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Isolation and Identification of Multifarious PGPR from Rhizosphere and its Effect on Plant Growth Promotion of *Vigna Radiata*

SAYLEE VILAS KALEKAR[1], NIRANJAN PRAKASHRAO PATIL[2] and RAJASHREE BHALCHANDRA PATWARDHAN[3]*

[1]Department of Microbiology, Fergusson College (Autonomous), Affiliated to Savitribai Phule Pune University, Shivajinagar, Pune, Maharashtra, India.

[2]Department of Microbiology, MES Abasaheb Garware College, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India.

[3]Department of Microbiology, Haribhai V. Desai College of Arts, Science and Commerce, Affiliated to Savitribai Phule Pune University, Maharashtra, India.

Abstract

Sustainable agricultural practices is a need of time to support the supply of agricultural produce for continuously growing global population and minimizing the dependence on chemicals. Plant growth promoting rhizobacteria (PGPR) invade the rhizosphere and use a variety of direct and indirect methods to promote plant growth. In this study, thirty-one PGPR were isolated and screened. Four isolates that had greatest number of characteristics that promote plant growth were selected. The 16S rRNA gene sequencing method was used to identify the selected isolates and they were identified as *Proteus vulgaris* PT2, *Pseudomonas putida* NP12, *Serratia rubidae* NW19 and *Pseudomonas fulva* TD4. *Proteus vulgaris* PT2 showed maximum IAA production of 127.65 µg/mL followed by *Pseudomonas fulva* TD4 showing 98 µg/mL. The impact of selected isolates on *Vigna radiata* plant seeds was examined by calculating vigour index using parameters like seed germination percentage, root length and shoot length. *Pseudomonas fulva* TD4 exhibited a notable increase in germination percentage 96.66 % and Vigor index of 2273.153 in comparison to control group. The isolation and characterization of potent rhizobacterial strains highlight their role as eco-friendly biofertilizers, promoting environmentally safe crop production. Also, the high IAA production by *Proteus vulgaris* PT2 and *Pseudomonas fulva* TD4 demonstrates their

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CONTACT Rajashree Bhalchandra Patwardhan dr.rbpatwardhan@gmail.com Department of Microbiology, Haribhai V. Desai College of Arts, Science and Commerce, Affiliated to Savitribai Phule Pune University, Maharashtra, India.

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potential in enhancing plant growth and root development, which is critical for nutrient uptake. Overall, *Pseudomonas fulva* TD4 emerged as a promising biofertilizer candidate with potential application in sustainable crop production system. The significant improvement in germination percentage and vigour index of *Vigna radiata* indicates the practical applicability of these isolates in seed treatment and crop yield enhancement.

Introduction

India's national economy and way of life are heavily dependent on Agriculture. According to the Annual report of Agriculture and Farmers welfare, published by Government of India in 2024-25, agriculture and its allied fields employ 54.6% of India's total workforce.[1] Agriculture faces several challenges in meeting the food demand of rapidly growing population. By 2050, the world population is projected to reach 9.77 billion. In addition to the in population increase the additional challenges are climate change, land degradation and reduction of natural resources. Crops are also subjected to various biotic and abiotic stresses that reduces the productivity of crops. In order to achieve sustainable agriculture, ecofriendly alternatives are required to be practiced and reducing the use of chemicals in the form of fertilizers and pesticides.

Vigna radiata, also known as green mung or green gram is one of the ancient pulse crops originated in Asia and Africa. It is an important legume species used in many parts of the World. It has a high nutritional value, drought tolerance and short life-cycle. It is a rich source of protein, dietary fibres, minerals, vitamins and variety of bioactive compounds However, its productivity is greatly affected due to various biotic and abiotic factors.[2] Studies indicate that salinity stress as low as even 50mM NaCl results in reduction of the yield by 60% and drought stress negatively affects its germination and reproductive ability.[3]

The rhizosphere of the plant is inhabited by plant growth promoting rhizobacteria (PGPR). They interact with plants and promote their growth.[4] They positively affect plant growth by direct and indirect mechanisms. The primary plant growth promoting attributes of PGPR include nitrogen fixation, phosphate solubilization, synthesis of plant hormones like Auxins, Gibberellins and ethylene, HCN production and ammonia production.[5] There are additional properties of PGPR that also contribute to plant growth and health like Potassium solubilization, zinc solubilization, siderophore production, ACC deaminase production, etc.[6] PGPR offer multiple agronomic advantages including enhanced germination, nutrient acquisition, increased biomass accumulation and tolerance to biotic and abiotic stresses, thus promoting sustainability as alternatives to chemicals.[7]

Multifarious PGPR are the bacterial strains that possesses various plant growth promoting attributes simultaneously.[8] The rationale of the present study in to isolate such strains from rhizosphere soil that can be a strategic approach to identify a superior organism as a bioinoculant with multiple plant growth promoting attributes and tolerating different types of stress conditions.[9] Our study focuses on isolation and identification of multifunctional PGPR, characterize their plant growth promoting traits through in vitro assays and evaluate their effects on growth promotion of *Vigna radiata*.

