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# A methodology of isolation for development of PGPR consortia

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**Abstract**
PGPRs offer an innovative solution for enhancing sustainable agriculture by promoting plant growth and acting as biocontrol agents. In this research, PGPRs from maize plant rhizospheres were isolated and assessed for their ability to boost plant growth through nitrogen fixation, nutrient solubilization, and indole-3-acetic acid production. They were also evaluated for their antagonistic potential against pathogens through siderophore, HCN, ammonia, cellulase, chitinase, and catalase production. The study explored the concept of microbial consortia, where combinations of PGPRs collaborate to improve agricultural productivity and combat soil-borne phytopathogens. To utilize these PGPRs effectively, the identification of bacterial isolates was a crucial step. This involved traditional culturing techniques, morphological and biochemical confirmation, followed by a rapid and efficient way for separating and classifing bacterial strains, together with phylogenetic analysis and GenBank submission. Subsequently, promising PGPRs were selected to form consortia using the cross-streaking method. This research contributes to the isolation, characterization, identification, and development of PGPR consortia, offering valuable insights for sustainable agriculture.

**Keywords:** PGPR, rhizosphere, consortia, culturing method, phylogenetic analysis

## Introduction
The ever-growing global population places significant pressure on modern agriculture. There's a need to produce more foodstuff from limited land per capita and diminishing natural resources, which is causing ecological harm and socio-economic challenges. To meet the increasing food demand, reliance on chemical fertilizers and pesticides has surged. However, their long-term use has been found to harm soil health and slow down biological activities (Guerrieri *et al*., 2020) [11]. This necessitates a shift towards sustainable agricultural practices that ensure "safe cultivation" without compromising the environment. The primary challenge for agriculture today is to maximize food production using available resources while maintaining environmental quality. In this context, microbial-based nutrient inputs (Bhattacharyya *et al*., 2020) [4] hold promise for enhancing food production, consistency, and sustainability within the global food chain. Plant growth and development process is a multifaceted progression, and a potential solution lies in harnessing the power of plant growth-promoting rhizo-bacteria (PGPR) found in the rhizospheric soil (Kumar *et al*., 2014) [16]. PGPRs possess diverse properties that benefit plant growth and development. In the rhizosphere, these microbes play pivotal roles in organic matter transformation, biogeochemical nutrient cycling, and interactions with host plants, all of which have favorable property on plant growth, development, differentiation and nutrition. These rhizo-bacteria add to plant development by fixing atmospheric nitrogen, solubilising essential macro and micro nutrients like phosphate, potash, iron, zinc etc. synthesising siderophores that enhance iron availability to plant roots, synthesizing plant growth regulators, phytohormones, and enzymes. They significantly contribute to crop bio-fertilization (Lihan *et al*., 2021) [18]. PGPRs also play a role in disease suppression and have been identified as effective biological control agents against various plant pathogenic fungi. These bacteria activate the Induced Systemic Resistance (ISR) of plants in opposition to a wide range of biotic stress. They combat plant diseases through the production of siderophores, ammonia, HCN, and biodegrading enzymes such as catalase, cellulase, and chitinase (Pirapak *et al*., 2022) [24]. Numerous research groups have successfully created microbial consortia using combinations of microbes, such as dual, triple, tetra, penta, and hexa mixtures, as biofertilizers.

These consortia promote both plant growth and biocontrol activities (Mukherjee *et al*., 2021) [22]. The employment of microbial consortia not only enhances disease suppression but also positively impacts plant growth promotion. Such consortia have the potential to reduce reliance on chemical fertilizers and pesticides, mitigating environmental damage (Stockwell *et al*., 2011) [31]. Therefore, the utilization of PGPR inoculants offers an attractive option to the excessive use of harmful agrochemicals.

## Materials and Methods

### Collection of Rhizospheric soil sample

Rhizospheric soil samples were assembled from various maize cultivation areas in Bihar, specifically from locations where maize plants exhibited consistent and robust growth. In order to capture the characteristics of the entire field, five samples were collected from each individual field, and these were combined to create a composite sample. These soil samples were then transported for the separation of rhizospheric bacteria to the laboratory.

### Selection of growth media

An array of growth media was employed for the isolation of rhizobacteria from maize rhizospheric soil. These included Nutrient agar, King's B agar, Jensen agar, N-free Okon's medium, Soil extract agar, T3 medium, Semisolid nitrogen-free bromothymol medium, Pikovskaya medium, Trypticase soy agar, and Kenknight agar.

