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Sustainable Rice Plant Growth Promotion by Bacteria Isolated from Rhizosphere Soil

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ABSTRACT

In the present study, sixteen bacterial isolates were collected and identified from the rhizosphere soil of the bean plant (Phaseolus vulgaris), named BB-1 to BB-16. Out of the sixteen bacterial isolates, six isolates showed positive activity of phosphate solubilization ability, three bacterial isolates were found positive for ammonia production, six were positive for Indole acetic acid (IAA) production, three could solubilize potash, three bacterial isolates produced cellulase, six exhibited positive for chitinase, five were tested positive for amylase and four bacterial isolates were positive for protease activity. Hydrogen Cyanide (HCN) production was noticed by the bacterial isolates BB-7only. The fungal pathogen such as Aspergillus terreus, and Penicillium rubidurum was resistant to most bacterial isolates, whereas bacterial isolate BB-3 showed sensitivity against Penicillium rubidurum. Their quantitative phosphate solubilization ability was 47.5-77.8 µg/ml, ammonia production was between 2.45 - 3.45 mg/l, and IAA production was 22.5 -29.5 µg/ml. Of the sixteen bacterial isolates, one bacterial isolate, BB-7, was positive for most of the tests and identified as Pantoea agglomerans. Plant growth-promoting activity of the isolate showed that in comparison to the control rice plant, higher root and shoot growth was achieved in rice pot inoculated with P. agglomerans culture.

Keywords: Indole acetic acid. PGPR, PSB, Phaseolus vulgaris, Rhizosphere.

INTRODUCTION

For more than 3.5 billion people on the planet, rice is a basic diet. With a rice-growing area of over 167 million hectares, the current worldwide paddy production is estimated to be roughly 755 million tonnes. With the current rice consumption pattern, there may be 4.6 billion people consuming it by 2025, necessitating a 20% increase in rice production to meet the demand (Muehe *et al.*, 2019). Due to a lack of proper water for irrigation, hikes in cultivation costs, and deteriorating water and soil quality, rice production and cultivated areas have remained stagnant for the previous 20 years. (Ray *et al.*, 2012). However, using chemical fertilizers to improve rice output not only costs farmers money but also has the

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potential to harm the environment by releasing nitrates and nitrogen oxides into the soil, groundwater, and atmosphere (Mir *et al.,* 2023). Additionally, they hurt human health, harm essential soil bacteria, decrease soil fertility, increase pest resistance, and remain in food grains. (Mir *et al.,* 2023). Alternative methods that increase soil fertility and increase rice yield without the use of chemical fertilizers must therefore be given priority. To meet the demands of society for a more significant and more sustainable food supply, sustainable agricultural practices, such as using microbial inoculants to accelerate rice plant growth and yield, are an excellent alternative (Harman *et al.,* 2021; Oliveira *et al.,* 2019). Plant growth-promoting rhizobacteria (PGPR) performed a crucial activity in rice plant growth and soil fertility. These PGPRs execute a critical role in agriculture and can accelerate plant growth through several mechanisms (Ahmed *et al.,* 2017). They directly solubilize the insoluble phosphate and potash compound in the soil pool, fix atmospheric nitrogen and supply it to the plant, produce Hydrogen cyanide (HCN) to control soil pathogens, and produce siderophore, plant growth regulators (IAA, gibberellins, and cytokinin) and vitamins. Besides, some of the other mechanisms of PGPR bacteria, such as the production of antibiotics, 1-aminocyclopropane-1-carboxylase (ACC) deaminase, and several enzymes, may not directly affect plant growth but indirectly play an essential role in plant metabolism (Aloo *et al.,* 2019; Parewa *et al.,* 2018). Several bacterial species, such as *Rhizobium, Pseudomonas, Azospirillium, Bacillus, Enterobacter, Gluconacetobacter, Acinetobacter,* and, *Burkholderia* were extensively studied earlier for their PGPR activities (Kahkahi *et al.,* 2021). The application of PGPR has been regarded as an essential method for sustainable agriculture because it successfully eliminates the need for pesticides and fertilizers without reducing production. Under conditions of insufficient water availability, the use of PGPR may also help promote water conservation and improve agricultural production productivity. (Ambrosini *et al.,* 2016).

Considering the above, the present study aims to isolate and identify some PGPR bacteria from rhizosphere soil and investigate the effect of isolated PGPR on Rice plant growth promotion.

Materials and methods

Collection of soil samples and Isolation of bacteria

Five different soil samples were collected from five locations of the rhizosphere soil deeper than 5 cm depth by uprooting the *bean* (*Phaseolus vulgaris*) plants from beans cultivated in an agricultural field at Patharagadia village of Khorda, Odisha, India. One gram of the collected rhizosphere soil sample was serially diluted (10^{-10} times) and pour-plated into nutrient agar (NA) media (g/l: NaCl 5.0, Peptone 5.0, yeast extract 3.0, pH 7.0) for isolation of bacteria. The plates were incubated at 37 °C for 72 h. The morphologically distinct colonies on the plate were picked up and re-streaked on the separate nutrient agar and slant for further study.

Phosphate solubilization activity

The phosphate solubilizing ability of the bacterial isolates was checked by growing them at 37°C for 72 hrs on Pikovaskayas-agar (g/l: Yeast extract 0.5; Dextrose 10.0; $Ca_3(PO_4)2$ 5.0; $(NH_4)_2SO_4$ 0.5; KCl 0.2; $MgSO_4$ 0.1; $MnSO_4$ 0.0001; $FeSO_4$ 0.0001; Agar 15.0). The bacterial colony-giving halo zones on Pikovaskayas-agar plates were considered phosphate solubilization positive (Wang *et al.,* 2017).

