

Plant growth-promoting *Pseudomonas* spp. from jasmine rhizosphere as a sustainable alternative to agrochemicals

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ABSTRACT

The global jasmine flower market has grown significantly over the decades, often relying heavily on synthetic agrochemicals to meet the increasing demand. However, growing concerns about the environmental impact of chemical-based floriculture have shifted attention towards green alternatives. Microbial plant growth promoters (PGP) offer promising bio-based and safe solutions. The present study focused on six PGP isolates belonging to the genus *Pseudomonas*, isolated from the rhizosphere soils of the geographical indication tagged Udupi Jasmine cultivated in Karnataka, India. This study involved isolation and functional characterization, followed by evaluation using bioassays and pot culture experiments. The isolates exhibited multiple beneficial traits. The green gram seeds treated with strains NPS-18 and NPS-6 exhibited 100% germination compared to only $6.66 \pm 0.28\%$ in the untreated control after 17 h. All isolates produced key phytohormones, including indole acetic acid and gibberellic acid, improving plant growth indices. The pot culture experiment showed that isolate NPS-18 was the most promising strain in terms of growth enhancement. This strain was identified as *Pseudomonas aeruginosa* using 16S *rRNA* gene sequencing. Overall, the versatile functional properties of the native PGP strains demonstrate their potential for use in sustainable jasmine cultivation.

1. INTRODUCTION

Globally, flowers play a substantial role in signifying numerous traditions. They also carry cultural and esthetic meanings that cross linguistic and religious boundaries, fostering social cohesion. In recent years, the phenomenal growth in floriculture has increased significantly. Market conditions of demand and supply have led to highly volatile pricing and the consideration of flowers as a market commodity [1]. Jasmine (*Jasminum* spp., family Oleaceae) is a flowering plant known for its fragrance [2]. Over 200 species of this genus exhibit an array of preferences and hierarchies. Some jasmine cultivars have received geographical indication (GI) tags because of their exceptional qualities from geo-specific growth conditions. Udupi jasmine [*Jasminum sambac* (L.) Aiton] locally known as Shankarpura mallige, received GI status on July 24, 2006 (GI No. 70; Certificate No. 36), documented at <https://search.ipindia.gov.in/girpublic/application/details/70> for its fragrance and exceptional shelf life owing to its geographical origin [3].

This variety, grown in a small geographical area of 4.22 km², has played a vibrant role not only in the cultural sphere but also in the

socioeconomic condition of the jasmine growers. Most importantly, the production and marketing of the Udupi Jasmine helped alleviate the cultivators' economic situation, impacting a better quality of life in about 6000 households [4]. There is a huge demand for these flowers on selected occasions and in the festival season, which makes them very expensive. Farmers are prone to use synthetic hormones, chemical fertilizers, and pesticides to meet soaring demand and gain profit. These practices have increased crop susceptibility to pests, diseases, and pollution. Moreover, women and youth mostly carry out cultivation, crop maintenance, harvesting, and marketing, making them vulnerable to the ill effects of chemical inputs. Increased global awareness of the adverse effects of chemical cultivation practices has shifted toward environmentally sound crop production. This has emphasized research favoring eco-friendly practices. There is a growing body of knowledge supporting plant growth-promoting rhizobacteria (PGPR) as a valuable alternative. PGPRs are microbes that inhabit the rhizosphere and improve plant health without any anomalies to the agroecosystem and human health [5].

Pseudomonas spp. are Gram-negative, rod-shaped, beneficial rhizobacteria found in the rhizosphere [6]. Although some species of rhizobacteria have been reported to cause plant diseases, most of them are known for plant growth-promoting (PGP) properties [7]. *Pseudomonas* spp. with PGP properties rapidly colonize by utilizing exudates secreted by plant roots [8,9]. The well-established

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mechanisms of plant growth promotion include the production of enzymes, metabolites, phytohormones, mineral phosphate solubilization, siderophores, and imparting resilience against biotic and abiotic stresses. Currently, other PGP microbes are not used to cultivate Udupi jasmine, mainly because of their nescience [3]. While PGP *Pseudomonas* are available in the local markets, they fail to bring about the desired effect as they are non-native to that location and fail to adapt to the soil conditions prevalent in jasmine-growing soils. The present study attempts to isolate native *Pseudomonas* strains that adapt easily to soil conditions, protecting them from the indiscriminate use of chemicals, which leads to decreased cultivation costs and soil degradation.