### Bacterial Isolation

A suspension mixture of soil sample was created by mixing 1 gram of soil with 99 milliliters of sterile water, after that a 10-times serial dilution. To isolate bacteria, 0.1 milliliters of each dilution were inoculated on nutrient agar plates and then these plates were incubated at 28 °C for 24 hours. To ensure the purity of bacterial strains, a series of streaking procedures on nutrient agar medium was conducted, typically repeated at least three times. The selection of distinct colonies was based on various morphological characteristics, including shape, color, size, regularity, as well as the curvature or flatness of the colonies. These purified bacterial strains were then preserved by cryopreservation at -70 °C in a solution of 30% glycerol.

For assessing bacterial abilities associated to encouraging plant growth and development, a single bacterial colony was chosen among the nutrient agar medium. This colony was cultured in nutrient broth at 30 °C under agitation at 180 rpm for a period of 24 hours. Following centrifugation at 10,000 x g for 5 minutes at 4 °C in addition to subsequent triple washing with sterilized water, the initial bacterial density was determined by adjusting the cell suspension to an optical density at 600 nm wavelength (OD600) of 0.85, roughly equivalent to 10^8 colony-forming units per milliliter. The relationship between the OD600 value with bacterial mass was established by quantifing the OD600 and conducting plating of the appropriate bacterial dilution on nutrient agar medium, then allowing overnight incubation at 30°C.

### Morphological characterization of bacterial Isolates

### Colony Morphology studies

During the purification process, each isolated bacterial culture underwent a thorough examination of its colony morphology. This examination was conducted by streaking a loop filled with the isolated culture onto agar plates. The evaluation encompassed characteristics like colony color, size, shape, texture, and margin.

### Gram staining

To identify the chosen microbial isolates, the standard Gram staining technique was employed. A clean slide devoid of any markings was selected, and a designated area for preparing the smear was marked. A drop of sterilized distilled water was located at the center of the glass slide. A loopful of inoculum from a young culture was mixed with the water on the slide and stretched into a thin smear by an inoculating needle. The smear was left undisturbed to dry in air and was then heat-fixed by passing the slide over a flame three to four times.

Subsequently, the spread was flooded with crystal violet solution for 30 seconds and gently washed with tap water. It was then soaked with iodine solution for 30 seconds, followed by rinsing with water. To decolorize, the slide was flooded with 95% ethyl alcohol for 10 to 15 seconds and rinsed by water. A stain of Safranin solution was put in the smear for 30 seconds, followed by rinsing with tap water. Following air-drying, the slide was examined with the help of a microscope at 100x magnification by oil immersion, also the relevant data were recorded (Gram, 1884) [9].

### Screening of isolates for PGPR characters

The isolated bacterial cultures underwent a comprehensive assessment for their PGPR characteristics, including the examination of nitrogen-fixing abilities, the solubilization of phosphorus, potassium, plus zinc, as well as the synthesis of Indole 3-acetic acid (IAA). Additionally, the screening encompassed the evaluation of siderophore production, ammonia generation, hydrogen cyanide (HCN) discharge, cellulose decomposition, catalase activity, and chitinase enzyme production.

### Nitrogenase activity

To assess nitrogenase activity, we employed the acetylene reduction assay as detailed by Hardy *et al*. in 1973. In this procedure, purified bacterial colonies were introduced into Nitrogen-Free Malate (NFM) semi-solid medium vials and then placed in incubation at 28 ± 2 °C for period of 48 hours. Next to this incubation period, acetylene (at a concentration of 10% v/v) was introduced into the vials. They were once again incubated at 28 ± 2 °C, this time for 16 hours.

To analyze the results, 100 µL of gas samples from the vials were collected and subjected to examination using a gas chromatograph (NUCON 5765 Gas Chromatograph). We took necessary precautions to prevent any unwanted interference or reactions. The reaction was stopped by introducing 0.2 mL of a 50% Trichloroacetic acid (TCA) solution. To quantify the acetylene reduction, 1 mL of gas was injected into a preheated gas chromatograph, where nitrogen (N2) served as the carrier gas. The operating temperatures for the injector, column, and detector were maintained at 100 °C, 80 °C, and 100 °C, respectively. The retention period of standard ethylene was used to calculate the amount of ethylene produced from the acetylene reduction. The acetylene reduction activity (ARA) was expressed in terms of nanomoles of ethylene produced per milligram of protein per hour. This testing was performed twice, with each recurrence having three replicates, and the mean value was computed.

