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IN VITRO SCREENING OF INDIGENOUS COMPETENT BACTERIAL STRAINS FOR THEIR MULTIPLE PLANT GROWTH PROMOTING ATTRIBUTES

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Abstract: *Aim:* The aim of this study is to isolate and screening of indigenous competent bacteria for their plant growth promoting attributes to formulate relevant microbial consortia which will further improve plant growth and soil health.

Place and Duration of Study: Division of Research and Development, Sahasra Crop Science, Hyderabad, between January 2021 and February 2021.

Methodology: Bacterial Isolates were initially screened and characterized for their plant growth promotion activities i.e. Nitrogen fixation, Phosphate solubilization, Potassium releasing activity, Siderophore, Exopolysachharides, IAA production, Ammonia production, HCN production and other biochemical tests.

Results: Among the sixty-six isolated bacteria 19 isolates were identified as competent. All the bacterial isolates were found to be positive for nitrogen fixation which will further improve plant growth by providing the required nitrogen during plant growth. As well as in vitro screening was done for different plant growth promotion activities, twelve bacterial isolates were positive for phosphate solubilization. Among these two bacterial isolates can solubilize the insoluble form of potassium. IAA production was shown by sixteen bacterial isolates. All the bacterial isolates can be producing exopolysaccharides, these strains were also further characterized with biochemical tests.

Conclusion: This study suggests that 19 bacterial isolates exhibited the highest nitrogen fixation, phosphate solubilization and potassium releasing activity under in vitro, these are screened and considered as potential isolates against other plant growth promoting characteristics. The potential promotes bacteria that can modulate physiological response for water shortage, enhanced water or nutrient uptake and transpiration, induction of plant growth hormone signaling activity and photosynthetic rate thereby ensuring plant growth under various conditions. In this regard isolates having PGP properties from the research work presented could be studied further under in vitro and in vivo conditions at different soils and crops for confirming their use as bioinoculants.

1. INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that colonize roots and promote the growth of plants. The term PGPR was first used by Kloepper and Schroth [1978] for the microorganisms closely associated with the rhizosphere region. It is well established that, in the rhizosphere, only 1–2% of bacteria promote plant growth. The rhizosphere is a hot spot of microbial interactions due to the exudates released by plant roots, which constitute the main food source for microorganisms, leading to efficient geochemical cycling of nutrients. Therefore, screening and selection of effective PGPRs and their utilization in integrated practices are of great importance

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for enhancing the growth and yield of crops along with maintaining the sustainability of the agroecosystems. The rhizosphere zone has been defined as the volume of soil directly influenced by the presence of living plant roots or soil compartment influenced by the root [Aeini and Khodakaramian, 2018].

The role of microorganisms in plant growth, nutrient management, and biocontrol activity is very well established. These beneficial microorganisms colonize the rhizosphere/ endo rhizosphere of plants and promote plant's development through various direct and indirect mechanisms. Furthermore, the role of microorganisms in the management of biotic and abiotic stresses is gaining importance [Prasanna *et al*., 2020]. The rhizosphere supports a large and active microbial population capable of exerting beneficial, neutral and detrimental effects on the plants. Rhizobacteria (root colonizing bacteria) that exert beneficial effects on the growth of the host plant via direct or indirect mechanisms are termed plant growth promoting rhizobacteria (PGPR). The plant-microbe interactions in the rhizosphere are responsible for increasing plant health and soil fertility [Turan *et al*., 2021].

PGPR strains use one or more direct or indirect mechanisms to enhance the growth and health of plants. These mechanisms can be active simultaneously or independently at different stages of plant growth [Al-Turki, A., 2021]. PGPR has been reported to directly enhance plant growth by a variety of mechanisms: fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores, and synthesis of plant growth hormones i.e. Indole-3- acetic acid (IAA), gibberellic acid, cytokinins, and ethylene. Indirect mechanisms involve the biological control of plant pathogens and deleterious microbes, through the production of antibiotics, lytic enzymes, hydrogen cyanide, catalase and siderophore or competition for nutrients and space can improve significantly plant health and promote growth, as evidenced by increases in seedling emergence, vigor, and yield [Khan, 2006]. After N_2 fixation, Phosphate (P) solubilization is a very important plant growth promoting activity. A large proportion of soluble inorganic phosphate added to the soil is fixed as insoluble forms soon after the application and becomes unavailable to the plants [Dadarwal *et al*., 1997]. Several soil bacteria particularly belonging to genera Bacillus and *Pseudomonas*, possess the ability to change insoluble forms into a soluble form by secreting organic acids like formic acid, acidic, propionic, lactic, glycolic, fumaric and succinic acid [Vazquez *et al*., 2000]. Biofertilizers such as microbial inoculants promote plant growth, productivity and increase the nutrient status of the host plant have internationally been accepted as an alternative source of chemical fertilizers. Significant increases in crop yields have been reported by applying PGPR microbial inoculants [Khalid *et al*., 2004]. So, keeping all this in view, the present study was carried out to isolate and characterization various indigenous competent plant growth promoting strains from the rhizospheric soil samples.