Materials and Methods

Sampling and Isolation

Four soil samples were collected from the rhizosphere of Triticum aestivum (Wheat) grown in Nashik region. 30 to 60 days old seedlings were collected with intact roots.[10] The roots were washed with sterile distilled water to remove bulk soil. The roots with rhizosphere soil were suspended in 100 mL sterile saline. Saline flasks were kept on shaker at 28^{o}C for one hour. Supernatant was collected and diluted serially. On sterile Nutrient agar plates, 100 µl diluted soil suspension was plated and the plates were incubated at 28oC for 24-48 hours to obtain isolated colonies. Colonies were examined for their morphological dissimilarities like colour, elevation, size, shape and surface and sub-cultured on sterile nutrient agar slants.

Screening for Indole Acetic Acid Production

Screening of isolates for IAA production was done in presence and absence of tryptophan using Salkowski method.[11,12] PGP isolates were grown in Luria broth (Himedia) and in tryptophan broth (Himedia) and incubated for 48 hours at room temperature. Centrifugation of broth at 10,000 rpm for 15 minutes was carried out following incubation for 48 hours incubation at room temperature. 1 ml of supernatant was mixed with 2 mL of Salkowski reagent, 2-3 drops of orthophosphoric acid was added and incubated at room temperature in dark for 1 to 2 hours and pink to red colouration was observed as positive test for IAA production.[13] The absorbance of pin colour was determined at 530 nm. A standard curve of pure IAA ranging from 1 to 100 µg mL^{-1} was used to calculate the IAA concentration.

Screening of Isolates for Nitrogen Fixing and Phosphate Solubilizing Ability

Isolates were streaked on nitrogen free Ashby's mannitol agar plates to screen for their nitrogen fixing ability.[14,15] Isolates were spot inoculated on Pikovskaya's agar plate containing tricalcium phosphate as an insoluble source of phosphate to screen for phosphate solubilizing ability.[16] The plates were incubated at 28°C for 5-6 days to check for clear halo surrounding the colony.[17]

Potassium Solubilization by PGP Isolates

Isolates were spot inoculated on modified Aleksandrov agar containing Bromo thymol Blue (BTB) (0.5% glucose, 0.05% $MgSO_4.7H_2O$, 0.0006% $FeCl_3$, 0.06% $CaCO_3$, 0.2% $CaPO_4$, 0.3% Mica, 3% agar pH 7.2 and bromo thymol indicator).[18] After 4 to 5 days of incubation at room temperature, the formation of yellow to orange halo around the colony was observed that indicate potassium solubilization.[19]

Zinc Solubilization by PGP Isolates

Isolates were spot inoculated on Bunt and Rovira medium containing insoluble zinc source i.e. zinc oxide. The plates were incubated at room temperature for 4 to 5 days to observe clear halo surrounding the colony that indicate zinc solubilization.[20]

Exopolysaccharide Production By PGP Isolates

Screening of PGP isolates was carried out using Sucrose salt medium (SSM) agar (1% sucrose, 0.1% $(NH4)_2SO_4$, 0.001% NaCl, 0.05% $MgSO_47H_2O$, 0.2% K_2HPO_4, 0.05% yeast extract, and 0.001% $CaCO_3$) supplemented with 0.5%w/v aniline blue.[21] Isolates ere streaked in quadrants on SSM agar plates. The plates were incubated at room temperature for 24 to 48 hours and observed for the formation of blue colonies.

Siderophore Production By PGP Isolates

Chrome Azurol S (CAS) agar plates were used to determine siderophore formation.[22,23] Quadrants were made on CAS agar plates and the overnight grown culture of PGP isolates was spot inoculated in the quadrants. The plates were incubated at room temperature for 5 to 6 days to observe for yellow to orange halos surrounding the colony.

