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Investigating The Agricultural Advancements: Harnessing The Power Of PGPR For Sustainable Plant Growth

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Abstract:

In agricultural landscapes, the rhizosphere serves as a dynamic niche where plant-microbe interactions profoundly influence soil health and plant growth. Plant Growth-Promoting Rhizobacteria (PGPR) is pivotal contributor to soil fertility, nutrient cycling, and plant health. In the context of Bihar, India, rice cultivation holds paramount importance for food security and economic stability but faces challenges related to soil degradation and nutrient deficiency. Harnessing the potential of indigenous PGPR strains presents a promising strategy to enhance rice productivity sustainably. This research endeavors to explore the microbial diversity in the rhizospheric soil of rice fields in Bihar, focusing on isolating and characterizing potential PGPR strains. Through molecular and microbiological techniques, indigenous PGPR candidates are identified for their plant growth-promoting attributes, including nitrogen fixation, phosphate solubilization, and biocontrol capabilities. Biochemical and molecular analyses reveal diverse metabolic capacities and genetic relatedness of the isolates, with Azotobactor spp. exhibiting a notable 98.61% similarity to Bacillus tropicus. These findings underscore the potential of microbial biotechnology to enhance agricultural sustainability, foster food security, and mitigate environmental impacts, heralding innovative strategies for rice cultivation in Bihar and beyond.

Keywords:

PGPR, rhizospheric soil, rice fields, isolation, characterization, functional assays, plant growth promotion *etc*.

1. Introduction:

In agricultural ecosystems, the rhizosphere serves as a vibrant microbial hotspot where intricate interactions between plants and microorganisms shape soil health and plant growth dynamics. Among the diverse array of microorganisms inhabiting the rhizosphere, Plant Growth-Promoting Rhizobacteria (PGPR) have emerged as key players in enhancing plant growth, nutrient uptake, and stress tolerance (Kashyap et. al., 2021). The exploration of PGPR communities holds immense promise for sustainable agriculture by reducing dependency on chemical fertilizers and pesticides while fostering eco-friendly farming practices.

Rice (Oryza sativa L.) is one of the world's most crucial staple crops, supporting the dietary needs of a significant portion of the global population. In the agroecological context of Bihar, India, rice cultivation represents a cornerstone of agricultural practices, contributing substantially to both food security and economic stability. However, the sustainability and productivity of rice farming systems face multifaceted challenges, including nutrient deficiency, soil degradation, and environmental stressors (Swarupa and Kiran, 2020).

The rhizospheric soil of rice fields harbors a rich reservoir of microbial diversity, with PGPR exhibiting the potential to revolutionize agricultural sustainability and productivity (Kour et al., 2019). Harnessing the beneficial attributes of indigenous PGPR strains offers a promising avenue for augmenting rice growth, yield, and resilience in the face of abiotic and biotic stresses. Through systematic screening and characterization of PGPR strains from the rhizospheric soil of rice fields in the Bihar region, valuable insights can be gleaned into the microbial dynamics underlying plant-microbe interactions and their implications for agricultural sustainability (Kumavat et al., 2022).

This research endeavor aims to undertake a comprehensive exploration of the microbial diversity within the rhizospheric soil of rice fields in Bihar, with a particular focus on isolating and characterizing potential PGPR strains. By employing state-of-the-art molecular and microbiological techniques, this study seeks to identify indigenous PGPR candidates endowed with multifaceted plant growth-promoting attributes, including nitrogen fixation, phosphate solubilization, phytohormone production, and biocontrol capabilities against phytopathogens.

2. Materials and Methods:

2.1 Sample Collection:

The process of collecting rhizosphere soil samples from rice fields entails the meticulous selection of sampling sites, emphasizing randomness to ensure comprehensive representation of the field's characteristics such as soil texture, pH levels, and plant health. Using soil augers or corers, samples are gathered from depths of 5-10 cm to capture diverse microbial populations. Upon collection, samples undergo controlled air-drying to prevent contamination and facilitate subsequent analyses, followed by sieving through a 2 mm mesh to remove debris and ensure homogeneity. Labeled containers preserve sample integrity during storage at 4°C, facilitating traceability and preventing confusion during further analysis (Das et al., 2019; Glick, 2016).

2.2 Isolation of PGPR

2.2.1 Screening of Symbiotic Nitrogen-fixing Microorganisms

Nitrogen fixation, a vital process in soil, is facilitated by organisms like Rhizobium and Azotobacter, crucial for meeting plants' organic nitrogen requirements. Symbiotic and non-symbiotic nitrogen fixation pathways are key mechanisms in this process (Haerani et al., 2023). Rhizospheric soil samples were serially diluted to 10^-8, and 0.1 ml aliquots were aseptically added to sterile YEMA Agar with Cycloheximide (200 mg/L) and YEMA Agar with Bromothymol Blue agar. Nitrogen-fixing microbes were screened based on colony characteristics on YEMA Agar and their acid or alkali production on YEMA agar with Bromothymol blue (Tan et al., 2014).

2.2.2 Screening of Non-symbiotic (Free-Living) Bacterial Species from Soil

Non-rhizospheric soil samples (100 mg) were serially diluted to 10^-8, and 0.1 ml aliquots were aseptically added to sterile Ashby's Mannitol Agar medium. Free-living nitrogen-fixing microbes were screened based on colony characteristics on Ashby Agar (Sherpa et al., 2021; Bandeppa et al., 2019).

2.2.3 Screening of Phosphate Solubilizing Microbes

Non-rhizospheric soil samples (100 mg) were serially diluted to 10^-8 using sterile saline water, and 0.1 ml aliquots were added aseptically to sterile Pikovskayas Agar medium. Phosphate solubilizing microbes were screened based on the visible appearance of zones of solubilization of inorganic phosphate around the colony (Gupta et al., 2022; Patel et al., 2022).