2. METHODOLOGY

2.1. Isolation of Bacteria

For isolation of Rhizobacteria, the method proposed by Vlassak *et al*. [1992] was followed. In this procedure, 10 g of soil from each soil sample was taken in a conical flask of 90 ml saline. The sample was agitated for 15 minutes on a vortex and serial dilutions of soil suspensions were prepared. 0.1 ml was spread on sterilized Petri plates containing Nutrient agar media the Petri plates were incubated at room temperatures (28 ^{o}C ± 2 ^{o}C) for 24-72 h. Two replicates were maintained for each dilution. The plates were examined daily for up to 3 days for bacterial colonies

2.2. Cultural Characterization

The plates incubated for a day at 30 ± 1 °C were observed for the growth of colonies on Nutrient agar media colonies were screened by enumeration. All the bacterial isolates were studied for their colony morphology, cell morphology (Gram reaction), pigmentation, spore production and biochemical characteristics according to the standard methods described in Bergey's Manual of Determinative Bacteriology [Holt *et al*., 1994 and Cappucino, 1983].

2.3. Maintenance of Isolates

All the isolates were maintained at 4 °C in equal volumes of nutrient broth and 30 % glycerol can be preserved as long as 12-15 months [Sundaram and Murali, 2018].

2.4. Screening of Bacterial Isolates for Plant Growth Promoting Traits

2.4.1. Nitrogen Fixation

Nitrogen free malate media (Glucose 5 g, Yeast extract 0.1 g, $CaCl_2$ 0.15 g, Na_2MoO_4 0.005 g, $MgSO_4.7H_2O$ 0.2 g, $FeSO_4$ $7H_2O$ 0.04 g, K_2HPO_4 0.5 g, Bromothymol blue (0.5% alcoholic solution) 5 ml, $CaCO_3$ 1.0 g, Agar 1.75 g, Distilled water 1,000 ml, pH 7.0), containing bromothymol blue (BTB) as an indicator [Gothwal *et al*., 2008] was used for preliminary screening and incubated at 37°C and 50°C up to 24 h. The blue coloured zone producing isolates were marked as nitrogen fixers in the solid culture conditions. The coloring zone was calculated by deducting the colony diameter from the coloring zone diameter. To determine whether these isolates were truly nitrogen fixers, they were further tested for their acetylene reduction activity (ARA) assay in liquid culture.

2.4.2. Phosphate Solubilization

Phosphate solubilization activity was determined using National Botanical Research Institute's phosphate growth media (NBRIP) contained: glucose, 10 g: $Ca_3(PO_4)_2$, 5 g; $MgCl_2 \cdot 6H_2O$, 5 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; KCl, 0.2 g and $(NH_4)_2SO_4$, 0.1 g, distilled water 1 L, pH 6.2 ± 0.2) medium containing 0.5 % (W/V) $Ca_3(PO_4)_2$ [Pikovskaya, 1948]. National Botanical Research Institute's phosphate growth medium devoid of yeast extract (NBRIY) media contained: glucose, 10 g; $Ca_3(PO_4)_2$, 5 g; $(NH_4)_2SO_4$, 0.5 g; NaCl, 0.2 g; $MgSO_4$ $7H_2O$, 0.1 g; KCl, 0.2 g; $MnSO_4$ H_2O, 0.002 g and $FeSO_4$ $7H_2O$, 0.002 g. Media plates were prepared and sterilized. The inoculums were spot inoculated on the pikovskayas plate. 24 old culture was used for the inoculation. The plates were incubated for 72-96 at room temperature. The clear zone was observed around the spotted area after the incubation period.

2.4.3. Potassium Releasing Activity

Potassium releasing activity was determined using Aleksandrov medium containing 0.3% potassium aluminum silicate [Prajapati and Modi, 2012]. KMB (Glucose 5 g/L, $MgSO_4$. $7H_2O$ 0.5 g/L, $CaCO_3$ 0.1 g/L, $FeCl_3$ 0.006 g/L, $Ca_3(PO_4)_2$ 2 g/L, Potassium aluminium silicate 3 g/L, Agar 20 g/L, distilled water 1 L, pH 7.2 ± 0.2) media was prepared and sterilized. The 24 hrs old culture was spot inoculated on the KMB plates and incubated for 72 hrs at room temperature. Plates were observed for the clear zone around the spotted area after the incubation period.

2.4.4. Indole Acetic Acid Production

The production of Indole acetic acid was done according to Dubey and Maheswari [2012]. LB broth (tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, distilled water 1000 ml, pH to 7.0 ± 0.2) was prepared and 24 hrs old cultures were inoculated into the broth and incubated at 28 °C for 72 hrs. After the incubation period, the cultures were centrifuged at recommended rpm and time. 2 ml of supernatant was collected into a test tube and two drops of Orthophosphoric acid were added. Salkowski reagent was prepared and added into the test tube double the amount of supernatant. To prepare the Salkowski reagent 0.4 gms of ferric chloride was added into 5 ml of distilled water and 17.5 ml of perchloric acid was added into 32.5 ml of distilled water and mixed the ratio of 1:150. Incubate the tubes for 30 min in dark. Development of pink color after the respective incubation period indicates the positive test for IAA production.

