Such practices have led to the plant being classified as highly threatened and vulnerable (Ravikumar and Ved, 2000; Saraf et al., 2013). *O. indicum* thrives well in the soils of Assam, and disease incidence on this plant is rare in its natural habitats. Its healthy growth may be attributed to the favorable soil conditions and the associated microbiome, which often promotes plant growth and provides protection against diseases and environmental stresses. The microbial population in rhizospheric soils is considered one of the key factors contributing to plant growth, as these microbes play a direct or indirect role in promoting the growth of their host plants.

A wide range of microorganisms is known to colonize rhizospheric soils (the soil surrounding the roots) due to the release of plant metabolites through the roots. These microorganisms facilitate the degradation of organic matter, nutrient cycling, mineralization, and the production of essential growth factors and antimicrobial compounds that benefit the host plants. Such mutual interactions between microorganisms and their respective host plants can lead to close associations among compatible microbes, which may vary from plant to plant. Therefore, rhizospheric microbes can have a direct or indirect influence on a plant's health through their beneficial or detrimental activities (Bertrand et al., 2001).

Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that inhabit the rhizosphere of plants and may grow in, on, or around plant tissues. They play a crucial role in enhancing plant growth and development through a variety of mechanisms. Depending on their physical interaction with the host plant, PGPR can be classified into two categories: (1) rhizospheric PGPR and (2) endophytic PGPR (Vessey, 2003).

PGPR may enhance plant growth by synthesizing plant hormones or by facilitating the uptake of nutrients from the soil through various direct mechanisms, such as atmospheric nitrogen (N) fixation, phosphorus (P) solubilization, siderophore production for iron sequestration, and ACC deaminase activity. PGPR also act as biocontrol agent by producing antibiotics, triggering induced local or systemic resistance, or degrading harmful substances (rhizoremediation) to prevent the adverse effects of xenobiotics (Ahemad and Kibret, 2014; Gupta et al., 2024). Although the exact mechanisms through which PGPR stimulate plant growth are not fully understood, the production of phytohormones, suppression of harmful organisms, activation of phosphate solubilization, and enhancement of mineral nutrient uptake are commonly suggested as possible mechanisms (Bowen et al., 1999; Liu et al., 1992).

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Table 1. Plant growth promoting activities of bacteria isolated from the rhizospheric soils of *Oroxylum indicum*. '+' and '-' indicates positive and negative results in qualitative tests. Values in parentheses indicate quantitative results.

Isolate code	Ammonia Production	HCN Production	Phosphate Solubilisation (Solubility index in mm)	IAA Production (Conc. in µg/ml)	Positive test count
Erog-1	+	-	+ (3.076 ± 0.292)	+ (5.75)	3
Bcer-3	+	-	-	-	1
Bthu-4	+	+ (weak)	-	-	2
Bcer-5	+	-	-	-	1
Bcer-6	+	-	+ (2.19±0.017)	-	2
Btro-7	+	-	+ (2.4±0)	+ (0.125)	3
Bcer-8	+	-	-	-	1
Bthu-9	+	-	+ (2.333±0.115)	-	2
Btro-11	+	-	+ (2.4±0)	-	2
Bcer-13	+	-	-	+ (0.125)	2
Bcer-14	+	-	-	-	1
Bthu-16	+	-	-	-	1
Bcer-17	+	-	-	-	1
Bthu-18	+	-	+ (2.333±0)	-	2
Bcer-19	+	-	+ (2.376±0.040)	-	2
Bcer-20	+	-	-	-	1
Bcer-21	+	-	+ (3.0±0)	-	2
Bcer-22	+	-	-	-	1
Bcer-24	+	+	-	-	2
Bcer-25	+	-	-	-	1
Bcer-27	+	-	-	-	1
Bcer-28	+	-	-	-	1
Bcer-29	+	-	-	-	1
Bcer-30	+	-	+ (2.376±0.040)	-	2
Bthu-32	+	-	+ (2.353±0.040)	-	2
Bcer-33	+	-	-	-	1
Bthu-35	+	-	+ (2.473±0.046)		2
Bcer-37	+	-	-	-	1
Bthu-38	+	+	+ (2.433±0.057)	-	3
Ptai-40	+	+	+ (3.916±0.144)	-	3
Bcer-41	+	-	+ (2.333±0)	-	2
Sarl-43	+	-	+ (3.166±0.288)	-	2
Bcer-47	+	-	+ (2.55±0.180)	-	2
Bvel-49	+	-	+ (2.533±0.075)	-	2
Bvel-50	+	-	+ (2.353±0.101)	-	2
Isolate count with positive results	35	4	18	3	

PGPR have been shown to effectively promote plant growth and development in a variety of crops, and research continues to explore the mechanisms underlying interactions between PGPR and their host plants (Singh et al., 2020). Fungal microbiota associated with *O. indicum* were studied earlier several times (Nath et al. 2016; Das and Narzary, 2017; Debi and Parkash, 2017; Juanjuan et al., 2023). Studies on bacterial microbiota associated with *O. indicum* are very limited and Debi and Parkash (2017) reported four isolates of bacteria without assessing their plant growth-promoting activities. In this study, we focus on the isolation and screening of bacteria associated with the rhizospheric soils of *O. indicum* to evaluate their plant growth-promoting activities.

2. Materials and methods

2.1. Collection of Rhizosphere Soil Samples

Soils adhering to the roots of healthy *Oroxylum indicum* plants (Figure 1) were collected from the Botanical Graden, Gauhati University Campus, Guwahati, Assam, India. The plant species was

confirmed in consultation with the plant taxonomists and the herbarium specimens available in GUBH (accredited with New York Botanical Garden Steere Herbarium), Department of Botany, Gauhati University. Roots of medium-sized plants (3–5 m in height) were traced below ground, and adhering soils were carefully removed (Shrivastava, 2015). The soil samples were collected in sterile polypropylene bags and stored immediately at 4 °C for further processing.

2.2. Isolation of Bacterial Pure Cultures

Bacterial pure cultures were obtained through soil dilution plating and subsequent sub-culturing on Nutrient Agar (NA) medium. Pure cultures were grown in NA slants and preserved at 4 °C for regular use and at −80 °C with 15% glycerol for long-term preservation.

2.3. Assay for Plant Growth-Promoting (PGP) Activity

All bacterial isolates were screened for different PGP activities as described below.