**Phosphorus solubilization ability assessment**

To qualitatively assess the phosphorus solubilization activity of endophytic bacteria, we employed Pikovskaya agar medium. This involved the observation of halo zones forming around bacterial colonies. The extent of phosphorus solubilization activity was quantified using two parameters: the Phosphorus Solubilization Index (P-SI) and the Phosphorus Solubilization Efficiency (P-SE). These measurements were based on the diameter of the halo zone and the diameter of the bacterial colonies after a 72-hour incubation period at 30 °C, as outlined by Pikovskaya in 1948.

$$\mathrm{P-SI} = \frac{\text{Colony diameter (mm)} + \text{Halo zone diameter (mm)}}{\text{Colony diameter (mm)}}$$

$$\mathrm{P-SE} = \frac{\text{Halo zone diameter (mm)}}{\text{Colony diameter (mm)}} \times 100$$

To quantitatively assess phosphate solubilization activity in endophytes, we employed the NBRI Phosphate Broth Medium (NBRI-PM). Tricalcium phosphate served as the sole phosphorus source for the bacterial isolates. The process involved inoculating the bacterial cultures and placing them in an incubator cum rotary shaker at 30°C for 9 days, with a rotation speed of 180 rpm, following the method by Mehta and Nautiyal in 2001.

After the incubation period, the bacterial broth was centrifuged at 12,000 rpm for 15 minutes. From the resulting supernatant, 1 mL was taken and diluted with 40 mL of distilled water. Additionally, 10 mL of ammonium molybdate solution (prepared by dissolving 12 g of ammonium molybdate in 250 mL of distilled water) was added to make a total volume of 50 mL, as per the method by Murphy and Riley in 1962. To enable quantitative estimation, 5 drops of ascorbic acid (prepared by dissolving 1.056 g of ascorbic acid in 200 mL of water) were added. The formation of a fluorescent blue color indicated the availability of phosphorus.

Finally, the available phosphate was measured using a spectrophotometer at an optical density (OD) of 600 nm and expressed in micrograms per milligram (µg/mg) through the creation of a standard curve using tricalcium phosphate.

**Potassium SolubilizationAbility Assessment**

The potassium solubilization activity of endophytic bacteria was qualitatively examined by using Aleksandrov agar plates by formation of halo zone around colonies. Thepotassium solubilization activity was expressed terms of potassium solubilization index (K-SI) and potassium solublization efficiency (K-SE) by measuring halo zone diameter and colony diameter after 72 hours of incubation at 30 $^0$c (Prajapati and Modi 2012) [25].

$$\mathrm{K-SI} = \frac{\text{Colony diameter (mm)} + \text{Halo zone diameter (mm)}}{\text{Colony diameter (mm)}}$$

$$\mathrm{K-SE} = \frac{\text{Halo zone diameter (mm)}}{\text{Colony diameter (mm)}} \times 100$$

The potassium solubilization activity of endophytic bacteria will be qualitatively examined by using Alexandrove both. The bacterial broth will be centrifuged at 12000 rpm for 15 minutes, the supernatant carried to estimate the potassium solubilization by flame photometer and expressed in terms of µg/ ml (Jabin *et al*., 2017) [12].

**Zinc Solubilization ability assessment**

The zinc solubilization activity of endophytic bacteria was qualitatively examined by formation of halo zone around colonies on Nutrient agar media blended with 0.1% of Zinc oxide. The zinc solubilization activity was expressed terms of zinc solubilization index (Zn-SI) and zincsolublization efficiency (Zn-SE) by measuring halo zone diameter and colony diameter after 72 hours of incubation at 30$^0$c (Saravanan *et al*, 2003) [30].

$$\mathrm{Zn-SI} = \frac{\text{Colony diameter (mm)} + \text{Halo zone diameter (mm)}}{\text{Colony diameter (mm)}}$$

$$\mathrm{Zn-SE} = \frac{\text{Halo zone diameter (mm)}}{\text{Colony diameter (mm)}} \times 100$$

The quantitative estimation of zinc solubilization activity examine by using liquid mineral salts medium (g/lit) as specified by Saravanan *et al*. (2007) [30]. Blended with 0.1% zinc oxide. According to the qualitative test, the positive bacterial isolates used to inoculate into the broth and keeping it incubator 3days at 30 $^0$C. Isolates were separately inoculated (10 µl of overnight bacterial culture) into 150ml conical flasks containing 50 ml of liquid mineral salt medium. Then the broth was incubated at 160 rpm for 72 hours in an incubator shaker. After the period of incubation, the culture was centrifuged and the supernatant was estimated in an atomic absorption spectrophotometer to know the concentration of Zn and expressed in terms of µg/ ml (Goteti *et al*., 2013).