2.4.5. Ammonia (NH_3) Production

The isolates were tested for ammonia production by inoculating the isolates into 10 ml of pre-sterilized peptone water in test tubes [Kang *et al*., 2020]. The tubes were incubated for 48-72 at 36 ± 2 °C. After that Nessler's reagent (0.5 ml) was added to each tube. Change in color of the medium from brown to yellow color was taken as a positive test for ammonia production.

2.4.6. Exopolysaccharides (EPS) Production

Exopolysaccharide production of bacterial strains were tested on YMG Agar (Peptone 5

g/L, Yeast extract 3 g/L, Malt extract 3 g/L, Dextrose 10 g/L, Agar 20 g/L, distilled water 1 L, pH 6.2 ± 0.2) medium were inoculated in YMG broth and preincubated at 25 °C for 24. 200 µl of culture broth was inoculated into 50 ml of YMG broth and incubated at 25 °C for 5 days at 120 rpm. Elimination of cells was followed by centrifugation (10,000 g for 20 min). The culture broth was mixed with 3 volumes of ethanol and after standing at 4 °C for 24, it was centrifuged (10,000 g, 4 °C, 20 min). The weight of the precipitated EPS was measured after drying at 80 °C for 3 days [Ali *et al*., 2013].

2.4.7. Siderophore Production

Siderophore production was estimated qualitatively. Chrome azurol sulphonate (CAS) agar medium (CAS-HDTMA solution: CAS-HDTMA solution was prepared by dissolving 121 mg chrome azurol sulfate (CAS) in 100 ml of distilled water, and to this, 20 ml of 1 mM $FeCl_3.6H_2O$ solution (1 mM $FeCl_3.6H_2O$ solution prepared in 10 mM HCl) was added. It was slowly added to 20 ml hexadecyltrimethylammonium bromide (HDTMA) solution (729 mg HDTMA in 400 ml distilled water) and autoclaved at 121 °C for 15 minutes. To a 900 ml sterilized King's B medium) [Schwyn and Neilands, 1987] was used for the detection of siderophores, isolates were grown in synthetic medium, containing 0.5 µM of iron and incubated for 24 hrs on a rotary shaker at room temperature. CAS assay is used to detect the siderophores. The CAS plates were used to check the culture supernatant for the presence of siderophores. Culture supernatant was added to the wells made on the CAS agar and incubated at room temperature for 24. The formation of yellow to orange colored zone around the wells indicates siderophore production.

2.4.8. Hydrogen cyanide (HCN) Production

KMB agar plates were prepared and 24 hours old cultures were streaked on the plates separately. A disc of Whatman qualitative filter paper No. 1 with a diameter equal to Petri plate was cut and placed in alkaline picric acid solution (0.5% picric acid (w/v) in 1% sodium carbonate) [Hyder *et al*., 2020]. The paper was placed on the lid of the Petri plate under aseptic conditions [Juanda, 2005]. The plates were incubated upside down at 28 °C for 48-72 hrs. Change in the color to light brown from yellow indicates the positive result for HCN production.

2.5. Biochemical Characterization

2.5.1. Indole production

Sterilized hydrogen sulfide-indole-motility agar (SIM agar) slants were inoculated with the overnight cultures of the isolates and incubated for 48 hours at 28 ± 2 °C [Isenberg and Sundheim, 1958]. Following incubation, 10 drops of Kovac'sindole reagent was added to each tube. The isolates showing the production of red color were recorded as positive for indole production.

2.5.2. Catalase test

This test was performed to study the presence of catalase enzyme in bacterial colonies. Pure isolates (24 hours old) were taken on glass slides and one drop of H_2O_2 (30%) was added [Rangaswami and Bagyaraj, 1993]. The appearance of the gas bubble indicated the presence of the catalase enzyme.

2.5.3. Oxidase test

The overnight cultures of the test isolate were spotted on plates poured with sterile Trypticase Soy Agar (TSA) and the plates were incubated for 24 hrs at 28 ± 2 °C [Collins and Lyne, 1970]. After incubation, 2-3 drops of N, N, N′, N′-tetramethyl-p-phenylenediaminedihydrochloride (Wurster's reagent) was added onto the surface of the growth of each test organism. The isolates showing the change of color to maroon were noted as oxidase positive.

2.5.4. Gelatin liquefaction

The overnight cultures of the test isolates were inoculated to sterilized nutrient gelatin deep tubes and incubated for 24 at 28 ± 2 °C [MacFaddin, 2000]. Then the tubes were kept in the refrigerator for 30 minutes at 4 °C. The isolates showing liquefied gelatin were taken as positive and those which resulted in the solidification of gelatin on refrigeration were recorded as negative for the test.

2.5.5. Methyl red test

Sterilized glucose-phosphate broth tubes were inoculated with the test culture and incubated at 28 ± 2 °C for 48 [Crown and Gen, 1998]. After incubation five drops of methyl red indicator were added to each tube and gently shaken. Red color production was taken as positive and yellow color production was taken as negative for the test.