2.2.4Screening of Sulfate-Reducing Bacteria from the Soil

Non-rhizospheric soil samples (0.1 gm) were serially diluted to 10^-8 using sterile saline water, and 0.1 ml aliquots were added aseptically to sterile Sulphate Reducing Agar medium. Sulfate-reducing microbes were screened based on the visible appearance of zones of solubilization of inorganic sulfate around the colony (Lin et al., 2010; Ouattara and Jacq, 1992).

2.3 Identification of Isolates

2.3.1 Assessment of Colony Characteristics

All bacterial isolates were cultured on selective agar media and assessed for colony characteristics including size, shape, color, margin, opacity, consistency, motility, and Gram characteristics (Hardiansyah, 2020; Naureen et al., 2005).

2.3.2 Assessment of IMViC (Indole, Methyl Red, Voges Prosker, and Citrate Utilization Assay)

IMViC test, comprising Indole, Methyl Red, Voges-Proskauer, and Citrate Utilization tests, was conducted to detect specific enzymatic activities indicating bacterial metabolic pathways (Das et al., 2019; Hamza et al., 2017; Mohite, 2013; Wagh et al., 2015).

2.3.3 Carbohydrate Fermentation Test

Bacterial isolates were inoculated into presterilized carbohydrate fermentation media containing different sugars and observed for fermentation patterns after incubation at 37°C for 24-48 hours (Deaker et al., 2008).

2.3.4 Catalase Production Assay

Catalase production, indicative of a bacterium's ability to neutralize hydrogen peroxide, was assessed by adding 3% H2O2 to bacterial suspensions and observing for immediate bubbles (Reiner, 2010).

2.3.5 Amylase Production Assay

Bacterial isolates were inoculated onto starch agar plates and observed for hydrolysis zones after iodine staining to detect amylase production (Visvanathan et al., 2020).Characterization of Isolated Strains:

2.3.6 Molecular Identification of Azotobactor spp. Isolate

The Azotobacter spp. isolate underwent molecular level identification and phylogenetic analysis at Mr. Biologist Institute in Pune. The isolate was subjected to 16S Conserved Region Gene Sequencing Service GBSEQ10 for nucleotide sequencing of the 16S rRNA gene. The obtained nucleotide sequence was subsequently analyzed through BLAST analysis using the MEGA software platform.

2.3.7 Phylogenetic Analysis of Azotobactor spp.

Phylogenetic analysis, which elucidates the evolutionary history of organisms through tree-like structures, was conducted. This analysis visualizes similarities among related biological sequences, achieved through sequence alignments. Evolutionary relationships are depicted in the form of phylogenetic trees, with correct alignments essential for accurate tree construction (Aquilanti et al., 2020).

Sequences of 16S ribosomal RNA (Bacteria and Archaea type strains) reference material genes were subjected to BlastN search program to determine homology, with closely related species exhibiting high levels of identity (97–100%) considered as the closest matches. These sequences, along with those from other genera, were retrieved from the NCBI GenBank. Pairwise alignment using ClustalW was performed before constructing the phylogenetic tree. A Maximum-Likelihood Tree was generated using the Kimura 2-Parameter model, and the reliability of the phylogenetic tree was assessed via bootstrap analysis with 1000 resamplings, utilizing Mega 11.0 software.

MEGA Software

The Molecular Evolutionary Genetics Analysis (MEGA) software serves as an integrated suite of tools for evolutionary analysis of DNA and protein sequence data. Developed to cater to biologists, MEGA includes features for sequence alignment, phylogenetic tree reconstruction, testing evolutionary hypotheses, estimating sequence divergences, acquiring sequence data online, and generating analysis descriptions (Kumar et al., 1994, 2008; Kumar and Dudley, 2007). MEGA encompasses maximum likelihood (ML) methods for molecular evolutionary analysis, aiding in the arrangement of DNA, RNA, and protein sequences to identify regions of similarity, indicative of functional, structural, or evolutionary relationships.

BLAST Analysis

The Basic Local Alignment Search Tool (BLAST) is employed for sequence similarity searches, accessible through a web interface or as a stand-alone tool. BLAST compares user queries to a database of sequences, finding short matches between sequences and initiating alignments from these 'hot spots'. Statistical information about alignments, such as the expect value or false-positive rate, is provided. The National Center for Biotechnology Information (NCBI) maintains a BLAST server, where queries submitted as sequences in FASTA format or sequence identifiers are compared to the database to find the most similar sequences (Gardy and Brinkman, 2006; Ye et al., 2006; Hamady et al., 2010).

2.4 In Vitro assessment of PGPR Characterization of isolates

All isolates underwent assessment for various Plant Growth Promoting Traits (PGPR) including indole acetic acid production, phosphate solubilization, and antimicrobial activity. Azotobactor, known for its ability to produce indole acetic acid crucial for plant root growth, was specifically evaluated for this trait using an in-vitro colorimetric analysis method (Negi et al., 2022).

2.4.1 Assessment of Indole Acetic Acid Production fromisolates

Indole acetic acid (IAA) serves as a physiologically active auxin, commonly produced during L-tryptophan metabolism by various microorganisms, including Azotobactor spp. IAA promotes cell elongation by altering cellular conditions such as increased osmotic contents, enhanced water permeability, decreased wall pressure, and increased cell wall synthesis.