2.3.1. Ammonia Production Test

Freshly grown cultures of bacterial isolates were inoculated into tubes containing 10 ml peptone water and incubated for 48–72 hours at 30 °C. Nessler's reagent (0.5 ml) was added to each tube, and the development of a yellow to brown color was considered a positive test, while no change in color was considered a negative test (Prasad and Dagar, 2014).

2.3.2. Phosphate Solubilizing Test

The bacterial isolates were inoculated into sterile Pikovskaya medium containing tri-calcium phosphate and incubated at 30 °C for 3 to 7 days. The formation of a clear zone around the colony indicates phosphate solubilization by the bacteria (Pikovskaya, 1948). The solubilization index was calculated following the method of Alam et al. (2002).

Phosphate Solubilization Index (PSI) = A/B
where, A = total diameter (colony + halo zone), and B = diameter of colony

2.3.3. HCN Production Test

Each bacterial pure culture was inoculated on NA plates supplemented with glycine (4.4 g/l). A Whatman filter paper soaked in sodium carbonate solution (2% w/v sodium carbonate dissolved in 0.5% w/v picric acid) was placed in the upper lid of the Petri plate to cover the inoculated plate. The plates were sealed properly with parafilm to prevent the escape of gases and incubated in a bacteriological incubator (Optics Technology, India) at 30 °C for 2 days to observe the color changes due to microbial action. A color change in the filter paper from yellow to orange or reddish-brown indicates HCN production (Bakker and Schippers, 1987).

2.3.4. Indole-3-Acetic Acid (IAA) Production Test

Active cultures of each bacterial isolate were grown in nutrient broth, from which a 50 µl volume was used as inoculum to further grow in 1.5 ml of nutrient broth medium supplemented with L-tryptophan (50 µg/ml). The cultures were incubated in a shaking incubator set at 28 ± 2 °C and 180 rpm. After 48 hours of incubation, the bacterial suspension in broth was centrifuged at 10,000 rpm for 10 minutes, and 1 ml of the supernatant was mixed with 4 ml Salkowski's reagent (prepared by adding 2 ml of 0.5 mol L^{-1} $FeCl_3$ to 98 ml of 35% $HClO_4$). After 1 hour of reaction, if the mixture turned pink to red, it confirmed IAA production. The quantity of IAA production was also estimated using a spectrophotometer (BioSpectrometer, Eppendorf, Germany) based on a standard curve of known IAA concentrations (1–10 µg/ml) measured at 530 nm absorbance (Patten and Glick, 1996).

2.4. Characterization and Identification of PGP-Positive Isolates

The bacterial strains showing positive PGP activity were characterized and identified using a polyphasic approach (morphological, biochemical, and molecular). Fresh cultures of each isolate were stained with Gram's reagents following the method described by Aneja (2007) and observed under a trinocular compound microscope (ZEISS AXIO Imager) at 1000× magnification. Colony morphology, cell shape and size, and Gram's reaction were recorded for each isolate. Additionally, the following biochemical tests were conducted to characterize the isolates:

3.4.1. Indole Test

The indole test was carried out using the Kovacs method as described by Aneja (2007). Freshly grown bacterial cultures were inoculated into tubes containing tryptone broth and incubated at 30 °C for 48 hours. After incubation, 1 ml of Kovac's reagent was added to each tube, and the mixture was gently shaken. After allowing the tubes to stand undisturbed for a few minutes, the formation of a cherry-red ring at the surface of the medium indicated a positive indole test, while no color change indicated a negative result.

2.4.2. Oxidase Test

The oxidase test was conducted following the method described by Kovacs (1956). A filter paper was saturated with a 1% solution of tetra-methyl-p-phenylenediamine dihydrochloride and allowed to dry. A bacterial colony was then picked with a sterile platinum loop and rubbed onto the treated filter paper. A color change to dark purple within 30 seconds indicated a positive oxidase reaction, signifying the presence of cytochrome c oxidase, while no color change was interpreted as a negative result.

2.4.3. Glucose Fermentation Test

For the glucose fermentation test, freshly grown bacterial cultures were inoculated into fermentation tubes containing glucose broth medium, with a Durham tube placed in an inverted position to detect gas production. The tubes were incubated at 30 °C for 24 to 48 hours. Acid production was indicated by a change in the color of the medium, and gas production was evidenced by the appearance of a bubble in the Durham tube (Collee et al., 1996).

2.4.4. Lactose Fermentation Test

Similarly, for the lactose fermentation test, bacterial isolates were inoculated into fermentation tubes containing lactose broth medium with an inverted Durham tube. The tubes were incubated at 30 °C for 24 to 48 hours. The formation of acid was determined by a color change in the medium, while gas production was observed as a bubble in the Durham tube, following standard microbiological methods (Cappuccino and Sherman, 2013).

2.4.5. Methyl Red-Voges-Proskauer (MR-VP) Test

The Methyl Red (MR) and Voges-Proskauer (VP) tests were performed using pre-sterilized tubes containing MR-VP broth as per the guidelines described by Clark and Lubs (1915).
Methyl Red Test**:** After incubation of the bacterial isolates in the MR-VP broth at 37 °C for 48 hours, 5 drops of methyl red indicator were added to the culture. A red coloration indicated a positive result, while a yellow color denoted a negative test.
Voges-Proskauer Test**:** For the VP test, to each test tube containing MR-VP broth, 12 drops of VP reagent I (alpha-naphthol) were added, followed by 2–3 drops of VP reagent II (potassium hydroxide). The tubes were gently shaken and left exposed to air. The development of a crimson-to-ruby pink color within 15–30 minutes signified a positive reaction, while no color change indicated a negative result (Barritt, 1936).

2.4.6. Urease Test

The urease test was conducted using urea broth as described by Christensen (1946). Freshly grown bacterial isolates were inoculated into the urea broth and incubated at 37 °C for 48 hours. A color change to pink indicated a positive result for urease activity, suggesting the hydrolysis of urea to ammonia and carbon dioxide.

2.4.7. Hydrogen Sulfide (H_2S) Production Test

To assess H_2S production, Sulfide Indole Motility (SIM) agar medium was used. Tubes were stab-inoculated with bacterial cultures and incubated at 37 °C for 48 hours, following the method of Edwards and Ewing (1972). A black coloration along the stab line was considered a positive result for H_2S production, while the absence of color change denoted a negative result.