Further, to confirm their phosphate solubilization activity, a quantitative phosphate solubilization assay was performed in 100 Pikovaskayas broth inoculated with the selected bacterial culture (Behera *et al.,* 2017). An uninoculated medium served as the control. The flasks were incubated at 37 ^{0}C for 168 hrs at 100 rpm. The pH of the culture medium was measured every day at specific intervals of the incubation period. The culture was harvested daily by centrifugation at 10,000 rpm for 10 minutes. The supernatant was separated from the bacterial cells by successive filtration through Whatman paper 42 # followed by 0.22 µm Millipore membranes and used to estimate the phosphate release in triplicate. The amount

of phosphate released was measured at 880 nm and compared with the standard curve, taking a specific amount of potassium dihydrogen phosphate as standard (Murphy & Riely, 1962).

NH_3 production activity

For qualitative screening of N_2 fixation, the individual bacterial isolates were grown in a semisolid nitrogen-free malate medium (Baldani *et al.,* 1986) containing K_2HPO_4 0.5 g/l; $MgSO_4$. $7H_2O$ 0.2 g/l; $FeCl_2.6H_2O$ 0.015 g/l; NaCl 0.1 g/l; DL-Malic Acid 5.0 g/l; KOH 4.8 g/l; Yeast Extract 1.0 g/l; 0.1% Bromothymol blue 5.0 mL; Bacto Agar 3.0 g/l and incubated at 30 ± 2 °C for 15 days. The bacterial isolate producing NH_3 shows a change in media color from yellow to blue and was considered positive for ammonia (NH_3) production.

Further quantitative analysis of ammonia production was analyzed using the method of Sasongko *et al.,* (2018). Bacterial cultures were grown in 500 mL of peptone water for 15 days at 30 ± 0.1 °C. Every three days at regular intervals, 50 ml of the bacterial culture was withdrawn aseptically and centrifuged at 5,000 rpm for 5 minutes. To the 50 ml of the culture supernatant, one drop of Ethylenediaminetetraacetic acid (EDTA) and 2 ml of Nessler's reagent (0.09 mol/L solution of potassium tetraiodomercurate(II) ($K_2[HgI_4]$) in 2.5 mol/L potassium hydroxide) were added and incubated for 30 minutes at 25°C. The amount of ammonia produced was measured at 420 nm by comparing the NH_4Cl standard curve (APHA, 1975).

Indole acetic acid (IAA) production

The IAA production activity of the selected bacterial isolates was evaluated by culturing them in nutrient broth containing 0.5% tryptophan and incubated at 37˚C for 48 h at 120 rpm in an incubator shaker. After incubation, 5 ml of bacterial culture was mixed with 1 ml of Kovac's reagent and kept in the dark for 24 hrs. The appearance of the red ring at the top indicates IAA production (Pant & Agrawal, 2014).

For quantitative IAA estimation, bacterial cultures were grown in a nutrient broth medium containing 5g/L of L-tryptophan at 37˚C for 168 h (Chandra *et al.,* 2018). At every 24 h, 5 ml of cultures were taken out aseptically and centrifuged at 8,000 rpm for 10 min at room temperature. 1 ml supernatant was pipetted out carefully, and 2 ml Salkowski reagent (2 ml of 0.5 M $FeCl_3$ in 49 ml 70% perchloric acid) was added. Further, two drops of orthophosphoric acid were added to this reaction mixture and kept in the dark to stimulate color formation for 30 min. to 2 hours. The appearance of a pinkish-red color was measured spectrophotometrically at 535 nm. The amount of IAA produced was calculated using a standard curve of IAA following the standard method described by Chandra *et al.,* (2018).

Qualitative screening of potassium solubilization activity

Potassium solubilization ability of the bacterial isolates was screened by streaking the bacterial colony on modified Aleksandrov medium (pH 7.0) containing g/L: glucose 5.0; $MgSO_4.7H_2O$ 0.5; $CaCO_3$ 0.1; $FeCl_3$ 0.006; $Ca_3(PO_4)_2$ 2.0; potassium aluminum silicate 3.0; and agar 18.0 and supplemented with Bromothymol blue (BTB) (Rajawat *et al.,* 2016) and incubated at 28 ± 2 °C for 72 hrs. The colonies exhibiting yellow zones are considered potassium solubilizers (Etesami *et al.,* 2017).

Screening for hydrolytic enzyme production

Bacterial isolates were screened for the production of hydrolytic enzymes such as amylase, protease, cellulase, and chitinase enzyme activity following the standard method.

Cellulase activity was detected by growing the bacterial colonies at 37°C ±1 °C for 48 hrs on 1% carboxymethyl cellulose agar medium. After bacterial growth, the bacterial colonies were flooded with 0.1% congo red solution, followed by washing with 1M NaCl for 5 min. The halo zones that appeared around the bacterial colonies were considered cellulase-positive (Behera *et al.,* 2014).

The amylase activity of the bacterial isolates was evaluated by growing the bacterial colonies on 1% starch agar medium at 37°C ±1 °C for 48 hrs. The bacterial

colonies that appeared were flooded with Gram's iodine (Gram's iodine- 250 mg iodine crystals added to 2.5mg potassium iodide solution and 125ml of water, stored at room temperature) solution. The hydrolysis zone that appeared around the bacterial colony was considered amylase-positive (Sundarapandiyan and Jayalakshmi, 2017)

The isolated bacteria were spot inoculated in Petri dishes with nutrient agar medium supplemented with 1% skim milk and incubated at 37 ±1 °C for 48 hrs. Following incubation, organisms secreting protease enzyme will exhibit a zone of proteolysis, which is demonstrated by a clear zone surrounding the bacterial growth (Rahman *et al.,* 2018)

The chitinolytic activity of the bacterial isolates was determined by growing the bacterial colonies on a chitin agar plate containing g/L; Na_2HPO_4 6.0; KH_2PO_4 3.0; NH_4Cl 1.0; NaCl 0.5; yeast extract 0.05; agar 15; colloidal chitin, 10; pH 7.0 at 37°C for 48-72 hrs (Shivalee *et al.*, 2016). Production of the halo zone around the bacterial colony was considered positive for the chitinase test (Shivalee *et al.,* 2016).