**Fe Solubilization Ability Assessment**

The Fe solubilization activity of endophytic bacteria was qualitatively examined by formation of orange color halo zone around colonies on Chromo azurol sulfonate (CAS) agar (adding 100 ml of Dark blue CAS mixture to 300ml of nutrient agar medium) plate. The Fe solubilization activity was expressed terms of Fe solubilization efficiency (%) (Fe-SE) by measuring orange color halo zone and colony diameter after 72 hours of incubation at 30 $^0$C (Schwyn and Neilands, 1987).

$$\mathrm{Fe-SE} = \frac{\text{Halo zone diameter (mm)}}{\text{Colony diameter (mm)}} \times 100$$

Quantitative estimation of Fe solubilization of bacterial isolate by using the nutrient broth. The broth were centrifuge at 12000 rpm for 10 mins. The clear supernatant was mixed with CAS dye. After 20 mins, the dye were settled in bottom and sample was centrifuge at 12000 rpm for 10 min, whereas, amount of solubilized Fe were measuring by Atomic absorption spectrophotometer. The final value was expressed in µg/ml (ppm).

**Siderophores Production Assay**

To assess siderophore production quantitatively, the Chrome Azurol S (CAS) assay was employed, following the methodology proposed by Arora and Verma in 2017. In brief, bacterial cultures aged 48 hours were subjected to

centrifugation at 10,000 rpm for 10 minutes, leading to the removal of cell pellets. The resulting supernatant was utilized to estimate the siderophore content. Subsequently, 100 µl of the supernatant from each bacterial culture was mixed with 100 µl of the CAS reagent. After a 20-minute incubation period, the optical density was measured at 620 nm using a spectrophotometer. Siderophore produced by strains was measured in percent siderophore unit (psu) which was calculated according to the following formula:

Siderophore production (%) = (Ar - As) X 100

Ar Where Ar is absorbance of reference and As is absorbance of sample

**Determination of theIAA Production**

For the qualitative assessment of IAA (Indole-3-Acetic Acid) production in isolated bacterial samples, we followed the method outlined by Gorden and Webber in 1951. We utilized Nutrient broth amended with 5µg/ml of tryptophan. The procedure involved inoculating the bacterial samples and then incubating them for three days.To determine the IAA production activity of the bacteria, the cultures were subjected to centrifugation at 10,000 rpm for 10 minutes. Subsequently, two drops of ortho-phosphoric acid, along with 4 mL of Salkowski reagent mixed with 35% perchloric acid, were added to 1 mL of the supernatant. This mixture was then incubated at room temperature for 20 minutes. The IAA production activity of the bacteria was indicated by a change in color to pink.For quantitative measurement, the intensity of the color development was read at 530 nm using a spectrophotometer. To quantify the IAA, a standard curve was prepared, and the results were expressed in micrograms per milligram (µg/mg) of protein.

**Ammonia Production Activity**

To qualitatively assess the ammonia production activity of the endophytic bacterial isolates, we employed a method involving the detection of a brown color formation on peptone medium upon the addition of Nessler's reagent. Here's how the procedure was carried out: Bacterial cultures were inoculated onto peptone medium in test tubes and then incubated for a period of 72 hours at 30°C.After the 72-hour incubation, Nessler's reagent was introduced into the tubes. The development of a brown color was indicative of ammonia production. To quantitatively measure the amount of ammonia produced, spectrophotometry was used to determine the optical density (OD) at 630 nm. This data was then converted into units of micrograms per milliliter (µg/ml) by creating a standard curve, following the method by Weise *et al*. in 2013 [33].

**Hydrogen cyanide production**

The HCN production ability of endophytic bacterial isolates by using 10% tryptone soy agar medium plates supplemented with glycine (4.4 g $l^{-1}$). The tryptone soy agar plates were spotted with an inoculation of 10 µl bacterial culture and the spot was spread using spreader. After spreading, Whatman filter paper No. 1 which was soaked in 2% sodium carbonate and 0.5% picric acid solution was placed to the underside of the lid of Petri plate, the plates were closed and sealed with parafilm and incubated for 5-7 days at 30 °C. The indicated the positive result for production of HCN was the change of filter paper color from yellow to reddish-brown (Castric, 1975) [5].