Ammonia Production By PGP Isolates

Sterile 10 ml peptone water added in tubes were inoculated with 24 hours old culture of selected PGP isolates. The tubes were incubated for 48–72 hours at 28°C. Development of yellow to brown color on addition of 0.5 mL Nessler's reagent was regarded as a positive test for ammonia production.[24]

Molecular Identification of Isolates using 16S rRNA Sequencing

DNA extraction method was used to isolate the genomic DNA of selected PGP isolates. Universal 16SrDNA primers were used to amplify 16S rRNA gene of selected isolates to be identified, at their specific annealing temperature. Sanger's DNA sequencing method was used to directly sequence the amplification products that were purified by enzymatic method. Sequencing analysis SeqA8 software was used to analyse the data. Nucleotide BLAST analysis was performed at NCBI server. Http://www.ncbi.nlm.nih.gov/BLAST.

Effect of Selected PGP Isolates on Seed Germination of *Vigna Radiata*

Seeds of *Vigna radiata* were surface sterilized by 0.1% $HgCl_2$ for 1 minute and ethanol for 30 seconds and 3 times with sterile distilled water. After drying the seeds in laminar for 1 hour, they were coated with PGP isolates in log phase by soaking the seeds in its suspension of the density equivalent to 0.5 McFarland standard for 30 minutes. The soaked seeds were placed on sterile Hoagland medium with 0.8 % agar.[25] The seeds were allowed to germinate for three days in dark after which the seedlings were subjected to 10 to 12 hours of photoperiod and 10 to 12 hours of darkness for about 7 days. Thirty seeds were placed in each plate. After 7 days Germination percentage and Vigor index were determined.

Statistical Analysis

Statistical analyses were performed using Minitab software version 17. Data from the plant trial was analyzed using One-way analysis of variance (ANOVA) and the post-hoc test Fisher was used to compare the significant differences ($p < 0.05$) of bacterial treatments in terms of features of plant growth under the influence of different PGP isolates.

Results

Based on the morphological differences between the colonies obtained on nutrient agar, Thirty PGP isolates were selected from the wheat rhizosphere soil samples (Fig.1). These isolates were maintained and stored at 4oC on sterile nutrient agar slants and at -15oC in glycerol stocks. The results of the screened PGP isolates for different plant growth promoting attributes like nitrogen fixation, phosphate solubilization, potassium solubilization, zinc solubili--zation, Indole acetic acid production, siderophore production and exopolysaccharide production is shown in Table 1.

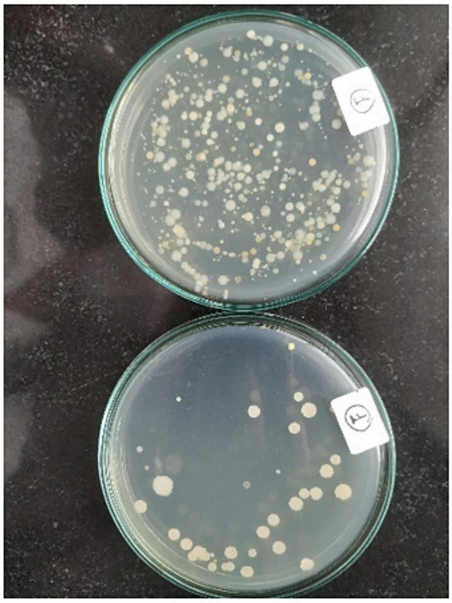


Fig. 1: Morphologically different colonies

Table 1: Plant Growth Promoting Attributes of Isolates

Isolate	Nitrogen fixation	Phosphate solubilization	IAA production	Siderophore production	EPS production	Potassium solubilization	Zinc solubilization	Ammonia production
NP7	+	+	-	-	+	-	-	-
NP10	+	+	-	+	++	-	-	-
NP12	+	+	++	+	-	++	-	+
NS1	+	+	-	-	++	-	-	-
NS10	+	+	-	-	++	-	-	-
NS11	+	+	-	-	++	-	-	-
NS17	+	+	-	+	++	-	-	+
NS19	+	+	-	-	++	-	-	+
NW3	+	+	-	-	++	-	+	-
NW7	+	+	-	-	+	-	-	-
NW9	+	+	-	-	+	-	-	-
NW11	+	-	-	-	+	-	-	-