Screening for hydrogen cyanide (HCN) production

All isolates were screened for the production of hydrogen cyanide following the method described by Ahmad (2008). Briefly, the nutrient agar was amended with 4.4 g glycine/l, and bacteria were streaked on a modified agar plate. A Whatman filter paper no. 1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed on the top of the Petri plate and incubated at 28°C for four days. The development of orange to red color indicated HCN production.

Antimicrobial activity

The antimicrobial activities of the selected plant growth-promoting bacterial isolates were detected against two fungal isolates, i.e., *Aspergillus terreus* (Accession no. KT222271) and *Penicillium rubidurum* (Accession no. MF144561) through agar well diffusion method (Jalan *et al*., 2022). Freshly prepared fungal spore suspensions were spread plated into potato dextrose agar (PDA) (Himedia) plates. Agar cup wells were prepared by punching the agar plate through an 8mm cork borer (Himedia) filled with 50µl supernatant of overnight bacterial culture and kept in a refrigerator for 2 hrs; then, the respective bacterial plates were incubated for 48 h at 28°C. The bacterial isolates' giving zone against the fungal culture was considered sensitive toward the fungal isolate (Asmerom *et al*., 2020).

Identification of bacteria

Morphological characteristics such as colony morphology, shape, and size and biochemical parameters such as Gram staining, oxidase, catalase, MR-VP, starch hydrolysis, citrate utilization, pigment, H_2S, motility, nitrate reduction, gelatine liquefication, acid production from lactose, maltose, raffinose, and arabinose of the selected strains were determined using standard procedures. The results were compared to Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). Based on their comparative ability to solubilize tricalcium phosphate and potash, production of NH_3, IAA, and HCN, and production of most of the hydrolytic enzymes, one of the bacterial isolates was selected as the most efficient plant growth-promoting bacteria and 16S rRNA gene of this most efficient isolates was amplified, and nucleotide sequences were determined using a BigDye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA) and submitted to the gene bank. A phylogenetic tree was generated in MEGA 4.0 using the neighbor-joining DNA distance technique (MEGA Inc., Englewood, NJ) (Behera *et al*., 2017).

Screening of Invitro plant growth promotion activity

The effect of plant growth promoting bacteria on root and shoot growth of *Oryza sativa* (Rice plant) was studied by growing the surface sterilized germinated rice seeds on a (10x10 cm) soil pot (n=3) filled with 150g of sterilized garden soil having pH 7.2, EC 0.256 DSM^{-1}, organic carbon 5.8g/kg, available nitrogen content 422 kg/h, phosphorus 137.12 kg/h and Potash 174 kg/h. Overnight

grown bacterial cultures (10^{-8} CFU/ml) of 100µl to 300 µl were added near the root region of the three different pots germinated with rice seed and observed for 30 days in greenhouse conditions (Day temperature 25 ±1°C, 14hrs light and night temperature 22±1°C with 10 hrs dark, and humidity 65±1%). The rice pot without bacterial inoculation was kept in control. The rice pots were watered at regular intervals. After 30 days, root length and shoot length were measured, and data was analyzed through Image J software.

Statistical analysis

Each set of experiments was performed in triplicate (n=3). The final result represented is the mean of the actual findings. The ± values in tables and error bars in graphs indicate the standard deviation among the replicates. ANOVA was performed using Graph Pad Prism version 5.01. A *p*-value ≤0.05 was considered to be significant.

Results and Discussion

Isolation of bacteria

Plant growth-promoting rhizobacteria (PGPR) are a diverse community of bacteria that live in the rhizosphere, on root surfaces, and in root associations; they have the potential to enhance plant growth in both direct and indirect ways. Phosphate solubilization, nitrogen fixation, synthesis of siderophore, HCN, ammonia, vitamins, and phytohormones (such as auxin, cytokinin, and gibberellins), among other processes, are examples of direct methods. In contrast, ACC deaminase activity, antibiotics production, hydrolytic enzyme production, etc., are examples of indirect methods (Mekonnen and Kibert, 2021). In the present study, based on their morphological characteristics on NA plates, sixteen different bacterial colonies were isolated from different rhizospheric soil samples collected from beans cultivated agricultural fields, hence named bean bacteria (BB-1 to BB-16). Heterotrophic groups of bacteria, including species of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligens, Arthobacter, Burkholderia, Bacillus,* and *Serratia* have been shown to promote plant development in a variety of setting (Kloepper *et al*.1989; Glick 1995).

Phosphate solubilization activity

PGPR can transform soil phosphate by releasing soluble phosphate from inorganic and organic pools of total soil phosphate through solubilization and mineralization (Hilda and Fraga, 2000). The phosphate solubilization test was carried out to determine the ability of the bacteria to dissolve inorganic phosphate. The results are presented in Figure 1a & Table 1**.** Out of the sixteen bacterial isolates, six bacterial isolates, BB-16, BB-5, BB-10, BB-13, BB-15, and BB-7, showed clear halo zone around their colony on Pikovaskaya's agar media and were considered positive for phosphate solubilization test. The quantitative phosphate solubilization ability of these six bacterial isolates was also checked up to 168 hrs in pikovaskaya's broth, and the data are presented in Fig.1b. The maximum phosphate solubilization was observed by the bacterial isolate BB-7 (77.8 µg/ml) with a maximum drop in pH 3.1 whereas, least phosphate solubilization was observed by the bacterial isolate BB-10 (47.5 µg/ml). In the present research investigation, the population of phosphate-solubilizing bacteria in rhizosphere soil was also reported earlier (Wang *et al.,* 2017). Quantitative phosphate solubilization observed in the present was supported by other researchers who also reported various ranges of tricalcium phosphate solubilization by different plant growth-promoting bacteria. Aliyat *et al.* (2022), observed a maximum Ca_3PO_4 solubilization of 174.33 ± 12.5 µg/mL which is higher than the present finding. In contrast, bacterial *sp*. reported from the mangrove soil of Chollangi, East Godavari exhibited a very similar range of phosphate solubilizing ability (80-100 ug/ml) supporting the present study (Audipudi *et al.,* 2012). As the amount of phosphate solubilization directly depends upon the amount of organic acid production by the individual bacterial isolates, hence along with phosphate solubilization, the decrease in pH of the medium was also observed. Other researchers also reported similar types of the phenomenon during phosphate solubilization (Perez

et al., 2007). Moreover, the hike in pH of the growth medium might be due to the utilization of organic acids or the production of alkaline compounds (Abusham *et al*., 2009). An earlier study found a similar negative association between pH decline and a rise in soluble phosphate in the bacterial growth medium. (Illmer & Schinner 1995).