**Cellulase enzyme production**

The cellulose-degrading activity of endophytic bacteria was qualitatively examined by formation of halo zone around colonies on cellulose Congo red agar media. The cellulose-degrading activity was expressed terms of hydrolysis capacity (HC) by measuring clear zone diameter and colony diameter after 72 hours of incubation at $30^0$ C (Pratima *et al*., 2011).

$$\text{Hydrolysis Capacity(HC)} = \frac{\text{Clear zone diameter (mm)}}{\text{Colony diameter (mm)}}$$

**Catalase enzyme production**

Examine the catalase production test by Slide method using a loop or sterile loop to transfer a small amount of colony growth or bacterial broth in the surface of a clean, dry glass slide. Place a drop of 3% $H_2O_2$ in the glass slide. Observe for the evolution of oxygen bubbles a positive result of catalase production (Apostol *et al*.,1989) [1].

**Chitinase enzymeproduction**

Chitinase production ability of the rhizobacterial isolates was was evaluated using on nutrient agar media plates which were supplemented with 0.1% chitin. The plates were divided into four parts and each part was streaked with bacterial culture. The plates were incubated for 48-96 hrs at 30 °C and observation of zone of clearance around the streaked colony which was an indicator of chitinase production (Frandberg and Schnurer, 1998).

**Molecular identification of PGPR isolates**

**Bacterial DNA Isolation**

Genomic DNA of the bacterial isolate was purified by a modified CTAB protocol derived from the process by Wilson *et al*. (2001) [34]. At first, 5 mL culture of the bacteria was centrifuged at 10,000 rpm for 1 minute. The supernatant was separated, and the bacterial pellet was suspended in 750 µl of TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0). Into this suspension, 20 µl of lysozyme (100 mg/ml) was supplemented and carefully mixed by inversion, followed by incubation at 37 °C for a period of 10 minutes.

Next, 40 µl of 10% SDS with 8 µl of proteinase K (10 mg/ml) were brought in to the lysate, thoroughly mixed, as well as incubated for 30 minutes at 56 °C. This step was followed by the adding up of 100 µl of 5 M NaCl, thorough mixing, and then 100 µl of preheated CTAB/NaCl solution. The CTAB/NaCl solution was prepared by dissolving 4.1g NaCl in 80 ml of water, adding 10g CTAB slowly while heating at about 65 °C, making up the final volume to 100 ml with nuclease-free water, sterilizing it by autoclaving, followed by incubating it at 65 °C for 10 minutes.

Post-incubation, an equal quantity of chloroform: isoamyl alcohol (1:1) solution was added to the bacterial lysate, mixed gently by inversion, and centrifuged at 1000 rpm for 5 minutes. The resulting aqueous solution was carefully transferred to a clean micro-centrifuge tube. DNA precipitation was achieved by adding 0.6 volumes of isopropanol, mixed thoroughly by inversion, incubated on ice for 10-15 minutes, and then centrifuged at 10,000 rpm for 10 minutes. The supernatant was disposed, and the DNA pellet was washed with 70% ethanol. Finally, the DNA pellet was

dissolved in 100 µl of nuclease-free water, assessed on an agarose gel, and used for PCR analysis.

**PCR Amplification and Sequencing**

The amplification of the 16S rRNA gene fragment was conducted using the 27F (5-AGAGTTTGATCCTGGCTCAG-3) and 1492R (5-TACGGTTACCTTGTTACGACTT-3) primers. The PCR reaction mixture containing 5 µl of 10x PCR buffer, 0.2 µM dNTPs, 1 µM of each primer, 5 µl of DNA template, 1 unit of Phusion® High-Fidelity DNA Polymerase (NEB), and sterilized de-ionized water to achieve a final volume of 50 µl. The PCR profile was set with an initial denaturation at 94°C for 3 minutes, followed by 25 cycles of denaturation (94°C, 30 sec), annealing (51 °C, 30 sec), extension (72°C, 1 minute), and a final step of extension at 72°C for 5 minutes.

The resultant PCR products were purified by agarose gel elution. The gel-eluted rDNA fragments were further sequenced using the 27F and 1492R primers.