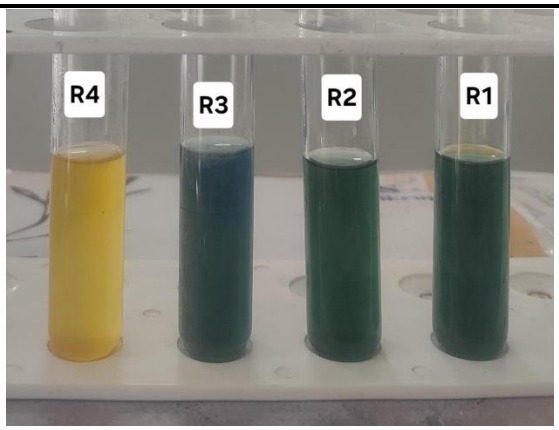
Indole acetic acid production was assessed using a colorimetric technique based on the Van UrkSalkowski reagent and Salkowski's method (Ehmann, 1977). Isolates were cultured in yeast malt dextrose broth (YMD broth) (ReadyMED, India) and incubated at 30°C for 4 days. Following incubation, the broth was centrifuged, and the supernatant collected.

Subsequently, 1ml of supernatant was mixed with 2ml of Salkowski's reagent and incubated in the dark. Optical density (OD) readings were recorded at 530 nm after 30 and 120 minutes using a spectrophotometer (Mohite, 2013).

2.4.2 Assessment of nitrogen fixation activity by deaminase assay

To evaluate the nitrogen-fixing capabilities of the isolates, they were inoculated onto Burk's medium, a nitrogen-free agar medium, with stringent aseptic techniques to prevent contamination. The inoculated plates were then incubated at 37°C for 48 hours in a microbiological incubator. Following incubation, growth observation was conducted, where the presence of discernible growth indicated proficient nitrogen fixation abilities in the isolates (Ha et. al., 2018).

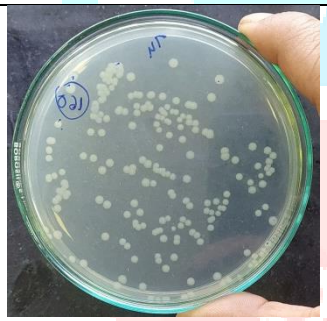
The manifestation of growth signifies the isolates' capacity to utilize atmospheric nitrogen and convert it into biologically usable forms, crucial for plant nutrition. This methodological approach serves as a reliable means to assess the nitrogen-fixing potential of the isolates, with Burk's medium providing a controlled environment conducive to accurate evaluation and validation of nitrogen-fixing capabilities (Hartono et. al., 2016).



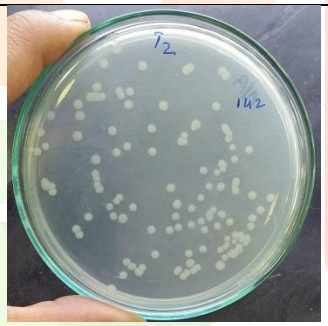
3. Result and Discussion

In present investigation, soil samples were initially diluted up to 10^{-10} after being mixed with sterile distilled water. Aliquots from the serial dilutions were spread onto Petri dishes containing Plate Count Agar (PCA) and incubated for 48 to 72 hours. Viable bacterial colonies were identified and counted, allowing for the determination of colony-forming units (CFUs) per gram of soil (Rani and Reddi, 2012). The results were recorded in Table 1 for further analysis, providing insights into microbial dynamics and ecological characteristics within the agricultural environment.

The microbial load was observed in the range of 45 Billion to 192 Billion/Gm of rhizosperic soil. Rhizosperic region was found to be highly dense in terms of microbial load. It may recorded to be varying with season where first month of summer.



Sample T3



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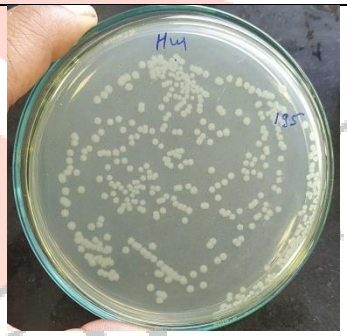
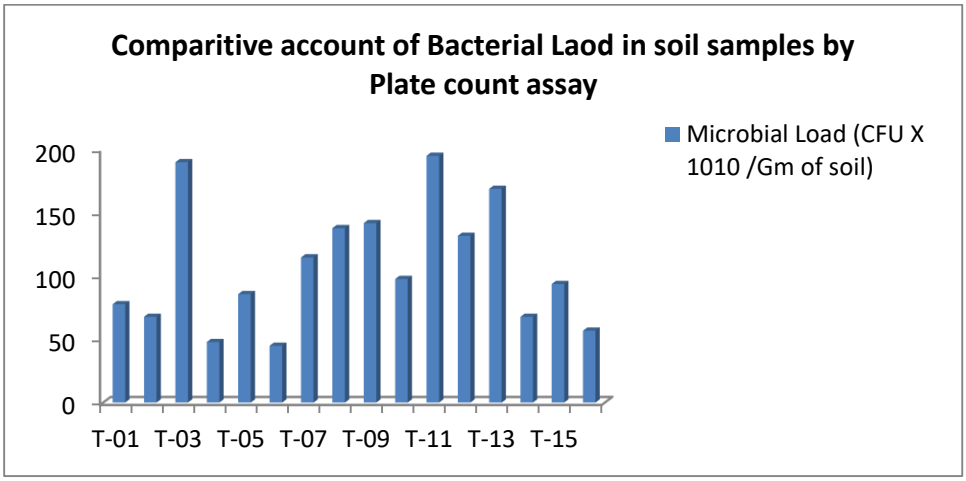


Sample T-11

Figure 1: Microbial Load of soil samples by spread plate assay



3.1.1 Screening of Symbiotic Nitrogen fixing microorganism

In the pursuit of isolating non-symbiotic bacteria, approximately 100 µL aliquots of 10^{-8} serially diluted soil samples were evenly spread onto Yeast Extract Mannitol Agar (YEMA Agar). Following inoculation, the Petri dishes were incubated for a period ranging between 48 to 72 hours. Well-isolated colonies were subsequently identified and recovered from the agar medium. To further analyze the isolated colonies, a portion of the well-isolated colony was diluted in sterile saline water. A loopful suspension of the diluted culture was then streaked onto sterile Nutrient Agar plates using the streak plate method. The morphology and characteristics of the isolated colonies were meticulously observed and documented, as illustrated in Figures 2 and 3.