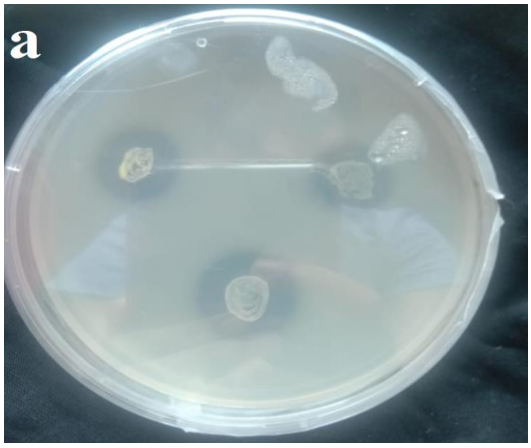

Fig.1 (a) Formation of halo zones by six bacterial isolates on Pikovskaya-agar medium

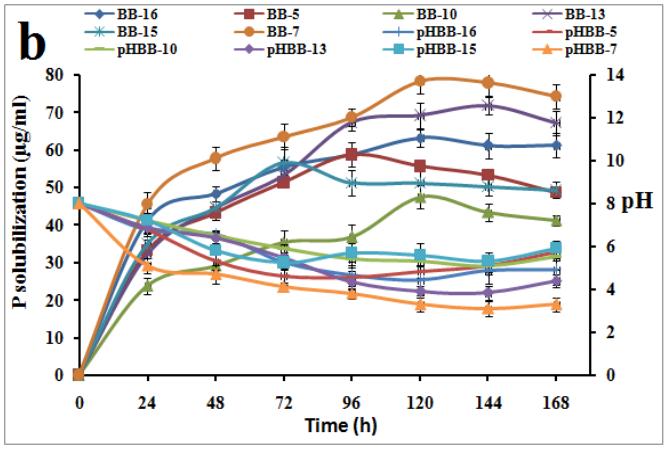

Fig.1 (b) Quantitative **phosphate** solubilization and dropping of pH by six bacterial isolates on Pikovskaya-broth medium.

Table 1: Response of bacterial isolates for different plant growth-promoting test

Test	**Bacteria positive for the test**
P solubilization	BB-1, BB-5, BB-7, BB-10, BB-13 and BB-15
NH_3 production	BB-7, BB-8 and BB- 16
K solubilization	BB-7, BB-10 and BB-16
IAA production	BB-1, BB-2, BB-4, BB-7, BB-10 and BB-11
Cellulase activity	BB-4, BB-7 and BB-13
Amylase activity	BB-1, BB-5, BB-6, BB-9, BB-10 and BB-11
Protease activity	BB-2, BB-6, BB-7 and BB-8
Chitinase activity	BB-1, BB-5, BB-6, BB-7, BB-9 and BB-12
Sensitivity towards *P. rubidurum*	BB-3
Sensitivity towards *A. terreus*	All resistant

Ammonia production activity

The PGPR's ability to produce ammonia is a crucial factor in fostering plant development. In general, PGPR-produced ammonia has been demonstrated to supply nitrogen to their host plants, hence enhancing root and shoot elongation and biomass. (Bhattacharyya *et al.*, 2020). Nitrogen-fixing bacteria can tether free nitrogen (N_2) from the air and convert it to ammonia (NH_3) (Arsita *et al*.2020). The ammonia generated by this process is excreted into the environment and is then available for either nitrification or assimilation (Kanamori, *et al*., 1989). The ability of the isolated rhizospheric bacterial isolate to fix nitrogen by producing ammonia was confirmed by growing the bacterial isolates on a nitrogen-

free malate broth medium supplemented with Bromothymol blue. Out of the sixteen isolates, three bacterial isolates, i.e., BB-7, BB-8, and BB- 16 could gradually change the color of the medium into blue within 15 days of incubation and hence considered as free-living nitrogen-fixing bacteria (Fig. 2a). In the present observation, the gradual change of color of the medium is due to gradual production of ammonia in the broth medium which increases the pH of the medium hence the dye (bromothymol blue) changed from green-yellow to blue gradually (Ishak *et al.,* 2018; Sukweenadhi *et al.,* 2019; Gothwal *et al.,* 2008).

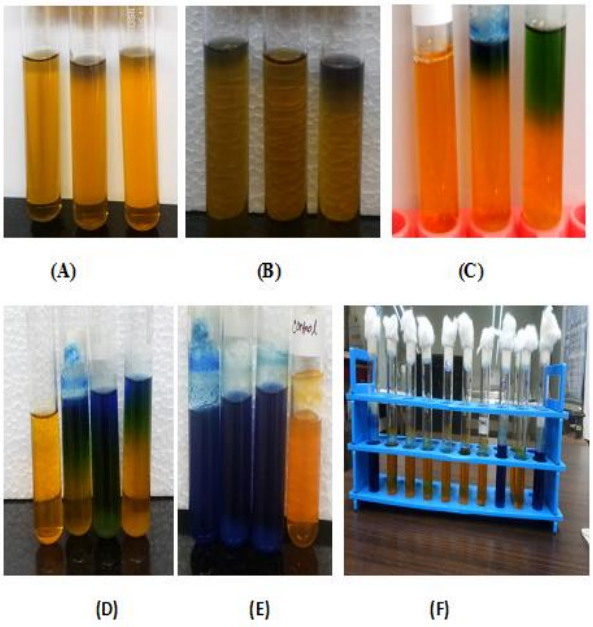

Fig.2 (a) Gradual change in color of the culture medium supporting the production of ammonia after 3 days (A), 6 days(B), 9 days(C), 12 days (D), 15 days (E), and after 18 days (F) by bacterial isolates