2.4.8. Acid-Fast Staining

The acid-fast staining procedure was performed as per the Ziehl-Neelsen method described by Aneja (2007). Air-dried and heat-fixed smears of bacterial isolates were flooded with carbol fuchsin and gently heated until steaming for 3–5 minutes to enhance penetration. The slides were then cooled, rinsed with distilled water, and decolorized with acid alcohol for 10–30 seconds. After

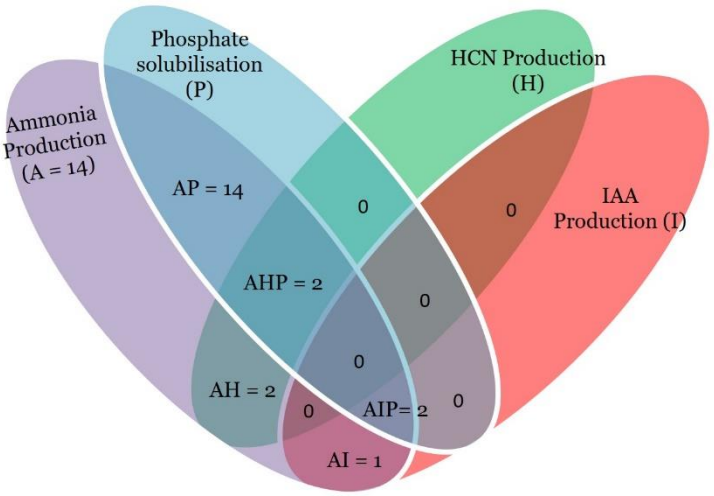

Figure 1. Distribution of plant growth promoting rhizobacterial isolates in Venn diagram representing four different PGP activities. AI – ammonia and IAA production; AIP – ammonia production, IAA production, and phosphate solubilisaton; AH – ammonia and HCN production; AHP – ammonia production, HCN production, and phosphate solubilisaton; and AP – ammonia production and phosphate solubilisaton.

washing again with water, the slides were counterstained with methylene blue for 1–2 minutes, rinsed, and dried. Acid-fast cells retained the red color of carbol fuchsin, while non-acid-fast cells appeared blue, indicating a negative result.

2.5. Molecular Characterization Based on 16S rDNA Amplification and Sequencing

The PGPR isolates were processed for genomic DNA isolation using the enzymatic-heat lysis method of Ahmed et al. (2014), with modifications in lysis conditions, washing steps, and centrifugation steps. Bacterial cells were harvested from 24-hour-old nutrient broth cultures by centrifugation (Centrifuge 5810 R, Eppendorf, Germany) at 5,000 rpm for 5 minutes. The cell pellets obtained were washed twice with 1 ml of sterile 0.5 M NaCl and once with sterile double-distilled water. Then, 490 µl of 1× TE buffer was added to the cells along with 10 µl of lysozyme (2 mg/ml) and incubated for 30 minutes at 37 °C. Heat cell lysis and enzyme denaturation were achieved by incubating the solution at 95 °C for 20 minutes. After incubation, the crude cells were separated from the lysates by centrifugation at 10,000 rpm for 10 minutes at 40 °C and allowed to cool to room temperature. Precipitation of the genomic DNA was assessed by adding an equal volume of chilled absolute ethanol to the lysates and incubating at –20 °C for 1 hour. The suspension was then centrifuged at 14,000 rpm for 30 minutes at 4 °C. The supernatant obtained was discarded, and the pellets remaining in the tubes were washed twice with 500 µl of chilled 70% ethanol and centrifuged for 5 minutes at 14,000 rpm at 4 °C. The DNA pellets were finally resuspended in 50 µl of 1× TE buffer. The quality of the extracted DNA was estimated by measuring absorbance at 230 nm, 260 nm, and 280 nm using a UV/VIS spectrophotometer (BioSpectrometer, Eppendorf, Germany).

2.5.1. PCR Amplification of 16S rDNA Marker

PCR amplification of the 16S rDNA gene marker was performed using universal forward and reverse primers, i.e., 8F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-ACCTTGTTACGACTT-3′), respectively (Youssef et al., 2009) procured from GCC Biotech, Kolkata. A total volume of 25 µl reaction mixture was prepared in grade-1 water (Merck, Millipore), containing 30 ng of DNA template, 1× PCR buffer (Genei), 0.3 U *Taq* DNA polymerase (Genei), 0.2 mM dNTP mix (Genei), and 10 µM each of the forward and reverse primers. The PCR cycle was carried out in a Mastercycler Nexus GSX1 (Eppendorf) under the following conditions: pre-denaturation at 94 °C for 5 minutes; followed by 40 amplification cycles consisting of denaturation at 94 °C for 1 minute, annealing at 50 °C for 30 seconds, and extension at 72 °C for 1 minute; and a final extension at 72 °C for 5 minutes. The amplification of the target sequence was confirmed by running it on a 1% agarose gel, and results were visualized and recorded using a gel imaging system (UVITEC Cambridge Fire Reader). Purification of the amplified PCR product was performed using the QIAquick Gel Extraction Kit (QIAGEN) following the manufacturer's recommendations. The purified PCR products were sent for Sanger sequencing to the service provider (Eurofins India).

2.5.6. DNA Sequence Analysis and Homology Search

DNA sequencing results received from the service provider were checked for quality and errors (if any) by viewing the ABI files in MEGA-X (Kumar et al. 2018). Noisy sequences from both ends were trimmed to retrieve quality sequences. Contig sequences were generated for each pair of forward and reverse sequences using CAP3 software (Huang and Madan, 1999). The contig sequences obtained were subjected to a BLAST homology search against the NCBI nucleotide database for species identification. All contig sequences were submitted to the NCBI database to obtain GenBank accession numbers.