This systematic approach aimed to identify and characterize non-symbiotic bacteria with potential nitrogen-fixing capabilities, contributing to the understanding of microbial interactions and nutrient dynamics within agricultural ecosystems.

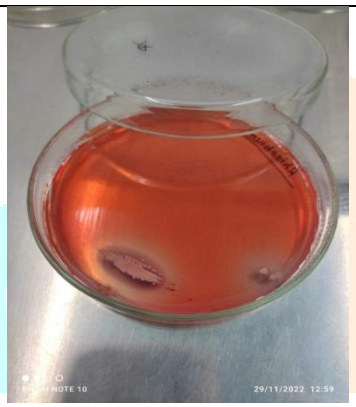


Figure 2: Isolate R1 on YEMA Agar

Figure 3: Isolate R1 on Nutirent Agar

3.1.2 Screening of Symbiotic Nitrogen fixing microorganism

In the endeavor to isolate symbiotic bacteria, approximately 100 µL aliquots of 10^{-8} serially diluted soil samples were evenly spread onto Azotobactor Agar plates. Following inoculation, the Petri dishes were incubated for a period ranging between 48 to 72 hours. Well-isolated colonies were subsequently identified and recovered from the agar medium.

To further scrutinize the isolated colonies, a portion of the well-isolated colony was diluted in sterile saline water. Subsequently, a loopful suspension of the diluted culture was streaked onto sterile Nutrient Agar plates using the streak plate method. The morphological characteristics and features of the isolated colonies were meticulously observed and documented, as depicted in Figures 4 and 5.

This methodical approach was employed to discern and characterize symbiotic bacteria with potential nitrogen-fixing capabilities, thereby contributing to the elucidation of microbial interactions and nutrient cycling dynamics within agricultural environments.

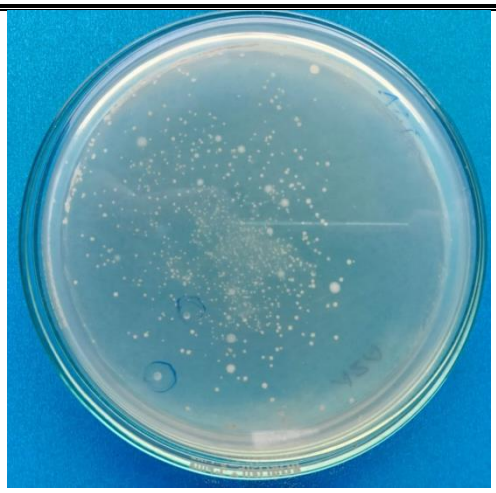


Figure 4: Isolate R2 , White Watery colony on Azotobactor Agar

Figure 5Isolate R2 on Nutirent Agar

3.1.3 Screening of Phosphate solubilizing microbes

In the pursuit of isolating symbiotic bacteria, approximately 100 µL aliquots of 10^{-8} serially diluted soil samples were evenly spread onto Pikovskaya's Agar Medium plates. Following inoculation, the Petri dishes were incubated for a period of 6-7 days at 32°C. Well-isolated colonies were subsequently identified and recovered from the agar medium.

The isolated colonies were meticulously observed and documented, as illustrated in Figures 6 and 7. The zone of solubilization surrounding the colonies was recorded, measuring approximately 3-4 mm after an incubation period of approximately 7 days.

This systematic methodology aimed to isolate and characterize symbiotic bacteria with potential phosphate-solubilizing capabilities, contributing to the broader understanding of microbial interactions and nutrient cycling mechanisms within agricultural ecosystems.

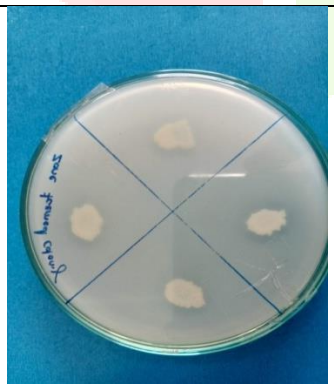


Figure 4: Isolate R3: Cream color colony with zone of solubilization on **Pikovskaya's Agar Medium**

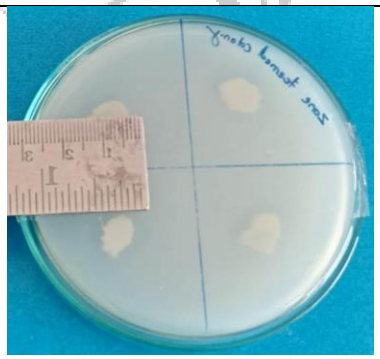


Figure 5: Isolate R 3: Colony Size appx 12 mm (after incubation of 6-7 days at 32 ^{0}C with Zone of solubilization of 3 mm

3.1.4 Screening of Sulphate reducing bacteria from the soil

In the quest to isolate sulfate-reducing bacteria, approximately 100 µL aliquots of 10^{-8} serially diluted soil samples were uniformly spread onto Sulphate Reducing Medium Agar plates. Subsequently, the Petri dishes were subjected to an incubation period of 6-7 days at 32°C. Following incubation, well-isolated colonies were meticulously observed and documented, as depicted in Figures 6 and 7.

The extent of solubilization surrounding the colonies was measured, yielding an average zone diameter of 3-4 mm after approximately 7 days of incubation.