Further**,** the bacterial isolates that could change the color of the medium were selected for quantitative ammonia production ability. The amount of ammonia production that took place by the three selected bacterial isolates in the broth medium without BTB up to 15 days of incubation is presented in **Fig. 2b**. Their maximum ammonia production was in the range of 2.45 mg/l, 3.45 mg/l, and 3.23mg/l by BB-16, BB-8, and BB-7 respectively after 15 days of incubation. Simultaneously, with an increase in ammonia concentration, the pH of the growth medium was gradually increased. In control, no ammonia production was detected, and no increase in pH was observed. The amount of ammonia production that took place by the three selected bacterial isolates in the broth medium is lower than the study conducted by Kang *et al.* (2020) and Bhattacharyya *et al.,* (2020), they reported higher production of ammonia by plant growth-promoting endophytic bacteria, *Klebsiella pneumoniae* YNA12 (7.75 ± 1.0 mg/L) and PGPR bacteria (7.54 µmol ml−1). In comparison to the present study, a very similar range of ammonia production by various plant growth-promoting bacteria was reported by Mazumdar *et al.* (2019).

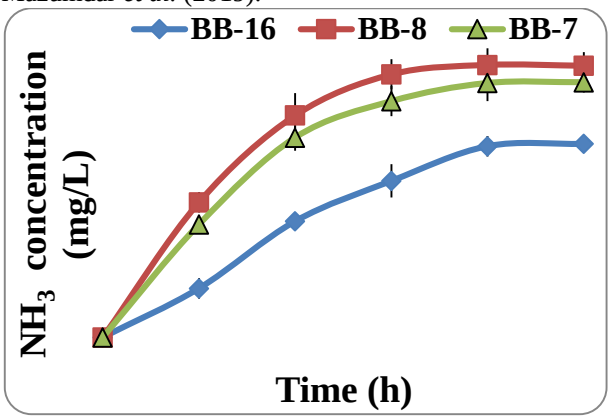

Fig.2b Comparative release of NH_3 (mg/l) by three bacterial Isolates

Screening for IAA production

The property of IAA production by rhizobacterial isolates is considered an effective tool for screening beneficial microorganisms, and they have a profound effect on plant growth (Wahyudi *et al.,* 2011). Previous studies also confirmed the involvement of rhizobacterial isolates in enhancing plant growth by synthesizing IAA (Chandra *et al.,* 2018). In the present study, out of sixteen bacterial isolates, six bacterial isolates, BB-1, BB-2, BB-4, BB-7, BB-10, and BB-11, exhibited a positive reaction by forming a pink color at the top of the culture broth (Fig. 3a) hence considered positive for IAA production. The

mechanism behind the color formation is the reaction between Kovac's Reagent and indole produced from the hydrolysis of tryptophan by the bacterial isolates in the growth medium. The indole produced in the culture broth is combined with the Kovacs reagent (which contains hydrochloric acid and para-Dimethylaminobenzaldehyde in amyl alcohol) added into the broth and turns the color of the solution from yellow to cherry red.

Further, quantitative estimation of indole acetic acid production in the culture supernatant was confirmed when the Salkowski reagent and orthophosphoric acid were added and kept in the dark for color formation for 30 min to 2 hrs. Isolates showing pink to red color (Fig.3b) were quantified at 535 nm. The amount of IAA released by each isolate was calculated from the standard curve of IAA. All six isolates were able to produce moderate to high amounts of IAA. Their maximum IAA production was observed in the range of 22.5 to 29.5 µg/ml (Fig. 3c). Maximum IAA production was observed with BB-10 (29.25 µg/ml) followed by BB-7 (25.29 µg/ml) whereas, significantly less IAA production was observed by the bacterial isolate BB-1 (22.5 g/ml) during 168 h of incubation. The amount of indole acetic acid produced by bacterial isolate in the present study (22.5 to 29.5 µg/ml) is found to be higher than Reetha *et al.,* (2014), who observed the highest IAA production by *B. subtilis* sp. and *P. fluorescens* of 15.38±0.537 and 12.67±0.325 µg/ml respectively. Similarly, Pant and Agarwal *et al.* (2014) obtained very low IAA production by rhizospheric bacterial isolate (5-11 µg/ml) in comparison to the current report. However, much higher Indole acetic acid production was also reported by other researchers (Chandra *et al.*, 2018; Shahab *et al.*, 2009).

Fig. 3 (a) Qualitative screening of IAA production

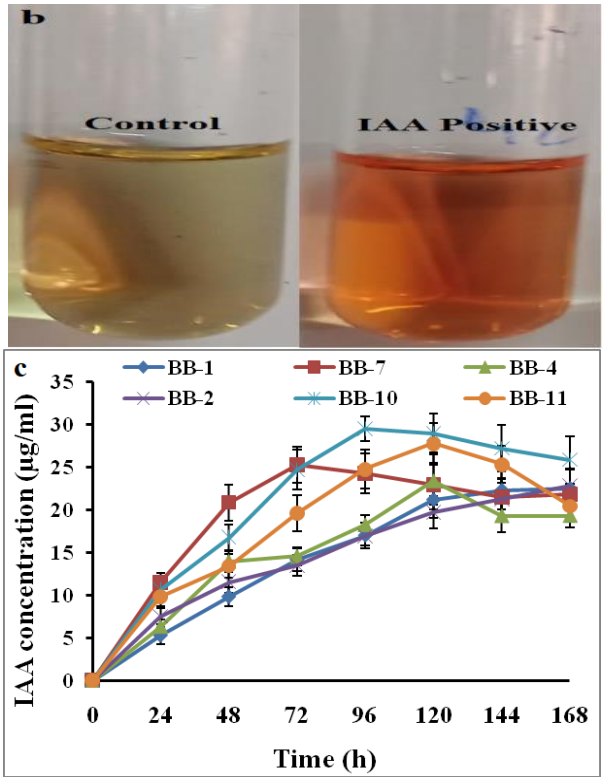

Fig. 3 (b) Pink color production after the **addition of Salkowski reagent and orthophosphoric acid (c) Comparative q**uantitative IAA production by six bacterial isolates at different periods.