3. Results

A total of 60 pure culture isolates were recovered from the rhizospheric soils of *O. indicum*. Out of these, 35 isolates tested positive for at least one or more plant growth-promoting (PGP) activities (Table 1, Figure 1). All 35 isolates showed positive results for ammonia production, 18 isolates were positive for phosphate solubilization with varied solubility indices (Figure 2A-D), 4 isolates were positive for HCN production (Figure 2E-F), and 3 isolates were positive for IAA production (Table 1). Only four isolates (*Erog*-1, *Btro*-7, *Bthu*-38, and *Ptai*-40) exhibited positive results for three different PGP tests. Approximately 50% of the isolates showed positive results for two different PGP tests, but none of the isolates tested positive for all four PGP tests (Table 1, Figure 1). The isolate *Ptai*-40 showed the highest phosphate solubility index (3.916 ± 0.144 mm), whereas the isolate *Bcer*-6 had the lowest solubility index (2.19 ± 0.017 mm) among the isolates. *Erog*-1 produced the highest concentration of IAA (5.750 µg/ml), compared to the other two isolates (*Btro*-7 and *Bcer*-13), which produced only 0.125 µg/ml of IAA (Table 1).

Gram staining and microscopic analysis revealed that two isolates (*Erog*-1 and *Ptai*-40) were Gram-negative rods, one isolate (*Sarl*-43) was a Gram-positive coccus, while the remaining isolates were Gram-positive rods (Table 2, Figure 3). None of the isolates tested positive for indole production, H_2S production, or acid-fast staining. Only two isolates (*Erog*-1 and *Ptai*-40) showed positive results in the citrate test. For the oxidase test, all isolates were oxidase-positive except for *Sarl*-43; *Erog*-1 exhibited a delayed oxidase-positive reaction. All the isolates tested positive in the glucose fermentation test, with bubble formation observed in the case of *Erog*-1. Only two isolates (*Erog*-1 with bubble formation and *Sarl*-43 without bubble formation) were positive in the lactose fermentation test. Three isolates (*Erog*-1, *Ptai*-40, and *Sarl*-43) were found urease positive, while twelve isolates (*Bthu*-4, *Bthu*-9, *Btro*-11, *Bcer*-14, *Bcer*-17, *Bthu*-18, *Bcer*-21, *Bcer*-29, *Bthu*-35, *Bcer*-37, *Bcer*-41, and *Sarl*-43) tested positive for the methyl red

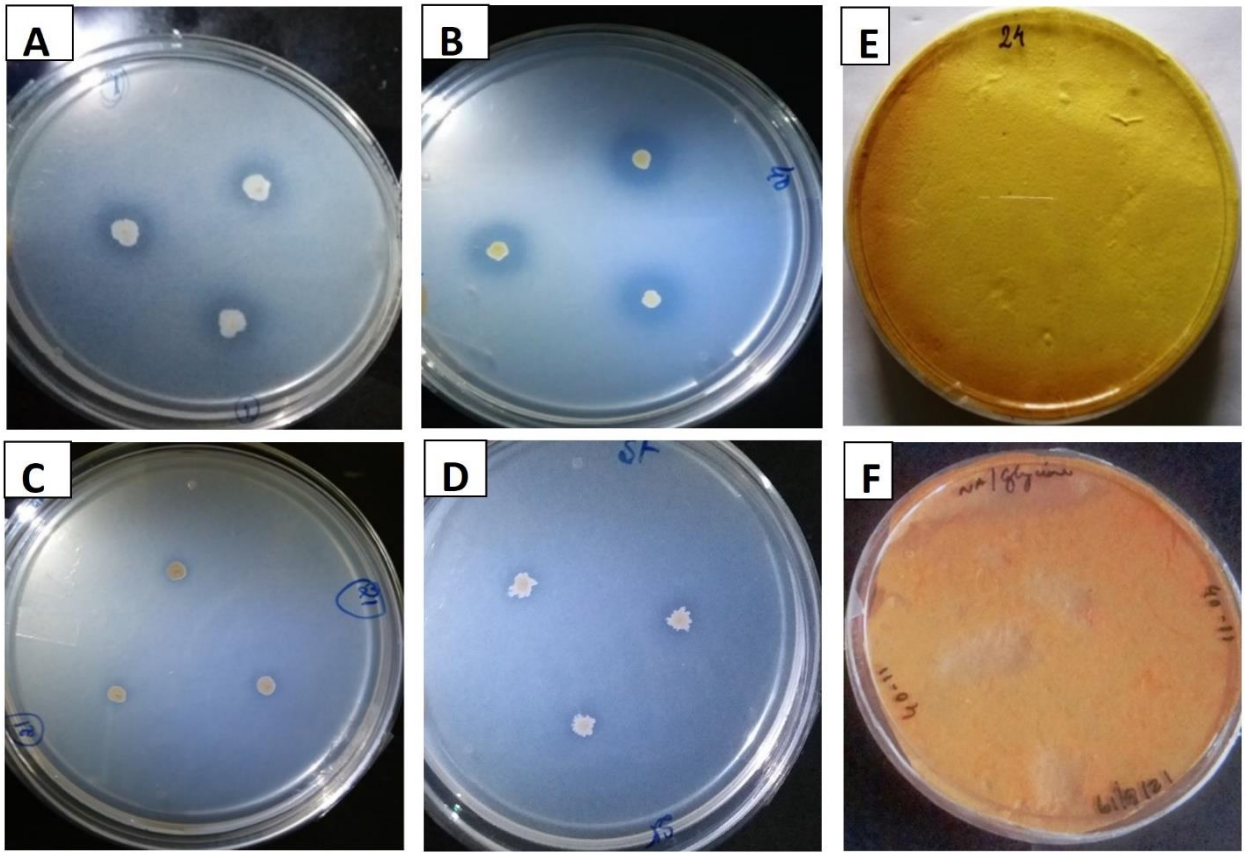

Figure 2. Representative photographs of some phosphate solubilizing and HCN producing bacterial isolates. A, B, C, D – with different degree of halo zone formation in Pikovskaya agar medium by the isolates *Erog*-1, *Bcer*-21, *Ptai*-40 and *Bcer*-41, respectively. E, F – positive HCN production test in nutrient agar medium by the isolates *Bcer*-24 and *Ptai*-40, respectively.

test. All isolates, except *Ptai*-40 and *Sarl*-43, were positive for the Voges–Proskauer test.

Molecular characterization based on 16S rDNA sequences identified seven species of bacteria (Table 3, Figure 4), with a relative abundance of 60% *Bacillus cereus*, 20% *Bacillus thuringiensis*, 5.71% each of *Bacillus tropicus* and *Bacillus velezensis*, and 2.86% each of *Enterobacter roggenkampii*, *Pseudomonas taiwanensis*, and *Staphylococcus arlettae* (Figure 5).