2.5.6. Voges prausker's test

To the pre-sterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37 °C for 48 [MacFaddin, 2000]. After incubation ten drops of Barritt's reagent-A was added and gently shaken followed by the addition of 10 drops of Barritt's reagent-B. The development of pink color in the broth was taken as positive for the test.

2.5.7. Citrate utilization

Isolates were streaked on Simmon's citrate agar slants and incubated at 28 ± 2 °C for 24 hrs [MacFaddin, 2000]. Change in color from green to blue indicates the positive reaction for citrate utilization.

2.5.8. Starch hydrolysis

Sterile starch agar plates were spotted with 10 µl overnight broth cultures of the isolates and incubated at 28 ± 2 °C for 24-48 [MacFaddin, 2000]. After incubation, the plates were flooded with an iodine solution. The formation of a transparent zone around the colony was taken as a positive reaction to the test.

2.5.9. Hydrogen sulfide (H_2S) test

Sterilized Hydrogen Sulfide-Indole-Motility agar (SIM agar) stabs were inoculated along the wall of the tubes with overnight cultures of the isolates and incubated for 48 hrs at 28 ± 2 °C (Beishir, 1991). Visualization of black color along the line of inoculation indicated a positive reaction to the test.

2.5.10. Urea Hydrolysis test

The slant of Christensen's urea agar medium was prepared and inoculated with isolated bacteria on the entire surface of the slant. The tubes were inoculated at 37 °C. The inoculated plants were observed for a color change at 6 hours, 24 hrs and every day up to 6 days (Dasgupta *et al*., 2015). Urease production was indicated by a bright pink (fuchsia) color on the slant. Any degree of pink color development was considered a positive reaction. Prolonged incubation was avoided as it might result in a false positive test due to the hydrolysis of proteins in the medium. To eliminate protein hydrolysis as the cause of a positive test, a control medium lacking urea was used.

2.6. Data Analysis

Data analysis was carried out using charts, tables, frequency, and percentiles to evaluate PGP traits, and biocontrol properties, of potential plant growth promoting rhizobacterial isolates.

3. RESULTS AND DISCUSSION

3.1. Isolation and primary screening of bacteria

A total of nine rhizospheric representative soil samples were collected and used for the isolation of different PGPR isolates. Out of 56 rhizobacterial isolates, 19 isolates were found coherent for different PGP traits like phosphate solubilization, production of siderophore, IAA, ammonia and H_2S (Table 1).

3.2. Nitrogen Fixation

The nitrogen fixing bacteria were preliminarily screened on nitrogen free malate agar media containing BTB as an indicator. Figure 1 depicts the highest and lowest zones of coloration developed by both of the plant associated rhizotypes. Isolates BS-8, 15, 16, 17, 18, 19, 20, 24, 28, 32, 33, 37, 40, 41, 47, 55, 57, 59 and 66 were found to grow maximally at 37 °C (Table 1). The maximum zone of coloration in the case of the isolates associated observed in B-40 and B-55 isolates.

3.3. Phosphate Solubilization

Bacterial isolates were screened for PO_4 solubilization potential in Pikovskayas broth supplemented with 0.1 % tricalcium phosphate. Among all the isolates 13 isolates were recorded

positively for phosphate solubilization qualitatively (NBRIP) among these the highest solubilization was recorded in the isolate B-55 (14 mm) followed by BS-8 (11 mm), 16, 18, 19, 20, 24, 47, 59 (11 mm) and least was observed in BS-15, 20, 57 (10 mm) (Table 1 & Fig 4). The bacterial isolates were recorded positively for phosphate solubilization by qualitatively (NBRIY), among these the highest solubilization was recorded in the isolate BS-40 (16 mm) followed by BS-55 (15 mm) and least was observed in BS-15, 16, 17, 18, 19, 20, 28, 57 and 59.

3.4. Potassium Releasing Activity

All the isolates recorded positively for potassium releasing activity by qualitatively among these the highest amount was recorded in the isolate BS-40 (16 mm), followed by the isolate BS-55 (14 mm) (Table 1).

3.5. Indole Acetic Acid Production

IAA is the most common phytohormone that is synthesized by bacteria. All the fifty one bacteria exhibited positive for IAA production. Among all the isolates BS-15, 16, 17, 18, 19, 20, 28, 32, 33, 37, 40, 41, 47, 55, 57 and 59, whereas remaining isolates showing weak (*) IAA production (Table 1 & Fig 4). IAA production by varying different physiological parameters such as pH, temperature, carbon and nitrogen sources of culture media. So that the intended conditions at which the IAA production is maximized.

3.6. Exopolysaccharide Production

The bacterial strains which are screened from drought tolerance were further tested for EPS production. Among fifty one isolates maximum amount of EPS production was observed in the isolate BS-37 (0.068 mg ml^{-1}) followed by BS-47 (0.044 mg ml^{-1}) and the least was observed in BS-15 (0.014 mg ml^{-1}) (Table 1 & Fig 4). The formation of EPS in bacteria is triggered by stress and EPS possesses unique cementing and water holding properties through the resulting biofilm formation.