Screening for potash solubilizing activity

After nitrogen (N) and phosphorus (P), Potassium (K) is a vital plant nutrient that aids in plant growth, metabolism, and development. Potassium is necessary to activate about 80 enzymes involved in plant and animal functions such as energy metabolism, nitrate reduction, starch synthesis, photosynthesis, and sugar degradation (Hussain *et al.*, 2016; Yang *et al.*, 2015). The Potash solubilizing ability of the bacterial isolates was screened on Aleksandrov medium, which showed that only three bacterial isolates (BB-7, BB-10, and BB-16) could solubilize the potash supplemented in the media by giving yellow halo zone on the plate (**Fig.4**).

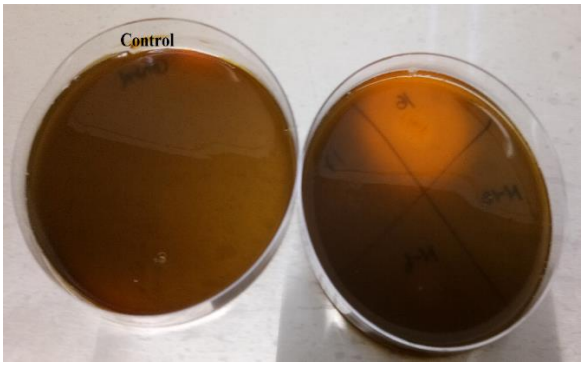

Fig. 4 Screening of potash (K) solubilizing activity of PGPR bacteria

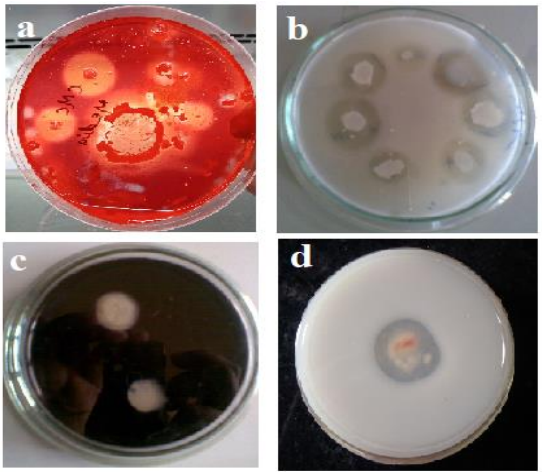

Fig. 5 Production of halo zone by different bacterial isolates on (a) CMC agar plate for cellulase assay, (b) Chitin agar plate for chitinase assay, (c) Starch agar plate for amylase assay and (d) Skim milk agar plate for protease assay.

Screening of hydrolytic enzyme activity

Urease, lipase, esterase, protease, amylase, chitinase, and cellulase are the lytic enzymes produced by bacteria that play a crucial role in the biological transformation of nitrogen, hydrogen, and carbon (Xun *et al.,* 2015). These enzymes deform pathogenic fungi's cell walls and act as critical mechanisms of eco-friendly soil-borne pathogen control (Tariq *et al.,* 2017). Out of the sixteen bacterial isolates, three bacterial isolates (BB-4, BB-7and BB-13) that produced halo zones around the bacterial colony on CMC congo red agar medium (Table-1), are considered positive for cellulase enzyme test (Fig. 5a) (Behera *et al.,* 2014). Six bacterial strains (BB-1, BB—5, BB-6, BB-7, BB-9, and BB-12) exhibited a halo zone around the colony on colloidal chitin agar plate, hence considered as positive for chitinase enzyme assay (Fig. 5b) (Shivalee *et al.,* 2016). Similarly, five bacterial isolates (BB-1, BB-5, BB-6, BB-9, BB-10, and BB-11) that produced a halo zone around the colony after flooding with iodine solution were considered positive for amylase test (Sundarapandiyan and Jayalakshmi, 2017) (Fig. 5c) and four bacterial isolates (BB—2, BB-6, BB-7, and BB-8) that produced halo zone on skim milk agar plate were considered positive for protease enzyme activity (Rahman *et al.,* 2018) (Fig. 5d).

The present findings support the findings of other researchers who have also observed hydrolytic enzyme activity by plant growth-promoting bacteria, including *Serratia marcescens, Bacillus cereus, Bacillus thuringiensis*, etc. (Jadhav and Sayyed, 2016; Rai and Nabti, 2017).

Screening for hydrogen cyanide (HCN) production

Production of HCN by certain strains has been involved in suppressing soil-borne pathogens (Sehrawat *et al.,* 2022). It has been observed that out of the sixteen bacterial isolates, only one bacterial isolate, BB-7, could produce HCN. A positive test of HCN production is indicated by a change of color of the filter paper from yellow to light brown, brown, or reddish-brown **(Fig.6)**.