4. Discussion

This study primarily aimed to evaluate the plant growth-promoting (PGP) potential of bacterial isolates from the rhizospheric soils of *O. indicum*. Out of 60 bacterial isolates recovered, 35 exhibited at least one PGP trait, underscoring the significant presence of beneficial microbes in the rhizosphere of this medicinal plant. These findings are consistent with previous research that has documented the occurrence of PGP bacteria in diverse rhizospheric environments, contributing to plant growth and soil health enhancement (Glick, 2012; Bhattacharyya and Jha, 2012).

All 35 PGP-positive isolates demonstrated ammonia production, highlighting the crucial role of these bacteria in nitrogen supply, especially in nitrogen-deficient soils. Ammonia production is a key trait associated with PGP activity, as it facilitates biological nitrogen fixation, indirectly benefiting plant growth (Kumar et al., 2019). The universal occurrence of ammonia production among the isolates in this study aligns with Sharma et al. (2017), who also reported a high frequency of ammonia-producing bacteria in rhizospheric soils. Notably, while Chaiharn et al. (2008) observed ammonia production in over 64% of phosphate-solubilizing bacteria from Northern Thailand's rhizosphere soils, our study found that 35 of 60 isolates, i.e. 58.33% of the tested isolates were positive for ammonia production.

Phosphate solubilization is another critical feature of PGP bacteria, enhancing the bioavailability of phosphorus, an essential nutrient for plant development. In this study, 18 isolates exhibited phosphate solubilization activity, with isolate *Ptai*-40 (*Pseudomonas taiwanensis*) showing the highest solubility index (3.916 ± 0.144 mm) (Table 1). This ability is pivotal for plant growth promotion under field conditions (Verma et al., 2001). Our results are comparable to those of Rodriguez et al. (2006), who reported similar phosphate solubilization abilities among *Bacillus* species isolated from different rhizospheric soils. Specifically, the presence of *Bacillus cereus* and *Bacillus thuringiensis* in our study supports the notion that *Bacillus* species are among the most effective phosphate solubilizers (Vessey, 2003). While previous research by Kannapiran and Ramkumar (2011) indicated that *Pseudomonas* and *Bacillus* were more efficient at solubilizing phosphates compared to other genera, our findings reveal that *Enterobacter* exhibited a higher phosphate solubility index than *Bacillus*. Kesaulya et al. (2021) also reported 13 rhizobacterial isolates obtained from maize rhizospheric soil, which demonstrated the ability to solubilize phosphate with varying solubility indices. Regarding indole-3-acetic acid (IAA) production, our study aligns with Nurmayulis et al. (2021), who reported that rhizospheric bacteria produced IAA levels of up to 5.467 mg/L. However, there was notable variability in IAA production among our isolates, with *Erog*-1 (*Enterobacter roggenkampii*) producing significantly more IAA (5.750 µg/ml) than *Btro*-7 (*Bacillus tropicus*) and *Bcer*-13 (*Bacillus cereus*). This suggests differing capacities for influencing plant growth among the isolates. Geetha et al. (2014) found that while all isolates from the green gram rhizosphere produced IAA, only a few solubilized inorganic phosphate or produced hydrogen cyanide (HCN). Similarly, in our study, 18 out of 35 PGPR isolates (51.43%) were phosphate solubilizers, and 4 isolates (11.43%) produced HCN, which is comparable to the observations of Geetha et al. (2014).

The low occurrence of HCN (4 isolates) and IAA (3 isolates) production in our study could be due to specific environmental conditions or microbial competition within the rhizosphere of *O. indicum*. Previous research has indicated that HCN production is often limited to certain strains of *Pseudomonas* and *Bacillus* under particular conditions (Ahmad et al., 2008). Similarly, IAA production levels can vary significantly among different bacterial species and environmental contexts (Patten and Glick, 2002).