NW15	+	+	-	-	-	-	-	-
NW17	+	-	-	-	-	-	-	-
NW19	+	+	+	+	+	++	+	+
PT2	+	+	+++	+	-	-	+	+
PT3	+	+	-	-	+	-	-	-
PT4	+	-	-	-	-	-	-	-
PT5	+	-	-	-	+	-	-	-
PT6	+	+	-	+	-	+	-	-
PT7	+	-	-	+	-	+	+	+
TD1	+	-	+	+	-	-	+	+
TD2	+	-	+	+	+	+	+	+
TD3	+	-	-	-	-	-	+	+
TD4	+	++	++	+	+	++	+	+
TD5	+	-	+	-	+	+	+	+
TD6	+	-	+	-	+	-	-	+
TD7	+	-	-	-	-	-	-	+
TD8	+	+	+	+	-	-	+	+
TD9	+	+	+	-	+	-	+	+

'+' : Positive test
'-' : Negative test

All the isolates could grow on nitrogen free Ashby's Mannitol agar (Himedia). Twenty isolates showed a visible halo when spot inoculated after 4 to 5 days of incubation at room temperature due to solubilization of insoluble Tricalcium phosphate that was added in Pikovskaya's medium (Fig 2).

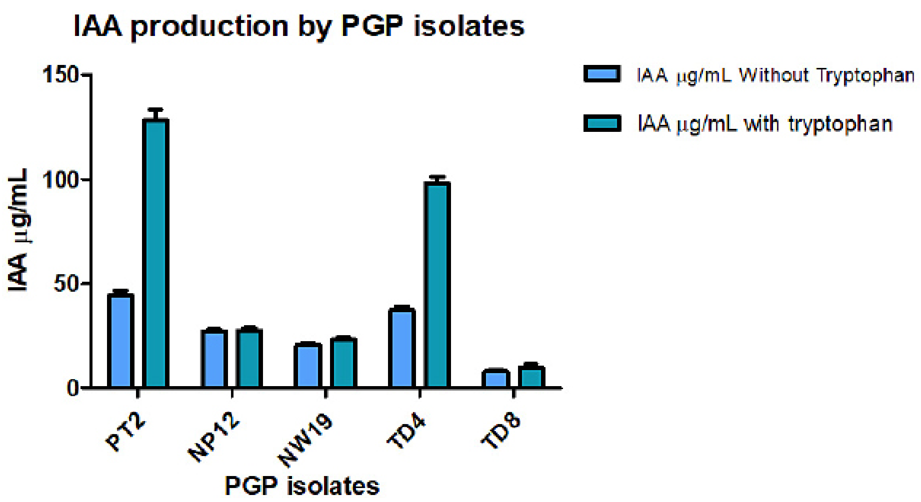
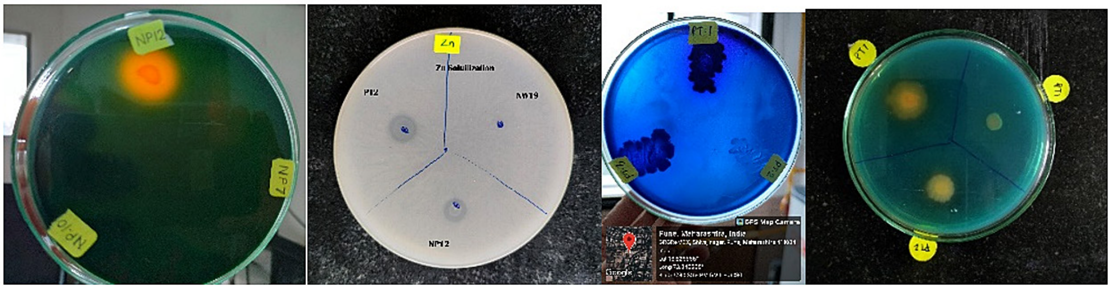


Fig. 2: IAA production by PGP isolates in presence and absence of tryptophan

Indole acetic acid (IAA) production was used as a primary method of screening the PGP isolates. Using Salkowski method IAA production was checked in both presence and absence of tryptophan. NP12, NW19, PT2, TD1, TD2, TD4, TD5, TD6 and TD8 showed pink colouration in presence of tryptophan, however, NP12, NW19, PT2, TD4 and TD8 showed IAA production even in absence of tryptophan. The amount of IAA produced by PGP isolates was quantified by measuring the absorbance of pink colour at 530 nm using spectrophotometer and comparing the values with a standard curve of IAA prepared using the range of IAA concentrations from 1 to 100 µgmL^{-1}. The highest IAA producer was found to be PGP isolate PT2 followed by TD4 as shown in Figure 2