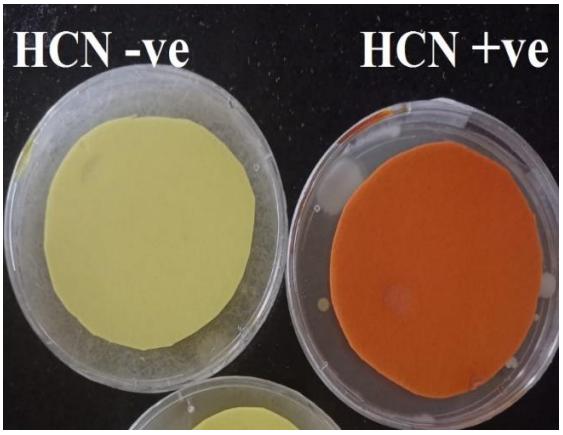

Fig.6 HCN production test by PGPR isolates

Antifungal activity

All sixteen bacterial strains were tested for their antagonistic activity against two fungal isolates such as *Aspergillus terreus* (KT222271) and *Penicillium rubidium* (MF144561). Most plant pathogens were resistant to the bacterial isolates, whereas the bacterial isolate BB-3 observed antifungal activity against *Penicillium rubidurum* (MF144561). Multiple studies have shown that plant root infections can be effectively managed by bacteria that produce HCN, secondary metabolites, and lytic enzymes (Nagrajkumar *et al.*, 2004).

Selection of most efficient PGPR bacteria

Based on the qualitative and quantitative analysis of P & K solubilization, production of NH_3, IAA, HCN, and various hydrolytic enzymes (Table-1), it has been observed that one of the bacterial isolate BB-7 exhibited positivity for most of the plant growth-promoting activities test, hence selected for identification and rice plant growth promotion test.

Identification of the bacterial strain

Morphologically, BB-7 was found to be a grey-yellowish, motile, convex, smooth, punctuate coccobacillus with a diameter of 0.5–0.9 m.

According to Biochemical test results, the bacterial isolate was found to be positive for yellow pigment, H2S, motility, nitrate reduction, gelatine liquefication, VP test, citrate, acid production from lactose, maltose, raffinose, and arabinose. These morphological and biochemical investigations led to the provisional identification of isolate BB-7 as belonging to the genus *Pantoea*. Additional identification was validated by analyzing the 16S rRNA gene sequence, of the bacterial strain followed by a BLAST and construction of a phylogenetic tree. Similarities between the strain BB-7 and the genus *Pantoea agglomerans* were found (Fig. 7). The 16S rRNA gene sequences of the isolates were submitted into the database maintained by the NCBI with the accession number MT071499. *P*lant growth-promoting *Pantoea agglomerans* strains isolated in the current investigation also supports the finding of several researchers (Shariati *et al.*, 2017; Luziatelli et al., 2020; Sergeeva *et al.*, 2007). The plant growth-promoting activity such as IAA production, siderophore production, phosphate solubilization activity, etc. observed by the identified *Pantoea agglomerans* strains is also supported by the previous report (Luziatelli *et al.*, 2020)

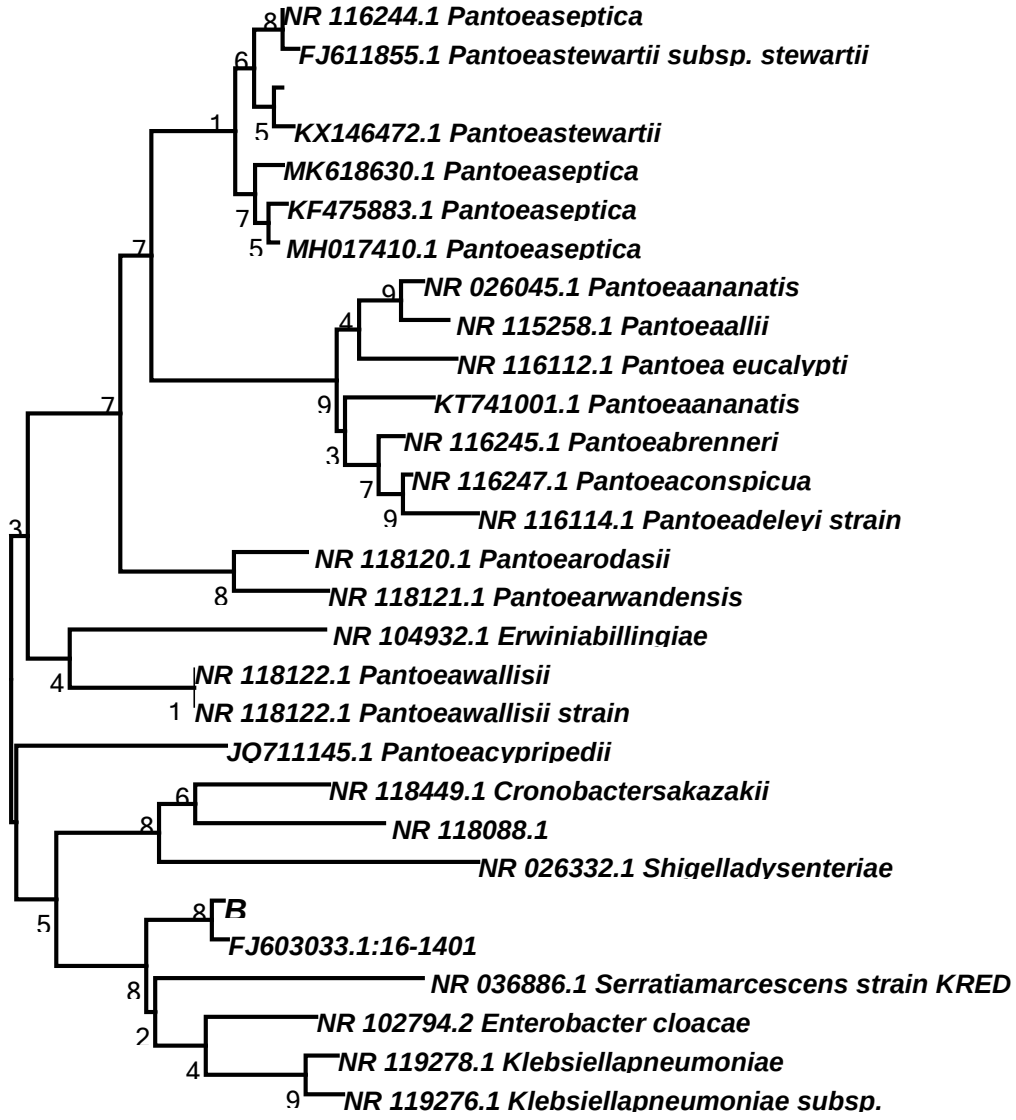

Fig. 7 Unrooted neighbor-joining tree demonstrating similarities of bacterial strain BB-7 with other *Pantoea* phylogeny.