This systematic approach was undertaken to isolate and characterize sulfate-reducing bacteria, thereby contributing to the comprehensive understanding of microbial diversity and biogeochemical processes within soil ecosystems.

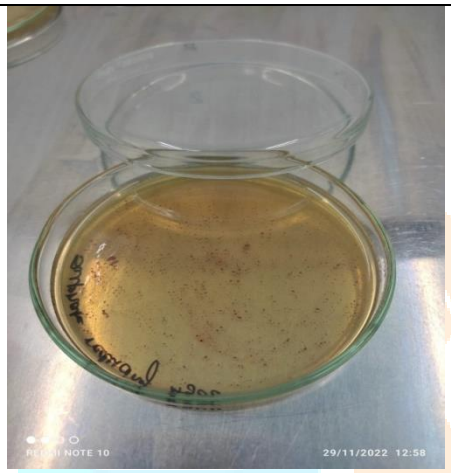


Figure 8:Isolate R4: Pin point, dark brown colored colonies on **Sulphate Reducing Medium Agar after incubation of 6-7 days at 32 ^{0}C**

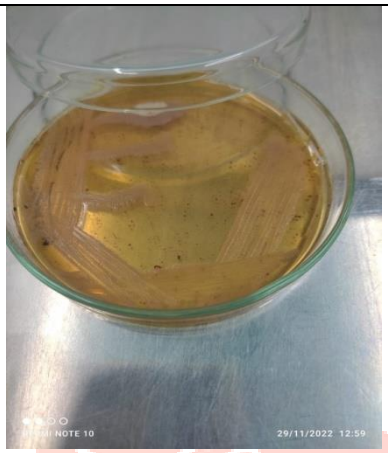


Figure 9: Isolate R4: Purified colonies on **Sulphate Reducing Medium Agar after incubation of 6-7 days at 32 ^{0}C**

3.2 Isolation and identification of isolates

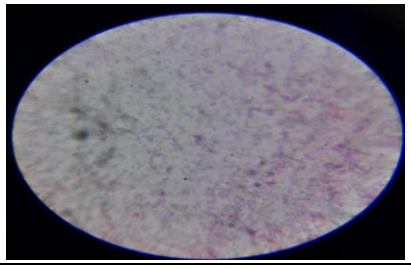
Isolation and Characterization of Agriculturally Important Bacteria

In this study, four agriculturally significant bacteria with distinct Plant Growth-Promoting Rhizobacteria (PGPR) traits were isolated, namely symbiotic nitrogen-fixing, non-symbiotic nitrogen-fixing, phosphate-solubilizing, and sulfate-reducing bacteria. The colony characteristics of these microbes were meticulously recorded for identification purposes.

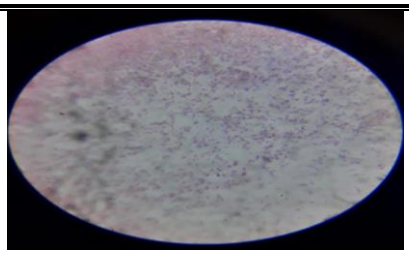
3.2.1 Colony Characteristic

Table 2: Colony characteristics of the isolates

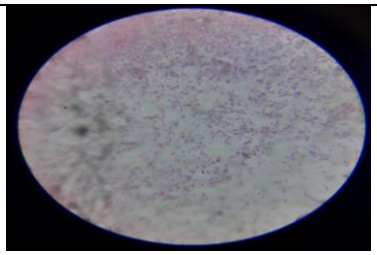
Name of Organism	Size	Shape	Colour	Margin	Elevation	Consistency	Opacity	Gram Nature
Isolate R1	1mm	Circular	White	Regular	Convex	Moist	Opaque	Gram negative
Isolate R2	1mm	Circular	White	Irregular	Convex	Moist	Opaque	Gram negative
Isolate R3	1mm	Circular	White	Regular	Convex	Moist	Opaque	Gram negative
Isolate R4	0.5mm	Circular	Brown yellow	Entire	Convex	Moist	Opaque	Gram negative



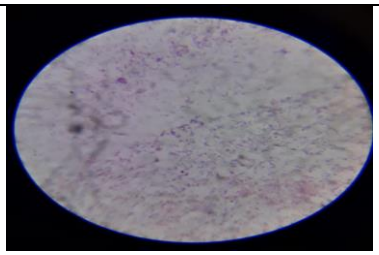
Isolate R1



Isolate R 3



Isolate R2



Isolate R 4

Figure 10: Gram Characteristics of isolates

The isolates were subjected to identification using the Bargery Manual assay technique. The following typical colony characteristics were observed and recorded for each isolated bacterium:

1. Isolate R1. Colonies cultivated on YEMA agar medium manifested as pinpoint white and convex with entire margins and moist consistency. They were identified as Gram-negative (Figure 10).
2. Isolate R2. Colonies grown on Azotobactor agar exhibited circular, watery white morphology with an irregular margin and moist consistency. The colonies were opaque and Gram-negative in nature (Figure 10).
3. Isolate 3. Isolates observed on Pikovskaya's agar medium presented as pinpoint, yellowish-creamy colonies with regular margins and moist consistency. They exhibited Gram-negative characteristics (Figure 10).
4. Isolate 4: Colonies grown on sulfate-reducing agar medium displayed circular morphology, dark brown-yellow color, and entire consistency. Gram-negative characteristics were observed (Figure 10).

The thorough characterization of these agriculturally significant bacteria provides valuable insights into their potential roles in plant growth promotion and soil nutrient dynamics within agricultural ecosystems.