**Identification of Isolates by 16S rRNA Sequence Analysis**

The sequence data obtained from both the forward and reverse primers were processed and analyzed using Bioedit version 7.2.5 (Hall *et al*., 1999) [34]. Consensus sequences were derived by aligning the sequences in pairs to identify the bacterial isolate. These nucleotide sequences were then compared against the NCBI non-redundant database (Nr) using BLAST (BlastN). The top 10 hits obtained from this search were utilized to create a phylogenetic tree for further analysis.

To preserve and share the identified sequence, it was submitted to the 16S rRNA submission portal at NCBI (https://submit.ncbi.nlm.nih.gov/) to acquire an accession number for future reference.

**Identification of Isolates by 16S rDNA Sequence Analysis**

Swift identification of bacterial isolates is essential for their proper utilization in agricultural applications. Utilizing 16S rRNA sequencing has emerged as a robust strategy for bacterial identification (Ayyaz *et al*., 2016; Kai *et al*., 2019) [3, 13]. The 16S rDNA was amplified from the genomic DNA of the identified isolates. The resulting PCR product was purified from the gel and sequenced employing both the forward and reverse primers as detailed in the materials and methods section. Utilizing BioEdit software, conserved sequences were extracted. These bacterial strain sequences were matched against publicly accessible databases such as NCBI using BLASTN. The top ten matches were employed to construct a phylogenetic tree using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) with default parameters.

**Development of PGPR Consortia**

To evaluate compatibility for the development of PGPR consortia, a method described by Raja *et al*. (2006) [26] was employed. Bacterial cultures were streaked on nutrient agar plates by vertically streaking in the center and horizontally at the plate's periphery. These cross-streaked plates were then placed in an incubator at 28 ± 1°C for 48-72 hours. The absence of inhibition zones surrounding the colonies indicated compatibility, signifying that these colonies did not exhibit antagonistic behavior. These compatible colonies were selected for the PGPR consortium development.

Broth cultures (2ml each) of the selected compatible isolates, grown in 72-hour-old cultures, were transferred into 250 ml conical flasks containing 100ml of nutrient broth. The consortium was incubated on a reciprocal shaker at room temperature for 48 hours to encourage optimal growth. After incubation, the cell count (cfu) of the consortia and their optical density at 600 nm (OD600) were measured for further analysis.

**Result and Discussion**

The nitrogen-fixing activity of the purified PGPR isolates was assessed using the acetylene reduction assay (ARA), revealing activity within the range of 17.6 to 42.14 µmol C2H4/mg chl/hr after a 14-day incubation period. These isolates exhibited phosphorus (P), potassium (K), zinc (Zn), and iron (Fe) solubilization activities, ranging from P-SI-2.2 to 4.1 & P-SE-122.7 to 309.1%, K-SI-2.0 to 3.8 & K-SE-178.6 to 282.4%, Zn-SI-2.0 to 4.5 & Zn-SE-103.4 to 350%, and Fe-SE-109.5 to 369.2%. Additionally, these isolates demonstrated siderophore and indole-3-acetic acid (IAA) production in the range of 37.22 to 96.51 µg mg-1 protein. Furthermore, they displayed ammonia production (0.49 – 1.28 ppm), as well as enzymatic activities including hydrogen cyanide (HCN), catalase, chitinase, and cellulase (HC- 2.0 to 9.75).

The isolation of potential PGPR from the rhizosphere often yields numerous isolates, as noted by various researchers (Deka *et al*., 2015; Kennedy *et al*., 2004) [6, 14]. Consortia, comprised of multiple microbes, synergistically interact to bolster plant growth and defend against phytopathogens, as highlighted in studies by Santoyo *et al*. (2021) [29], Kumar *et al*. (2018, 2021) [16-18]. Nitrogen, crucial for plant growth, can be facilitated by biological nitrogen fixation (BNF) through microorganisms (Santi *et al*., 2013) [28]. Additionally, the solubilization of P, K, Zn, and Fe by soil bacteria helps restore soil nutrient balance and health for sustained agricultural productivity, a process attributed to the secretion of organic acids and phosphatases (Pastore *et al*., 2020; Mpanga *et al*., 2020) [23, 21].