Screening of Invitro plant growth promotion activity

In the present investigation, different concentrations (100µl, 200µl, and 300µl) of *P. agglomerans* culture were inoculated into the rice pot, and comparative root and shoot growth patterns of rice plant were recorded in triplicate from germination of seeds up to 30days of growth of the plant (**Table 2**). Compared to the control rice plant, a 2 to 4 cm increase in root length and a 1 to 5 cm increase in shoot growth has been observed in rice pots inoculated with different inoculum sizes of *P. agglomerans* culture (**Fig. 8**). It has been observed that with an increase in inoculums size, higher root, and shoot growth has been observed. The present finding is very similar to Abd El-Mageed *et al.,* (2022), who observed higher shoot length after treatment of PGPR in rice plants compared to untreated ones. Accordingly, Mir *et al.,* (2022) observed similar differences between root and

shoot growth in PGPR treated rice plants, as observed in the present study. Positive impacts of PGPR on rice plant growth may be due to the enhanced production of plant hormones like indole acetic acid, which might have stimulated the activity of enzymes like α-amylase that enhanced early germination by improving the levels of soluble sugars from starch breakdown (Duarah *et al.*, 2011), nitrogen fixation and phosphate solubilization capability of the bacterial strain, and any others PGPR activities in favor of plant growth response. Besides, the metabolites secreted by *P. agglomerans* contain molecules that act in synergy with auxins and maintain an optimal gradient of this hormone, which positively affects the temporal pattern of de novo root formation, as well as root morphology and efficiency (Luziatelli *et al.*, 2020).

Table 2 Effect of *P.agglomerans* on rice plant growth

Experiment specification	**Shoot length (cm)**	**Root length (cm)**	**Shoot-by-root ratio**
Rice pot without inoculation	19.22±1.01	2.13±0.075	9.02
Rice pot with 100µl inoculation	21.05±0.97	3.33±0.9	6.32
Rice pot with 200µl inoculation	22.92±0.98	5.08±0.98	4.51
Rice pot with 300µl inoculation	23.79±2.33	5.84±0.98	4.07

Fig. 8 Effect of different concentrations of *P. agglomerans* culture on rice shoot and root length.

Conclusion

Plant growth promotion is the most critical factor that sustains our agriculture. Various plant developmental processes are controlled by internal signals that depend on the soil's adequate supply of mineral nutrients to roots. Therefore, the availability of nutrient elements can be a significant constraint to plant growth in many world environments, particularly in hot, arid areas where the soils are deficient in nutrients. The potential plant growth-promoting bacteria can provide an improved solution in these areas to promote plant development. In the present investigation, out of the sixteen bacterial isolates, six isolates isolated from the rhizosphere area of the bean plant showed positive activity toward different plant growth-promoting activity. However, the bacterial isolate *P. agglomerans* was positive for most of the plant growth-promoting tests such as NH_3 production, IAA production, phosphate solubilization, potash solubilization, HCN production, cellulase, and chitinase test. The use of this bacterium in correct bio-formulations provides a remarkable solution for sustainable rice cultivation, and depending on the obtained results, it could be summarized that the treatment of 300µl of *P. agglomerans* culture to the 10x10 cm rice planted soil pot filled with 150g of sterilized garden soil is the most suitable parameters for obtaining the highest rice root and shoot growth. Hence, application in the form of this bioformulation could potentially improve the sustainable growth and production of rice.

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Conflict of Interests

The authors declare no competing interests.

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تعزيز نمو نبات الأرز المستدام بواسطة البكتيريا المعزولة من تربة الجذور

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ملخص

في هذه الدراسة تم جمع وتشخيص ستة عشر عزلة بكتيرية من تربة الجذور لنبات الفاصوليا ((Phaseolus vulgaris، سميت من BB-1 إلى .BB-16من بين العزلات البكتيرية الستة عشر، أظهرت ست عزلات نشاطًا إيجابيًا لقدرة إذابة الفوسفات، وثلاث عزلات بكتيرية إيجابية لإنتاج الأمونيا، وستة عزلات بكتيرية إيجابية لإنتاج حمض الإندول أسيتيك ((IAA، وثلاث عزلات بكتيرية قادرة على إذابة البوتاس، وثلاث عزلات بكتيرية أنتجت السليوليز. أظهرت ستة عزلات بكتيرية إيجابية لإنزيم الكيتيناز، وخمسة منها إيجابية للأميليز وأربع عزلات بكتيرية إيجابية لنشاط الأنزيم البروتيني. وقد لوحظ إنتاج سيانيد الهيدروجين ((HCN بواسطة العزلات البكتيرية BB-7 فقط. كانت الممرضات الفطرية مثل Aspergillus terreus و Penicillium Rubidurum مقاومة لمعظم العزلات البكتيرية، في حين أظهرت العزلة البكتيرية BB-3 حساسية ضد Penicillium Rubidurum.كانت قدرتها الكمية على إذابة الفوسفات في حدود 47.5-77.8 ميكروغرام/مل، وكان إنتاج الأمونيا بين 2.45-3.45 ملغ/لتر، وكان إنتاج IAA 22.5-29.5 ميكروغرام/مل. من بين العزلات البكتيرية الستة عشر، كانت عزلة بكتيرية واحدة، BB-7، إيجابية بالنسبة لمعظم الاختبارات وتم تحديدها على أنها Pantoea agglomerans.اظهرت العزلة نشاطا معززا لنمو النباتات مقارنة مع نبات الأرز الضابط، حيث حققت نموا أعلى للجذور والبراعم في أصيص الأرز الملقّح بمزرعة P. agglomerans..

الكلمات الدالة: IAA, PGPR، PSB، الفاصولياء، ريزوسفير

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