One of the three vital macro nutrients that plants need is Potassium. Seven PGP isolates were capable of solubilizing potassium that was indicated by the colour change of bromo thymol blue indicator to yellow indicating the production of acid for solubilization (Fig 3A). Zinc solubilization was checked by spot inoculation on sterile Aleksandrov agar and observing a clear halo surrounding the growth of zinc solubilizing PGP isolate indicating the solubilization of insoluble zinc oxide (Fig 3B). Exopolysaccharide production was assessed on SSM medium (Sucrose salt medium) containing aniline dye. The isolates that take up blue colour of aniline was selected as exopolysaccharide producers (Fig 3C). Twenty isolates showed exopolysaccharide production which is considered as one of the important properties of PGPR for protection of plants against desiccation, reducing the uptake of sodium ions by forming a protective barrier and helping plants to combat salt stress. Twelve PGP isolates showed siderophore production when inoculated on CAS agar producing purple to orange halos around the colony (Fig. 3D). The type of siderophore produced by the PGP isolate is indicated by the variation in the colour of halo. Ammonia production by PGP isolates was checked by addition of Nessler's reagent in peptone water tubes inoculated with PGP isolates. Ammonia producing ability is one of the important properties of PGPR to expand the accessibility of essential nutrients to plants. 15 PGP isolates showed yellow to brown coloration after addition of Nessler's reagent indicating the production of ammonia.



(A) (B) (C) (D)

Fig. 3: (A) Potassium solubilization, (B) Zinc solubilization, (C) Exopolysaccharide production, (D) Siderophore production

Selected PGP isolates were identified by molecular identification method using 16S rRNA gene sequencing. The BLAST analysis from NCBI database was carried out for all the selected isolates to find out their relationship with already known isolates. Morphologically all isolates were Gram negative rods except TD8 which was Gram positive rod The percentage of 16S rRNA sequence identity and their obtained NCBI Accession numbers of isolates are indicated in Table 2.

Table 2: Molecular identification of PGP isolates and their obtained NCBI accession numbers

Sr. No.	PGP Isolate	Isolate identified as	Percent similarity (%)	NCBI Accession number
1	PT2	*Proteus mirabilis*	100	PP177352
2	NP12	*Pseudomonas putida*	100	PP159175
3	NW19	*Serratia rubidae*	99.79	PP177351
4	TD4	*Pseudomonas fulva*	100	PV249042
5	TD8	*Bacillus subtilis*	100	PV249043