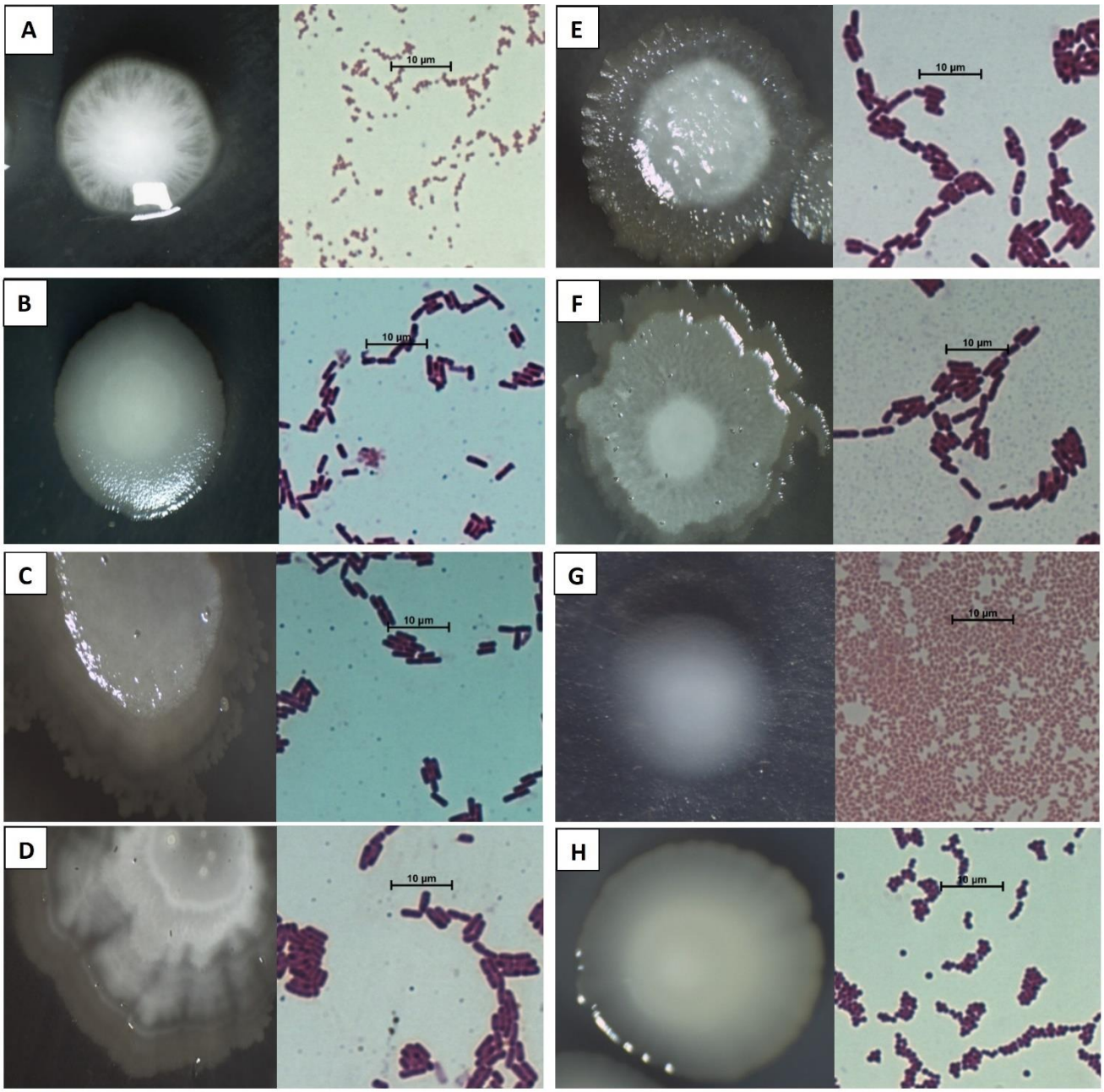

Figure 3. Colony morphology (front view) and microscopic view (at 1000× magnification after Gram staining) of the PGPR recovered from the rhizospheric soils of *Oroxylum indicum* after 24 hrs of incubation at 30°C on nutrient agar medium. A – *Enterobacter roggenkampii* (*Erog*-1), B – *Bacillus cereus* (*Bcer*-3), C – *B. thuringiensis* (*Bthu*-16), D – *B. cereus* (*Bcer*-22), E – *B. thuringiensis* (*Bthu*-32), F – *B. thuringiensis* (*Bthu*-38), G – *Pseudomonas taiwanensis* (*Ptai*-40), H – *Staphylococcus arlettae* (*Sarl*-43). Scale = 10 µm for all the microscopic images.

Biochemical characterization revealed a predominance of Gram-positive rods among the isolates, consistent with the identification of *Bacillus* species, which are commonly found in soil environments (Priest, 2013). The presence of Gram-negative rods, such as *Pseudomonas taiwanensis*, indicates a diverse microbial community within the rhizosphere, contributing to various aspects of plant growth promotion. Notably, the absence of indole and hydrogen sulfide (H_2S) production in all isolates may suggest a reduced potential for these activities in the rhizosphere of *O. indicum*, aligning with findings from Khan et al. (2015).

Species-level identification identified seven bacterial species, with *Bacillus cereus* (60%) and *Bacillus thuringiensis* (20%) being the most dominant (Figure 4). This high relative abundance of *Bacillus* species is consistent with their known resilience and ability to thrive in diverse soil conditions (Shivaji et al., 2015). The identification of minor populations such as *Enterobacter roggenkampii* and *Staphylococcus arlettae* suggests the presence of specialized roles within the rhizosphere, potentially contributing to niche-specific interactions and overall plant health. The dominance of *Bacillus* species in this study aligns with Idris et al. (2007), who reported that *Bacillus* species are prevalent in the rhizosphere of many plants due to their versatile metabolic capabilities and stress tolerance. However, the relatively low number of isolates positive for IAA and HCN production contrasts with studies by Ghosh et al. (2018), where these traits were more commonly observed in rhizospheric bacteria of different plant species. This discrepancy might be attributed to variations in soil composition, plant species, and environmental conditions influencing microbial behavior.

The biochemical characterization of the isolates also revealed their metabolic adaptability to the rhizosphere environment. For instance, all isolates showed positive results for glucose fermentation, indicating their ability to utilize available carbon sources efficiently. This adaptability is essential for survival and functionality within the competitive rhizosphere ecosystem.

5. Conclusion

Effective screening and selection of PGPR strains are crucial for their application in sustainable agriculture. Although limited

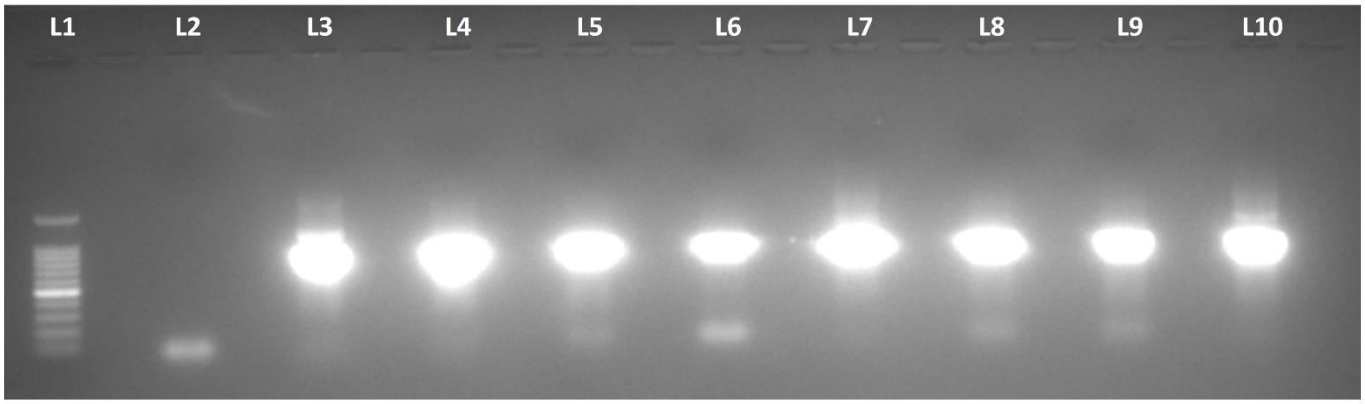

Figure 4. Representative photograph of 16S rDNA amplified PCR products in agarose gel electrophoresis. Wells were loaded alternately to keep the bands apart from each other. L1 – DNA ladder (100 bp DNA Ladder, Genei); L2 – negative control; L3 to L10 represent strain *Erog*-1, *Bcer*-5, *Bcer*-6, *Bcer*-8, *Bthu*-9, *Bcer*-13, *Bthu*-16 and *Bthu*-18, respectively. Each PCR amplified product of 25 µl volume was mixed with 2 µl of tracking dye for loading in the respective lane, and each prominent band was excised with surgical blade for further DNA purification using QIAquick Gel Extraction Kit (QIAGEN).