3.7. Siderophore Production

Among all the fifty one isolates siderophore production was observed in the isolates of BS-28, 32, 33, 40 and 55 (Table 1).

Table 1: Plant Growth Promoting Attributes of Bacterial Isolates

Isolate Code	*Nitrogen Fixation*	*P. Sol (mm)*		*K. Rel (mm)*	*Siderophore*	*EPS (mg ml^{-1})*	*IAA*	*NH_3 Production*	*HCN*
		NBRIP	*NBRIY*						
BS-8	+	11	-	-	-	0.030	-	-	-
BS-15	+	10	10	-	-	0.014	+	-	-
BS-16	+	11	10	-	-	0.026	+	-	-
BS-17	+	-	10	-	-	0.021	+	+	-
BS-18	+	11	10	-	-	0.035	+	-	-
BS-19	+	11	10	-	-	0.029	+	-	-
BS-20	+	10	10	-	-	0.017	+	-	-
BS-24	+	11	-	-	-	0.038	-	-	-
BS-28	+	-	10	-	+	0.029	+	+	-
BS-32	+	-	-	-	+	0.027	+	+	-
BS-33	+	-	-	-	+	0.032	+	+	-
BS-37	+	-	-	-	-	0.068	+	+	-
BS-40	+	-	16	15	+	0.018	+	-	-
BS-41	+	-	-	-	-	0.030	+	-	-
BS-47	+	11	-	-	-	0.044	+	-	-
BS-55	+	14	15	14	+	0.033	+	-	-
BS-57	+	10	10	-	-	0.032	+	+	-
BS-59	+	11	10	-	-	0.020	+	+	-
BS-66	+	-	-	-	-	0.026	-	-	-

P: Phosphorus; K: Potassium; EPS: Exopolysachharide; IAA: Indole Acetic Acid: NH_3: Ammonia; HCN: Hydrogen Cyanide, + Positive Result; - Negative Result

3.8. Hydrogen Cyanide Production

All the bacterial isolates were also tested for hydrogen cyanide production. The isolates which are tested for hydrogen cyanide production are all showing negative results (Table 1).

3.9. Biochemical Characterization

All the isolates were motile rods, individual cell morphology, among all 16 isolates were tested positive for methyl red test, 37 isolates were found positive for Voges prausker test, 28 isolates were having the ability of starch hydrolysis, 9 isolates showed able to utilize citrate, 33 isolates were giving positive for urea hydrolysis test, 34 isolates were exhibiting catalase test, 7 isolates were giving positive results for oxidase test, 15 isolates were able to liquefy the gelatin. All the isolates tested negative for the hydrogen sulphide test. The morphological and biochemical characterization of bacterial isolates and their results are tabulated (Table 2).

4. DISCUSSION

PGPR colonizes the roots of the plant and promotes plant growth and development through a variety of mechanisms. The exact mechanism by which PGPR stimulate plant growth is not known, although several mechanisms such as the production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization and promotion of the mineral nutrient uptake are usually believed to be involved in plant growth promotion [Glick, 1995 and Lalande *et al*., 1989]. In the present study, beneficial bacteria were screened against plant growth promoting characteristics. More bacteria are found in the rhizosphere of a plant due to exudates secreted by the plant which could have made rhizosphere samples richer in nutrients thereby increasing the diversity of microbes isolated. Specific constituents of soil nutrients and pH may impose physiological constraints on microorganism's survival and growth, thereby directly altering the bacterial community composition of these constituents. The rhizosphere is a dynamic region governed by complex interactions between plants and organisms that are in close association with the root. The composition and pattern of root exudates affect microbial activity and population numbers, which in turn have an impact on the soil environment [Hillel and Hatfield, 2005]. Beneficial or harmful relationships exist between rhizosphere organisms and plants,

Table 2: Biochemical characterization of Screened Bacterial Isolates

Isolate Code	*Methyl red*	*VP*	*Starch*	*Citrate*	*Urea hydrolysis*	*Catalase*	*Oxidase*	*Gelatin*	*H_2S*
BS-8	-	+	+	+	+	+	-	-	-
BS-15	-	+	-	-	+	+	-	-	-
BS-16	-	+	-	-	-	+	-	-	-
BS-17	-	+	+	-	-	+	-	-	-
BS-18	-	+	+	-	+	+	-	-	-
BS-19	-	+	-	-	+	+	+	-	-
BS-20	-	+	-	-	+	+	-	-	-
BS-24	-	+	+	-	-	+	-	+	-
BS-28	+	+	+	+	+	+	-	+	-
BS-32	+	+	+	+	+	+	-	+	-
BS-33	-	+	+	-	+	+	-	-	-
BS-37	+	-	+	-	+	-	-	-	-
BS-40	+	-	-	+	-	-	-	+	-
BS-41	-	+	+	+	+	+	+	-	-
BS-47	+	+	+	-	+	-	-	-	-
BS-55	+	-	-	+	-	-	-	+	-
BS-57	-	+	-	-	+	-	-	-	-
BS-59	-	+	-	-	-	+	-	-	-
BS-66	-	+	+	+	+	+	-	-	-