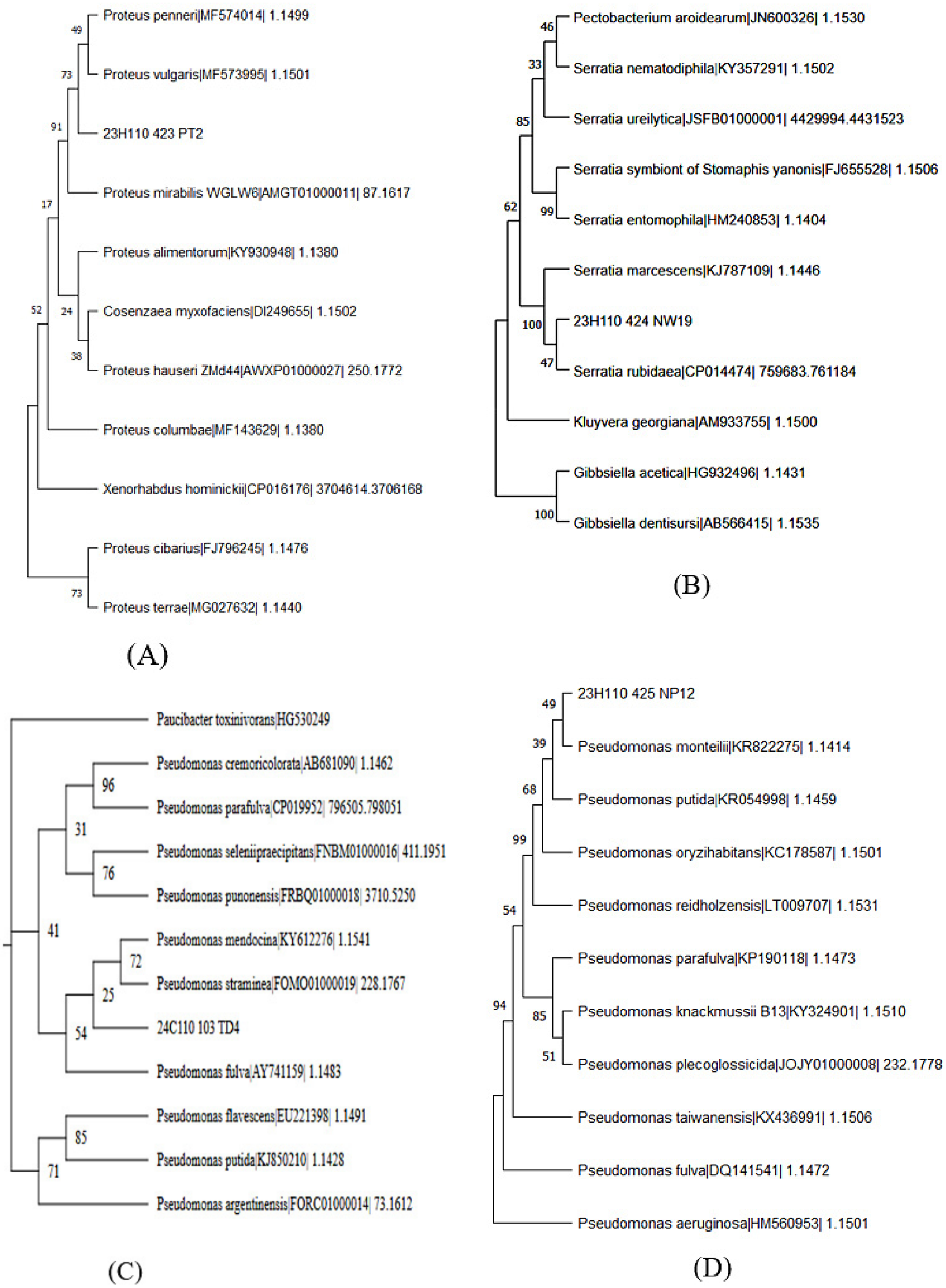
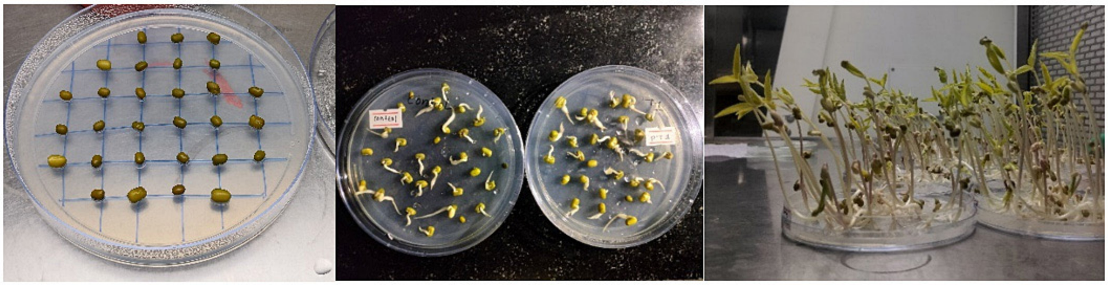


Fig. 4: Phylogenetic tree of (A) *Proteus vulgaris* PT2, (B) *Serratia rubidae* NW19, (C) *Pseudomonas fulva* TD4, (D) *Pseudomonas putida* NP12

Phylogenetic analysis was done to find out the evolutionary relationships among the isolates and already known species based on the 16S rRNA sequence reports and phylogenetic tree was obtained as depicted in the Figure 4

Based on the plant growth promoting properties, PGP isolates PT2, NP12, NW19and TD4 were selected for seed germination assays of *Vigna radiata*. Green moong *(Vigna radiata)* has a high nutritional value, drought tolerance and short life-cycle. The seeds were given different treatments by soaking them in the suspension of selected PGP isolates and allowed to germinate on sterile Hoagland medium as shown in the Figure 5.



(A) (B) (C)

Fig. 5: Seed germination studies (A) Arrangement of seeds on Hoagland medium plates, (B) Seed germination, (C) Emergence of seedlings

Growth parameters like germination index, root length, shoot length and Vigor index was calculated and shown in Table 3. The experiment was performed in triplicates with proper control. The data was analysed by Fisher pairwise comparison test using Minitab software version 17. *Pseudomonas fulva* TD4 showed significant increase in Vigor index compared to control.