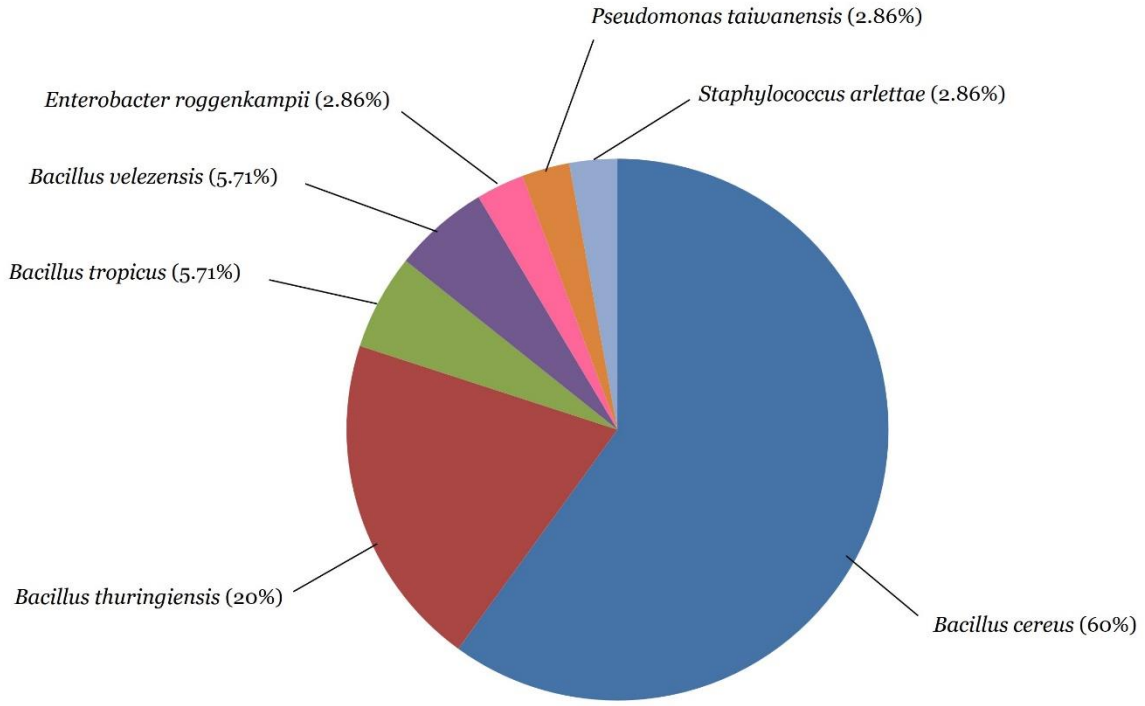

Figure 5. Relative abundance of PGPR species isolated from the rhizospheric soils of *Oroxylum indicum*

research has been conducted on PGPR strains associated with *O. indicum*, this study represents an initial effort to explore the plant growth-promoting capabilities of its rhizobacterial community. Documenting multiple traits such as nitrogen fixation, phosphate solubilization, HCN production, and IAA production provides a foundation for future research in this area.

Further investigations are essential to fully understand the specific mechanisms underlying PGPR-mediated plant growth promotion. The PGPR strains identified in this study could potentially serve as bacterial inoculants to enhance the growth of *O. indicum* and other crops. Field trials are necessary to assess their effectiveness under real-world conditions and to develop optimized strategies for their application in sustainable agricultural practices. Additionally, exploring the synergistic effects of these isolates on plant growth and nutrient acquisition could further enhance their utility in agricultural systems.

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Author contributions
Both authors equally contributed to the concept and design of the study. DS generated the data and contributed to the manuscript draft. DN supervised the research work and evaluated the draft manuscript. All the authors extensively reviewed and approved the final manuscript.

Conflict of interests
The Authors declared no conflict of interest

Table 2. Biochemical characteristics of plant growth promoting rhizobacteria (PGPR) recovered from the rhizospheric soils of *Oroxylum indicum*. '+' indicates positive test; '-' indicates negative test.

Sl. No.	Isolate code	Gram's reaction	Indole test	Citrate test	Oxidase test	Glucose fermentation test	Lactose fermentation test	H_2S test	Urease test	Methyl Red test	Voges–Proskauer test	Acid fast stain
1	*Erog*-1	-	-	+	+ (delayed oxidase positive)	+ (with bubble)	+ (with bubble)	-	+	-	+	-
2	*Bcer*-3	+	-	-	+	+	-	-	-	-	+	-
3	*Bthu*-4	+	-	-	+	+	-	-	-	+	+	-
4	*Bcer*-5	+	-	-	+	+	-	-	-	-	+	-
5	*Bcer*-6	+	-	-	+	+	-	-	-	-	+	-
6	*Btro*-7	+	-	-	+	+	-	-	-	-	+	-
7	*Bcer*-8	+	-	-	+	+	-	-	-	-	+	-
8	*Bthu*- 9	+	-	-	+	+	-	-	-	+	+	-
9	*Btro*-11	+	-	-	+	+	-	-	-	+	+	-
10	*Bcer*-13	+	-	-	+	+	-	-	-	-	+	-
11	*Bcer*-14	+	-	-	+	+	-	-	-	+	+	-
12	*Bthu*-16	+	-	-	+	+	-	-	-	-	+	-
13	*Bcer*-17	+	-	-	+	+	-	-	-	+	+	-
14	*Bthu*-18	+	-	-	+	+	-	-	-	+	+	-
15	*Bcer*-19	+	-	-	+	+	-	-	-	-	+	-
16	*Bcer*-20	+	-	-	+	+	-	-	-	-	+	-
17	*Bcer*-21	+	-	-	+	+	-	-	-	+	+	-
18	*Bcer*-22	+	-	-	+	+	-	-	-	-	+	-
19	*Bcer*-24	+	-	-	+	+	-	-	-	-	+	-
20	*Bcer*-25	+	-	-	+	+	-	-	-	-	+	-
21	*Bcer*-27	+	-	-	+	+	-	-	-	-	+	-
22	*Bcer*-28	+	-	-	+	+	-	-	-	-	+	-
23	*Bcer*-29	+	-	-	+	+	-	-	-	+	+	-
24	*Bcer*-30	+	-	-	+	+	-	-	-	-	+	-
25	*Bthu*-32	+	-	-	+	+	-	-	-	-	+	-
26	*Bcer*-33	+	-	-	+	+	-	-	-	-	+	-
27	*Bthu*-35	+	-	-	+	+	-	-	-	+	+	-
28	*Bcer*-37	+	-	-	+	+	-	-	-	+	+	-
29	*Bthu*-38	+	-	-	+	+	-	-	-	-	+	-
30	*Ptai*-40	-	-	+	+	+ (weak)	-	-	+	-	-	-
31	*Bcer*-41	+	-	-	+	+	-	-	-	+	+	-
32	*Sarl*-43	+	-	-	-	+ (weak)	+ (weak)	-	+	+(strong)	-	-
33	*Bcer*-47	+	-	-	+	+	-	-	-	-	+	-
34	*Bvel*-49	+	-	-	+	+ (weak)	-	-	-	-	+	-
35	*Bvel*-50	+	-	-	+	+ (weak)	-	-	-	-	+	-
Isolate count with positive tests		33	0	2	34	35	2	0	3	12	33	0