VP: Voges Prauskers; H_2S: Hydrogen Sulphide; + Positive Result; - Negative Result

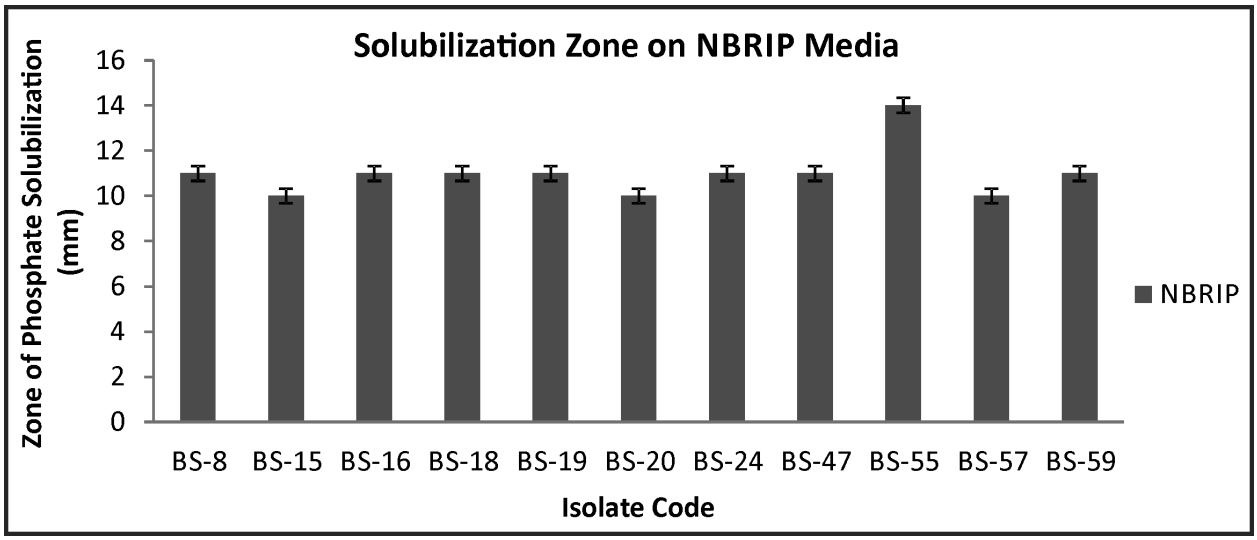


Figure 1: Phosphate Solubilization of Potential Isolates on NBRIP Media

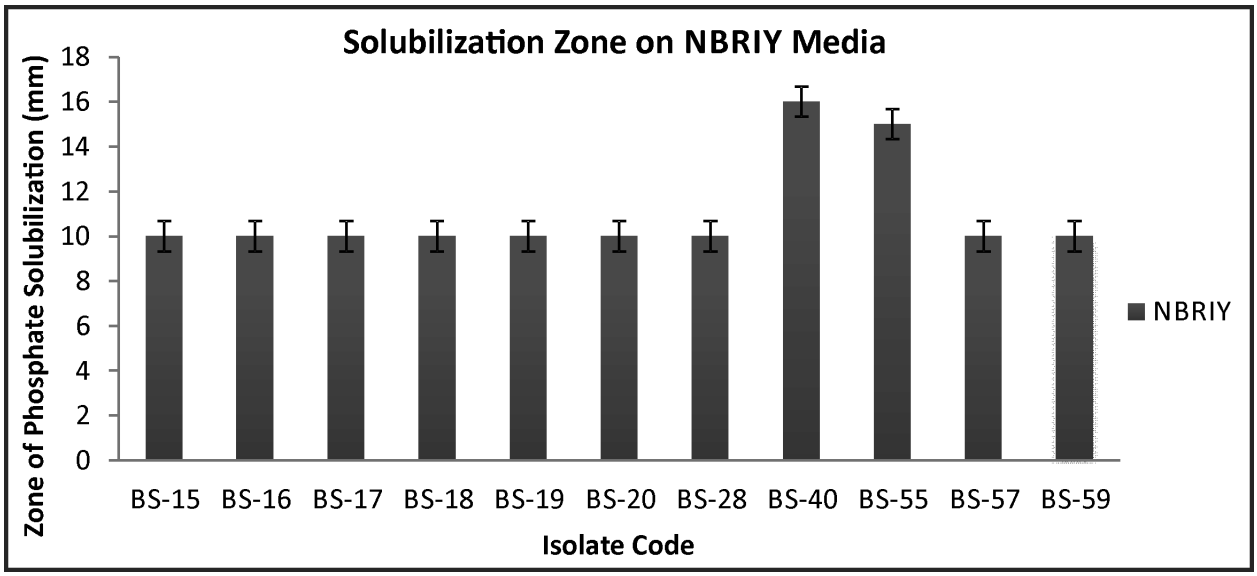


Figure 2: Phosphate Solubilization of Potential Isolates on NBRIY Media

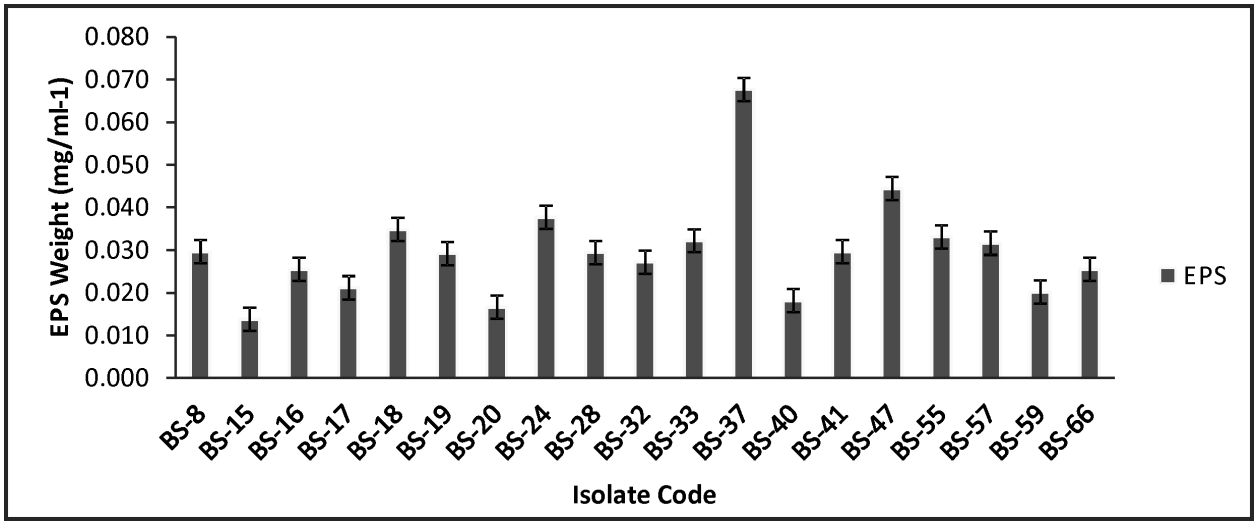
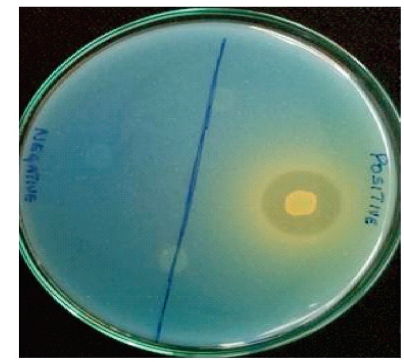
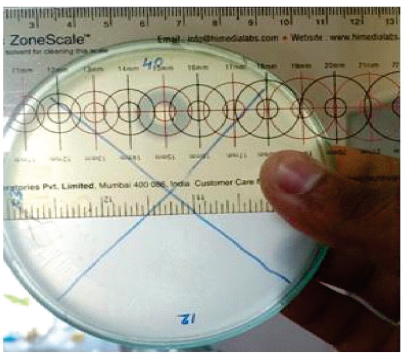


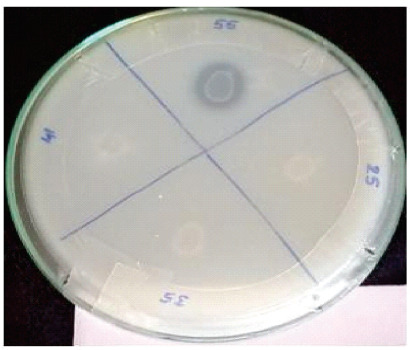