Table 3: Seed Germination Indices

Treatments	Percent Germination after 48 hours (%)	Mean root length (cm)	Mean shoot length (cm)	Mean Wet weight (g)	Mean Dry weight (g)	Vigor Index
Control	80.64	14.854^{CD} (±3.017)	4.948^{AB} (±2.619)	0.21^{D} (±0.067)	0.084^{E} (±0.03)	1596.833
Proteus vulgaris PT2	86.66	17.502^{AB} (±4.915)	5.304^{AB} (±2.869)	0.3459^{C} (±0.0799)	0.09^{DE} (±0.03)	1976.368
Pseudomonas putida NP12	96.42	14.019^{D} (±5.023)	4.758^{AB} (±2.912)	0.3724^{BC} (±0.107)	0.122^{AB} (±0.04)	1810.478
Serratia rubidae NW19	82.35	16.948^{ABC} (±4.518)	5.232^{AB} (±3.424)	0.2552^{D} (±0.128)	0.09^{CDE} (±0.04)	1826.523
Pseudomonas fulva TD4	96.66	17.637^{A} (±3.656)	5.880^{AB} (±2.777)	0.4008^{B} (±0.083)	0.110^{BCD} (±0.04)	2273.153

Standard deviations are indicated by values in brackets. The parameters, root length, shoot length, wet weight and dry weight were recorded after 8 days of treatment. Fisher pair-wise comparison test indicates that the values superscribed with the same letter do not significantly differ ($p \leq 0.05$). Total 30 seeds were used per treatment.

Discussion

Plant growth promoting rhizobacteria (PGPR) play a crucial role in sustainable agriculture by enhancing plant productivity through multifaceted mechanisms involving nutrient mobilization, phytohormone production, and improvement of soil physicochemical properties.[20] In the present study, four efficient PGPR isolates; Proteus vulgaris PT2, *Pseudomonas putida* NP12, Serratia rubidae NW19, and *Pseudomonas fulva* TD4 were identified and characterized for their plant growth promoting potential. These genera are well documented in the rhizosphere for their beneficial interactions with plants,[26,27] however, previous investigations have largely emphasized *Pseudomonas putida* and *Pseudomonas fluorescens*. Notably, our findings highlight *Pseudomonas fulva* TD4 as a comparatively underexplored yet highly efficient PGPR, thereby expanding the repertoire of potential bioinoculant candidates.

Nitrogen availability remains a primary limiting factor for plant growth as a constituent of proteins, nucleic acids, enzymes, chlorophyll, etc. Biological nitrogen fixation represents an ecologically sustainable strategy to supplement nitrogen inputs in agroecosystems. The ability of all selected isolates to grow on nitrogen free medium suggests their potential diazotrophic capability, which is a fundamental trait associated with PGPR mediated plant growth promotion. This attribute is particularly significant in reducing dependence on synthetic nitrogen fertilizers and justifying associated environmental impacts. Phosphorus, despite being abundant in soils, predominantly exists in insoluble forms, limiting its bioavailability. The capacity of PGPR to solubilize phosphate through the secretion of low molecular weight organic acids constitutes a critical mechanism for enhancing phosphorus uptake.

In addition to nutrient mobilization, phytohormone production, particularly indole-3-acetic acid (IAA) is a key determinant of PGPR efficacy. IAA modifies the plant auxin pool to either optimal or suboptimal levels, directly enhancing plant development.[28,29] IAA modulates root architecture by promoting lateral root formation and elongation, thereby increasing the absorptive surface area. In this study, Proteus vulgaris PT2 exhibited the highest IAA production, followed by *Pseudomonas fulva* TD4, indicating their strong potential to influence plant developmental processes. The observed IAA production in the presence of tryptophan further confirms the tryptophan dependent pathway as a dominant mechanism in these isolates

Siderophore production represents another critical trait contributing to both nutrient gain and biocontrol activity. By chelating ferric ions under iron limiting conditions, siderophores enhance iron availability to plants while simultaneously restricting its accessibility to competing phytopathogens.[24] The positive siderophore activity observed in *Pseudomonas putida* NP12 and Proteus vulgaris PT2 underscores their dual role in promoting plant growth and suppressing pathogen proliferation, thereby contributing to plant health.