Table 3. Summary of thirty-five isolates representing plant growth promoting rhizobacteria associated with *Oroxylum indicum*. The table presents GenBank accession numbers and sequence similarity with closest species for each strain.

Sl. No.	Isolate code	Strain no.	Gram's reaction	Cellular morphology	GenBank Accession (16S)	Species name	% Similarity (NCBI Reference Sequence)
1	*Erog*-1	PGPR_1	-	Coccobacillus	PQ223386	*Enterobacter roggenkampii*	99.93% (CP133578)
2	*Bcer*-3	PGPR_3	+	Rod	PQ223387	*Bacillus cereus*	99.93% (MG027671)
3	*Bthu*-4	PGPR_4	+	Rod	PQ223388	*Bacillus thuringiensis*	99.93% (CP053934)
4	*Bcer*-5	PGPR_5	+	Rod	PQ223389	*Bacillus cereus*	99.93% (KP121395)
5	*Bcer*-6	PGPR_6	+	Rod	PQ223390	*Bacillus cereus*	99.93% (MG027633)
6	*Btro*-7	PGPR_7	+	Rod	PQ223391	*Bacillus tropicus*	99.93% (ON422091)
7	*Bcer*-8	PGPR_8	+	Rod	PQ223392	*Bacillus cereus*	100% (OR243825)
8	*Bthu*- 9	PGPR_9	+	Rod	PQ223393	*Bacillus thuringiensis*	99.93% (MN809398)
9	*Btro*-11	PGPR_11	+	Rod	PQ223394	*Bacillus tropicus*	100% (ON422091)
10	*Bcer*-13	PGPR_13	+	Rod	PQ223395	*Bacillus cereus*	99.86% (OM967117)
11	*Bcer*-14	PGPR_14	+	Rod	PQ223396	*Bacillus cereus*	100% (KP121395)
12	*Bthu*-16	PGPR_16	+	Rod	PQ223397	*Bacillus thuringiensis*	99.93% (MK968768)
13	*Bcer*-17	PGPR_17	+	Rod	PQ223398	*Bacillus cereus*	99.93% (MK757979)
14	*Bthu*-18	PGPR_18	+	Rod	PQ223399	*Bacillus thuringiensis*	100% (PP152281)
15	*Bcer*-19	PGPR_19	+	Rod	PQ223400	*Bacillus cereus*	100% (KP121395)
16	*Bcer*-20	PGPR_20	+	Rod	PQ223401	*Bacillus cereus*	100% (KP121395)
17	*Bcer*-21	PGPR_21	+	Rod	PQ223402	*Bacillus cereus*	100% (CP053954)
18	*Bcer*-22	PGPR_22	+	Rod	PQ223403	*Bacillus cereus*	100% (KP121395)
19	*Bcer*-24	PGPR_24	+	Rod	PQ223404	*Bacillus cereus*	100% (KP121395)
20	*Bcer*-25	PGPR_25	+	Rod	PQ223405	*Bacillus cereus*	100% (KP121395)
21	*Bcer*-27	PGPR_27	+	Rod	PQ223406	*Bacillus cereus*	99.79% (KP121395)
22	*Bcer*-28	PGPR_28	+	Rod	PQ223407	*Bacillus cereus*	100% (KP121395)
23	*Bcer*-29	PGPR_29	+	Rod	PQ223408	*Bacillus cereus*	99.79% (MH595933)
24	*Bcer*-30	PGPR_30	+	Rod	PQ223409	*Bacillus cereus*	100% (OR243825)
25	*Bthu*-32	PGPR_32	+	Rod	PQ223410	*Bacillus thuringiensis*	99.86% (PP152281)
26	*Bcer*-33	PGPR_33	+	Rod	PQ223411	*Bacillus cereus*	100% (KP121395)
27	*Bthu*-35	PGPR_35	+	Rod	PQ223412	*Bacillus thuringiensis*	99.93% (PP152281)
28	*Bcer*-37	PGPR_37	+	Rod	PQ223413	*Bacillus cereus*	100% (MK757979)
29	*Bthu*-38	PGPR_38	+	Rod	PQ223414	*Bacillus thuringiensis*	99.93% (PP152281)
30	*Ptai*-40	PGPR_40	-	Rod	PQ223415	*Pseudomonas taiwanensis*	99.93% (KF668481)
31	*Bcer*-41	PGPR_41	+	Rod	PQ223416	*Bacillus cereus*	100% (MN068934)
32	*Sarl*-43	PGPR_43	+	Coccus	PQ223417	*Staphylococcus arlettae*	99.93% (AP019698)
33	*Bcer*-47	PGPR_47	+	Rod	PQ223418	*Bacillus cereus*	100% (MN068934)
34	*Bvel*-49	PGPR_49	+	Rod	PQ223419	*Bacillus velezensis*	99.93% (CP053377)
35	*Bvel*-50	PGPR_50	+	Rod	PQ223420	*Bacillus velezensis*	99.93% (CP053376)

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