Figure 3: Exopolysachharide Production of Potential Bacterial Isolates



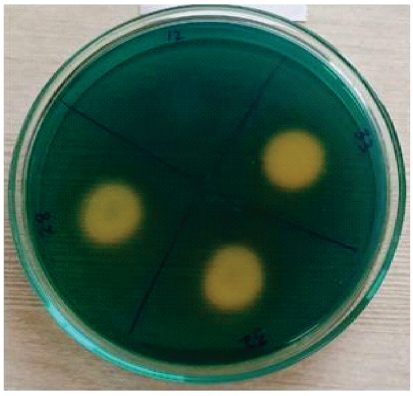
N-Fixation



P-Solubilization

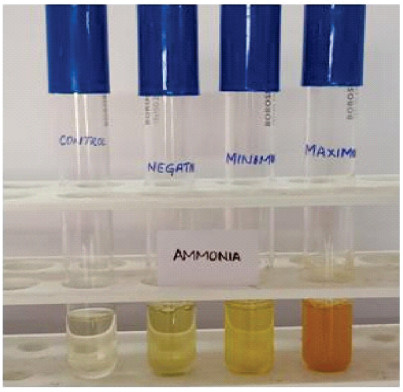


K-Solubilization



Siderophore

Indole Acetic Acid



Ammonia Production

Figure 4: Plant Growth Promoting Attributes

which ultimately affect root function and plant growth. In addition, the rhizosphere may include organisms that do not directly benefit or harm plants but influence plant growth and productivity.