In addition to nitrogen and phosphorus, potassium and zinc are essential nutrients influencing plant metabolic processes and overall productivity. Potassium solubilizing bacteria facilitate the release of potassium from insoluble silicate minerals through acidolysis and chelation mechanisms. Consistent with previous reports, *Pseudomonas fulva* TD4 and *Pseudomonas putida* NP12 demonstrated significant potassium solubilization capacity. Similarly, zinc solubilization by PGPR enhances micronutrient availability, which is crucial for enzymatic activities, chlorophyll synthesis, and hormonal regulation. Interestingly, isolates exhibiting zinc solubilization also showed elevated IAA production, suggesting a potential synergistic relationship between micronutrient mobilization and phytohormone synthesis, warranting further molecular investigation. Exopolysaccharide (EPS) production is increasingly recognized as a critical determinant of rhizobacterial functionality under stress conditions. EPS contributes to soil aggregation, improves water retention, and enhances soil structure, thereby facilitating root growth and microbial colonization. Moreover, EPS mediated biofilm formation on root surfaces provides a protective microenvironment that mitigates abiotic stresses such as drought, salinity, and nutrient deficiency as well as indirectly preventing fungal infections of plants by competing with pathogens for iron acquisition.[25] The ability of the selected isolates to produce EPS highlights their potential role in enhancing plant resilience under adverse environmental conditions, further supporting their applicability as bioinoculants.

The practical implications of these functional traits were validated through seed germination and plant growth assays using *Vigna radiata*. Inoculation with PGPR isolates significantly improved germination parameters and seedling vigour compared to untreated controls. Among the isolates, *Pseudomonas fulva* TD4 exhibited the highest vigour index, indicating its superior plant growth–promoting efficiency. This enhanced performance can be attributed to the cumulative effect of multiple traits, including nutrient solubilization, phytohormone production, and stress mitigation mechanisms. These findings are in agreement with earlier studies demonstrating the effectiveness of Pseudomonas spp. as biofertilizers and bio-stimulants.

Overall, the present study underscores the importance of exploring diverse and indigenous rhizobacterial populations for sustainable agricultural applications. The identification of *Pseudomonas fulva* TD4 as a potent PGPR with multifarious growth promoting qualities provides a promising path for the development of effective bioinoculant formulations. Future studies focusing on genomic characterization, field level validation, and formulation development are warranted to fully exploit its agricultural potential.

Conclusion

The isolation, screening, and characterisation of diverse plant growth promoting rhizobacteria (PGPR) from wheat rhizosphere soil were successfully demonstrated in this work, along with their possible use in stimulating *Vigna radiata* growth. Among the thirty-one isolates obtained, four strains—Proteus vulgaris PT2, *Pseudomonas putida* NP12, Serratia rubidae NW19, and *Pseudomonas fulva* TD4 exhibited multiple plant growth promoting attributes, including nitrogen fixation, phosphate solubilization, phytohormone (IAA) production, siderophore production, potassium and zinc solubilization, ammonia production, and exopolysaccharide synthesis.

Quantitative analysis revealed that Proteus vulgaris PT2 was the highest IAA producer, whereas *Pseudomonas fulva* TD4 combined substantial IAA production with several additional beneficial traits, reflecting its multifarious PGPR nature. Seed germination and seedling growth assays further confirmed that inoculation with selected PGPR significantly enhanced germination percentage, root and shoot growth, biomass accumulation, and vigor index compared to the uninoculated control. Notably, *Pseudomonas fulva* TD4 consistently outperformed other isolates, recording the highest germination percentage and vigor index, thereby emerging as the most promising bioinoculant candidate.

Overall, the findings highlight the importance of exploiting indigenous, multifunctional PGPR as eco-friendly alternatives to chemical fertilizers for sustainable agriculture. The superior performance of *Pseudomonas fulva* TD4 suggests its strong potential for development as a biofertilizer to improve productivity of *Vigna radiata* and possibly other crops. However, further studies involving greenhouse and field trials, formulation development, and evaluation under diverse agro-climatic conditions are warranted to validate its large-scale applicability and long-term benefits in sustainable crop production systems.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This research manuscript contains all the datasets produced and examined throughout the study.

Ethical Statement

This research did not involve human subjects, animal subjects or any material that requires ethical committee approval.

Informed Consent Statement:

This study did not involve human participants and therefore, informed consent was not required.

Permission to Reproduce Material from other Sources

Not applicable

Author Contributions

- **Saylee Vilas Kalekar:** Data collection, Experiment performing, Analysis of Data, Compilation of the results and Writing Original Draft.
- **Niranjan Prakashrao Patil:** Supervision, Visualization, Review and Analysis
- **Rajashree Bhalchandra Patwardhan:** Supervision, Visualization and Review.

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