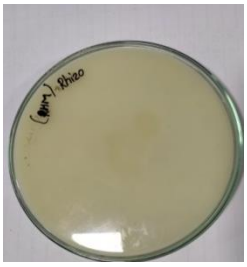
3.2.2 Biochemical Characterization of isolates

Biochemical testing is a fundamental method in microbiology, crucial for identifying and characterizing bacteria based on their metabolic attributes. Leveraging the diverse metabolic capabilities of bacteria, including nutrient utilization and enzymatic activity, this method allows microbiologists to discern the identity of unknown bacteria and differentiate them from others. Key tests such as the IMViC test, catalase test, oxidase test, urease test, nitrate reduction test, and carbohydrate fermentation test play vital roles in bacterial identification. These tests enable the detection of specific enzymes and metabolic pathways, providing valuable insights into bacterial characteristics. Biochemical testing serves as a pivotal tool across clinical, environmental, and food microbiology domains, offering a cost-effective and efficient means of unraveling bacterial metabolic profiles and aiding informed decision-making in various scientific and practical contexts.

	Table 3: Biochemical characteristics of isolates			
Biochemical Tests	**Isolate R1**	**Isolate R2**	**Isolate R3**	**Isolate R4**
Catalase	Negative	Positive	Positive	Positive
Starch Hydrolysis	Positive	Positive	Positive	Positive
Caseinate	Positive	Positive	Positive	Negative
Indole	Negative	Positive	Negative	Positive
Methyl Red	Negative	Negative	Negative	Negative
VP	Positive	Negative	Negative	Negative
Citrate	Positive	Positive	Positive	Negative

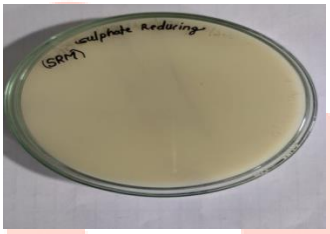
Figure 11: Amylase Assay of isolates

Isolate R1 (Amylase Positive)



Isolate R2(Amylase Positive)

Isolate R3(Amylase Positive)



Isolate R4(Amylase Positive)

Figure 12: Catalase Assay inferences

Sr. No.	Decription of Isolate	Catalse Assay inference
1.	Isolate R1	Positive
2.	Isolate R2.	Positive
3.	Isolate R3	Positive
4.	Isolate R4	Positive

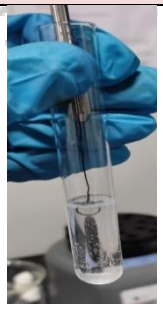


Figure 12: Catalase Test positive inference

Figure 13: indole Assay Inferences

Isolate R1 – negative

Isolate R2: Positive

Isolate R3 – negative

Isolate R4 -Positive

Methyl Red Test Inferences

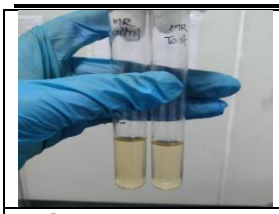
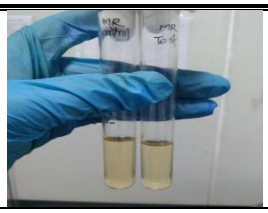
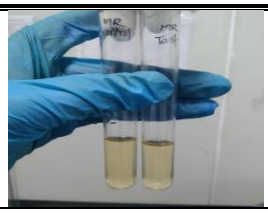
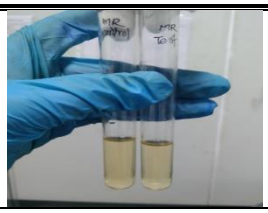
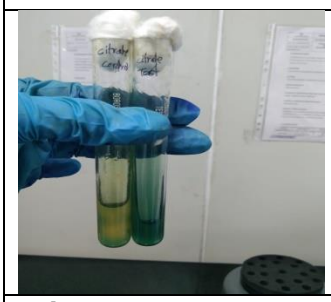
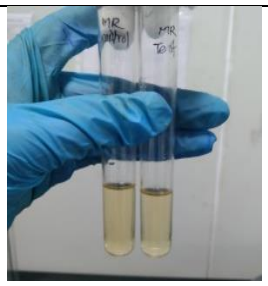
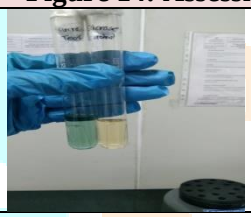
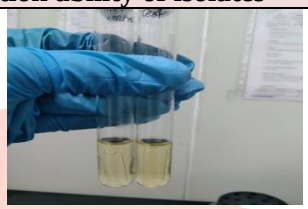
			
Isolate R1 – negative	Isolate R2 – negative	Isolate R3 – negative	Isolate R4 – negative
			
Isolate R1: Positive	Isolate R2: Positive	Isolate R3: Positive	Isolate R4: Negative

Figure 14: Assessment of Sugar formation ability of isolates



Sugar Fermentation: Positive Inference



Sugar Fermentation: Negative Inference

Sugar fermentation Inferences

Name of Isolate	Isolate R1	Isolate R2	Isolate R3	Isolate R4
Glucose	Negative	Positive	Negative	Negative
mannitol	Positive	Positive	Positive	Negative
lactose	Negative	Positive	Negative	Negative
Sucrose	Negative	Positive	Negative	Negative
Maltose	Positive	Negative	Negative	Negative
Galactose	Positive	Negative	Negative	Negative

The biochemical characterizations of bacterial isolates were conducted, employing IMViC tests, enzyme production assays, and sugar fermentation analyses. The IMViC test, comprising Indole, Methyl Red, Voges-Proskauer, and Citrate assays, facilitated the differentiation of gram-negative bacteria, notably within the Enterobacteriaceae family. Isolate R1, R2 and R3 exhibited positive results for the catalase test, indicating catalase enzyme presence. Furthermore, all three isolates tested positive for starch hydrolysis and caseinate breakdown. Isolate R2 showed positive indole test results, while isolate R1 and Isolate R3 were positive for the VP test. Isolate R4, uniquely, tested positive for the indole test but negative for the citrate test, and it was negative for all sugar fermentation tests. Among the isolates, Isolate R1, R2 and R3 were capable of fermenting mannitol, while R1 and R3 could also ferment maltose and galactose. Notably, none of the isolates were able to ferment sucrose or lactose, and R4 tested negative for the catalase test. These results elucidate the diverse metabolic capacities of the bacterial isolates, shedding light on their biochemical properties and potential ecological roles.