Isolation and screening for potential plant growth promoting bacteria are crucial steps for the preparation of biofertilizers. To select efficient nitrogen fixing, phosphate solubilizing and potassium releasing bacteria, there is a need to develop simple, inexpensive and quick procedures with repeatable and reliable results [Dobereiner, 1988]. Such as an in vitro screening procedure and the combinations of which provide rapid and repeatable results. Insoluble phosphate compounds can be solubilized by organic acids and phosphatase enzymes produced by plants and microorganisms. Phosphate solubilization also has been shown by most of the PGPR. Phosphorous is essential for plant health and is typically insoluble or poorly soluble in soils under salt stress conditions. Some of the bacteria improve the solubilization of unavailable phosphorous and applied phosphates, resulting in higher yields even under stress conditions. Potassium releasing activity is the most important mechanism for the microorganisms to solubilize a fixed form of potassium in the soil. The main mechanism of potassium releasing bacteria is acidolysis, chelation, exchange reactions, complexolysis and production of organic acids. The utilization of potassium releasing bacteria to increase the soluble form of potassium and has been regarded as a desirable pathway to increase plant yields [Dong *et al*., 2019]. The predominance of these genera both in the soil and in the root zone may be due to low nutritional requirements, its capacity to utilize numerous complex organic substrates [Krotzky and Werner, 1987] and high tolerance to low pH [Eckford et al., 2002]. Several mechanisms can be involved in microbial P and K solubilization, with the most common one being via media acidification, as shown by our experiment. The acidification is usually attributed to the production of several organic acids from the fermentation of organic compounds such as citric, gluconic, lactic, malic,

and oxalic acid. The decrease in pH enhances rock phosphate dissolution by removing Ca from rock phosphate, thus releasing P into the solution [Kumari and Phogat, 2008]. Several literature reports suggest that the solubilization of mineral phosphate by microorganisms might also be due to the production of chelating substances (e.g., siderophores) that bind with metal cations (aluminum, iron, and calcium), thus preventing phosphorus complexation [Verma *et al*., 2016; Verma *et al*., 2019].

Exopolysaccharides possess unique water holding and cementing properties, thus playing a vital role in the formation and stabilization of soil aggregates and regulation of nutrients and water flow across plant roots through biofilm formation [Tisdall and Oadea, 1982]. The EPS production of these selected isolates was higher under stress than under no stress conditions, indicating that EPS production in bacteria occurs as a response to the stress [Roberson and Firestone, 1992]. Siderophores are produced and utilized by bacteria as iron (Fe)-chelating agents which are produced in response to iron deficiency which normally occurs in neutral to alkaline pH soils. Although siderophore production is mainly achieved under iron deficiency, other factors such as carbon source, nitrogen source, pH, and temperature are essential to the synthesis of siderophores. It has been reported that higher concentrations of phosphate solubilizing bacteria are commonly found in the rhizosphere soil as compared to nonrhizospheric soil [Reyes and Valduz, 2006]. IAA is one of the most important phytohormones and functions as an important signal molecule in the regulation of plant development. It has been reported that IAA production by PGPR can vary among different species and strains and is also influenced by culture conditions, growth stage and substrate availability [Mirza *et al*., 2001]. The outcome obtained from this study provided insights into the relationship between the rhizosphere bacteria and their plant growth promoting characteristics. Hence, in this regard, further characterization by molecular level and invivo multilocational trials must be needed to finally commercialize the coherent isolates in various types of soils under different agro climatic regions zones to prove the identified bacterial strains.

5. CONCLUSION

On a global scale, the effects of continuous use of agrochemicals for improving agricultural productivity and production can cause serious damage to human and animal health as well as the environment. It can be concluded from the above discussion that plant growth promoting rhizobacteria are increasingly used for agricultural productivity and production improvement. In the same context, it is inferred from the present study was focused on the isolation, screening, PGP and biochemical characterization of bacterial strains inhabiting rhizosphere having excellent PGP traits such as Nitrogen fixation, Phosphate solubilization, Potassium Releasing Activity, Indole acetic acid and ammonia production, biocontrol properties such as siderophore, hydrogen cyanide, exopolysaccharide production was considered for the present study. Isolates with good plant growth promoting potentialities were characterized and the best 15 efficient isolates among them were identified. The results are promising for the design of potentially active plant growth promoting PGPR strain based formulation which would be beneficial for crop improvement and crop protection. For more specific identification of bacteria from the results of this study, it is recommended to carry out 16S rRNA sequencing can also be done to get a complete sequence for molecular identification. Moreover, the bacterial strains and their consortium formulation require field evaluation and validation before being confirmed as bio-inoculants to combat multiple plant growth promoting traits in various agroecosystems.

DATA AVAILABILITY

The data that support the findings in this study are available from the corresponding author upon reasonable request.

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