Siderophores produced by PGPR serve both as a direct and indirect mechanism for plant growth enhancement. These low molecular weight organic compounds aid in providing plants with iron nutrition under low-iron conditions while concurrently restricting iron availability, hindering the proliferation of fungal pathogens. Siderophores facilitate the scavenging and transport of iron, converting it into a biologically relevant form (Rengel *et al*., 2005; Wang *et al*., 2021) [27, 32]. Ammonia production by PGPR is pivotal for promoting plant growth by providing essential nitrogen, thereby supporting root and shoot elongation and biomass (Marques *et al*., 2010) [20].

Hydrolytic enzymes like chitinases and cellulases play a vital role in combating fungal infections in plants. These enzymes act through direct lytic action on fungal cell walls or by inducing plant defense mechanisms through the release of signaling molecules (Glick *et al*., 2007; Lugtenberg *et al*., 2009; Grover *et al*., 2012) [7, 19, 10].

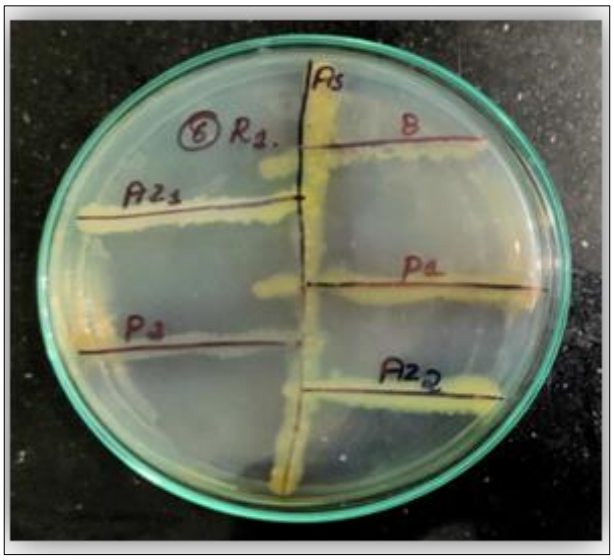


**Fig 1:** Cross streak plate for compatibility test

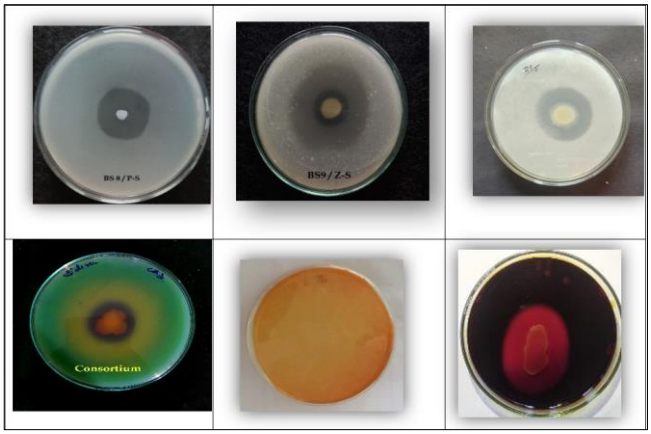


**Fig 2:** P, K, Zn and Fe solubilizing zone and HCN and Chitinase produced by PGPR isolates

## Conclusion

Conventional agricultural practices have heavily relied on chemical fertilizers to provide essential nutrients for soil-plant systems. However, due to concerns regarding their cost, availability, and environmental impact, alternative solutions are being explored. Among these alternatives, Plant Growth-Promoting Rhizobacteria (PGPR) have emerged as a promising avenue for stimulating plant growth by releasing growth-promoting compounds. PGPR play a pivotal role in supporting plant health through multiple mechanisms, such as increasing nutrient availability, enhancing disease resistance, and promoting overall plant growth. The utilization of microbial consortia, where diverse PGPR strains work synergistically, further amplifies these advantages, positively impacting crop growth. Comprehending native bacterial populations, their characterization, and identification of specific strains are fundamental steps in understanding the distribution and diversity of indigenous rhizospheric bacteria. In our present study, we outline a detailed procedure for bacterial isolation, gram staining, 16S rDNA amplification, sequencing, and subsequent submission of 16S rDNA sequences to GenBank to acquire accession numbers. Our research highlights that while biochemical tests for bacterial identification and characterization are valuable, they might have limitations in distinguishing closely related strains. In contrast, full-length 16S rRNA sequencing offers a more comprehensive and precise understanding of the distinct characteristics of each isolate within the rhizosphere. To summarize, this outlined procedure provides researchers with the necessary tools to isolate, characterize, identify, and cultivate bacterial consortia, harnessing their potential to augment crop growth.

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