3.2.3 Molecular identification of Isolate R2:

The phylogenetic analysis conducted in this study revealed a close relationship between all examined species and members of the Bacillus spp. genus. Using the maximum likelihood method with the Kimura 2-parameter model in MEGA 11.00, a phylogenetic tree was constructed, affirming the aforementioned findings. Furthermore, the analysis identified potent isolates clustered into distinct groups alongside their closest relatives obtained from the NCBI GenBank

database. Specifically, isolates of Azotobactor exhibited a striking 98.61% similarity with Bacillus tropicus (NR157736.1), constituting a cohesive group within the phylogenetic tree. This underscores the genetic relatedness and evolutionary affinity between the studied isolates and their Bacillus spp. counterparts, providing valuable insights into their taxonomic classification and ecological significance.

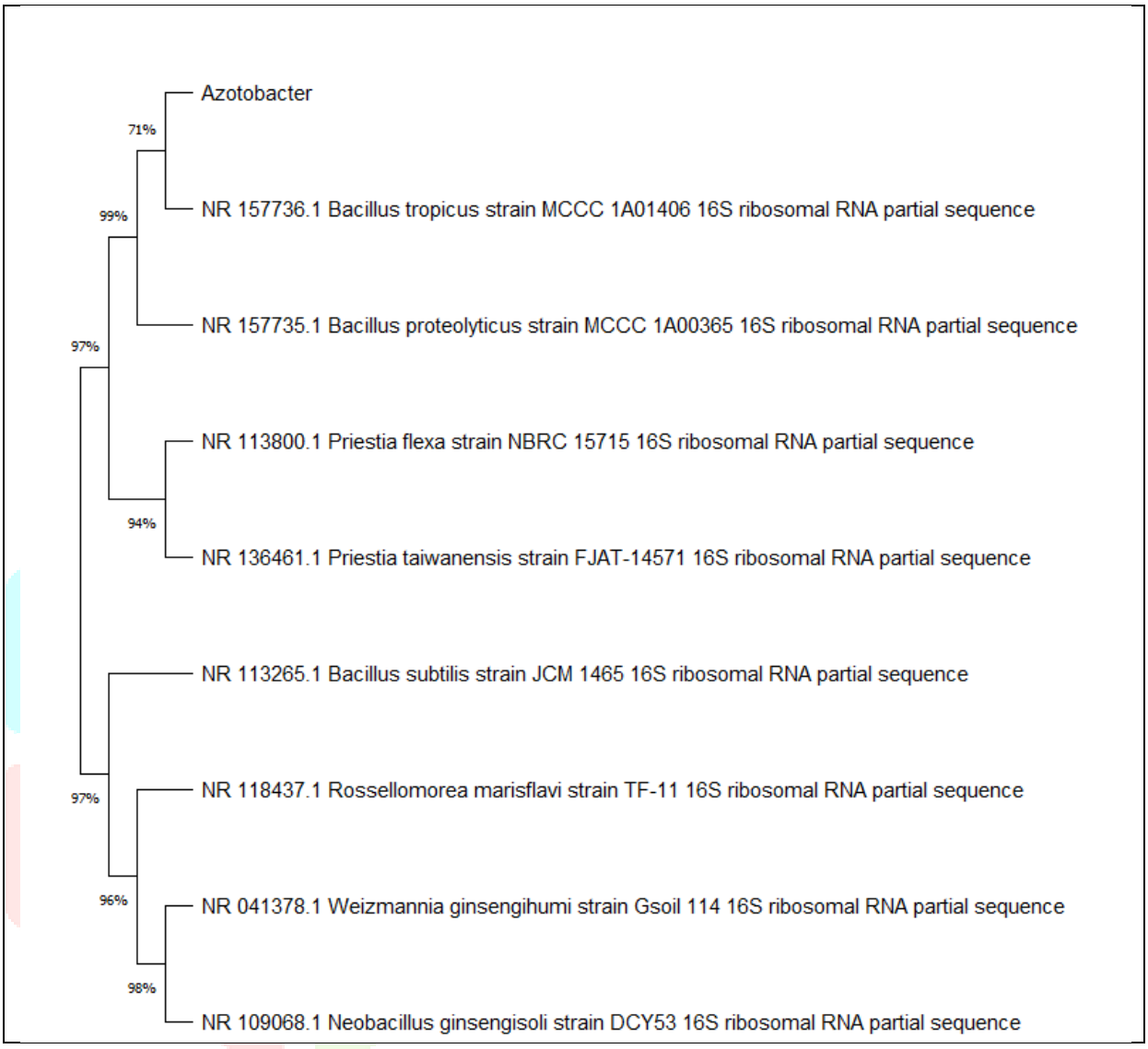


Figure 1:Isolate 2 sample Maximum-Likelihood phylogenetic tree generated by Kimura 2 parameter

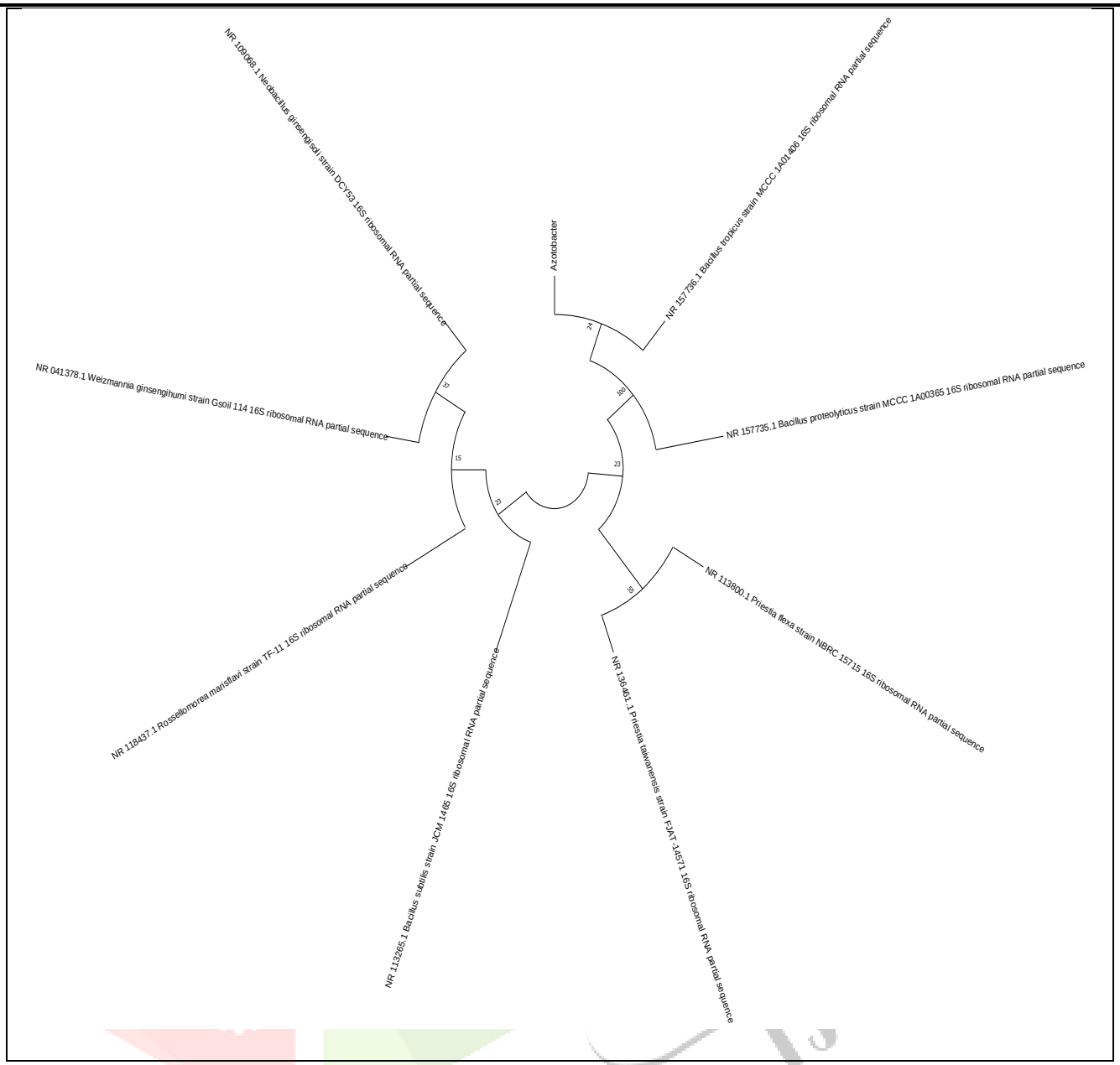


Figure 2: Circular tree combined Maximum-Likelihood phylogenetic tree generated by Kimura 2 parameter model of *Bacillus tropicus*

3.3 In Vitro assessment of PGPR Characterization of isolates

All 4 isolates underwent assessment for PGPR traits to determine their potential in exhibiting multiple PGPR activities, including the production of indole acetic acid and nitrogen fixation. The outcomes are discussed as follows:

2.4.1 Assessment of Indole Acetic Acid Production from isolates

Isolate R1, R2 and R3 observed for acetic acid production with activity. Out of which R2 was recorded as significance for indole acetic acid production. The activities are discussed in figure 15.

Figure 15: Indole acetic acid activity				
Isolate	**R1**	**R2**	**R3**	**R4**
Indole Acetic Acid Activity	+	+++	++	-
Nitrogen Fixation	++	+++	+	-

"+++: Strong Positive" "++: Fair Positive" "+: weak Positive" "–: Negative"

2.4.2 Assessment of Nitrogen Fixation Activity

Isolates R1, R2, and R3 exhibited positive results for nitrogen fixation as detected by the deaminase assay explained in figure 15. Further validation of this activity through in vivo experimentation is necessary to substantiate the significance of this trait.

Conclusions

The study delved into the microbial diversity of rhizospheric soil in Bihar's rice fields, employing screening assays targeting key Plant Growth-Promoting Rhizobacteria (PGPR) traits such as nitrogen fixation and phosphate solubilization. The results unveiled a diverse array of agriculturally significant bacteria, including Azotobacter spp., Rhizobium spp., Pseudomonas spp., and Desulfovibrio spp. These isolates showcased promising PGPR attributes, reflecting their potential to improve soil fertility, nutrient cycling, and plant health. The biochemical and molecular analyses yielded insights into their metabolic capacities and genetic relatedness, notably identifying Azotobacter Bacillus tropicus as a significant PGPR strain. Phylogenetic analysis elucidated evolutionary relationships among the isolates and their taxonomic classification, emphasizing their ecological significance. This research contributes to understanding microbial diversity dynamics in agricultural ecosystems, paving the way for developing biofertilizers and biocontrol agents to promote sustainable agriculture practices in Bihar and beyond.

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