2. MATERIALS AND METHODS

2.1. Collection of Rhizosphere Soil Samples

Rhizosphere soil samples were collected from five well-maintained gardens (labelled as sites 1, 2, 3, 4, and 5) in Shankarpura, Udupi District, Karnataka, India. The geographic coordinates of sites 1, 2, 3, 4, and 5 are 13.259139 N, 74.771672 E; 13.256178 N, 74.774898 E; 13.261779 N, 74.767479 E; 13.261507 N, 74.767177 E, and 13.260136 N, 74.773157 E, respectively [Table 1]. Three healthy and high-yielding plants were identified from each location, and the adhering soils from the roots were aseptically drawn without damaging the plant. The collected soil samples were mixed, and representative soils were brought to the lab in sterile polyethylene bags and maintained at 4°C until analysis. An outline of the method is presented in Figure 1.

2.2. Isolation of PGP *Pseudomonas* spp. from Rhizosphere Soils

Pseudomonas strains were isolated from the soil samples by dilution plating on King's B agar medium [10]. The composition per liter of the medium used in the study was: proteose peptone 20 g; magnesium sulfate heptahydrate 1.5 g; dipotassium hydrogen phosphate 1.5 g;

glycerol 15 mL; distilled water 1000 mL; pH 7.2, and agar 15 g. The plates were incubated at $28 \pm 2^\circ\text{C}$ for 24 h. Representative colonies were purified and maintained at 4°C [11].

2.3. Morphological and Biochemical Characterization

The morphological properties of the isolated bacterial colonies on Luria–Bertani agar were observed after incubation for 24 h at $28 \pm 2^\circ\text{C}$. After incubation, colony size, shape, elevation, and color were recorded. Isolates were characterized for Gram staining, endospore formation, and motility. The biochemical properties constituting gelatin liquefaction, starch hydrolysis, utilization of different sources of carbohydrates, oxidase test, and catalase test were determined [12,13].

2.3.1. Siderophore production

All *Pseudomonas* isolates were assessed for siderophore production using a standard method [14]. One milliliter of 2% FeCl_3 solution was added to one mL of culture filtrate, and the appearance of an orange or red–brown color indicated siderophore production.

2.3.2. Phosphate dissolving ability

The bacteria were streaked onto Modified Sperber's medium [15]. The inoculated plates were incubated at $28 \pm 2^\circ\text{C}$ for 24 h, and the formation of clear zones around the growth confirmed phosphate solubilization.

2.3.3. Indole acetic acid (IAA) producing ability

The IAA-producing ability of *Pseudomonas* spp. was evaluated using the Salkowski method [16]. The isolates were inoculated into King's B broth medium supplemented with 0.5 mgL^{-1} of L-tryptophan and incubated at $28 \pm 2^\circ\text{C}$ for 3 days with continuous agitation at 120 rpm. The liquid cultures were centrifuged, and the supernatant was treated with Salkowski's reagent. This mixture was incubated for 20 min, resulting in the development of a pink color. IAA production was estimated by measuring the intensity of the colored complex at 530 nm using a double-beam PC-based ultraviolet-visible spectrophotometer (Model 2202, Systronics India Ltd., Gujarat, India).

2.3.4. Gibberellic acid (GA) producing ability

The *Pseudomonas* strains isolated were screened for the production of GA [17]. The isolates were inoculated into King's B broth and incubated for 7 days at $28 \pm 2^\circ\text{C}$. Broth cultures were centrifuged at 10,000 rpm for 20 min. The supernatant was extracted using ethyl acetate 2:1 (v/v) after adjusting the pH to 2.5 using 1M HCl. The optical density of the resulting solution was measured at 254 nm using a spectrometer. Unknown concentrations were determined based on the GA standard curve.

Table 1: Details of sampling locations chosen in the present study.

Sampling location	Latitude	Longitude
1	13.259139	74.771672
2	13.256178	74.774898
3	13.261779	74.767479
4	13.261507	74.767177
5	13.260136	74.773157

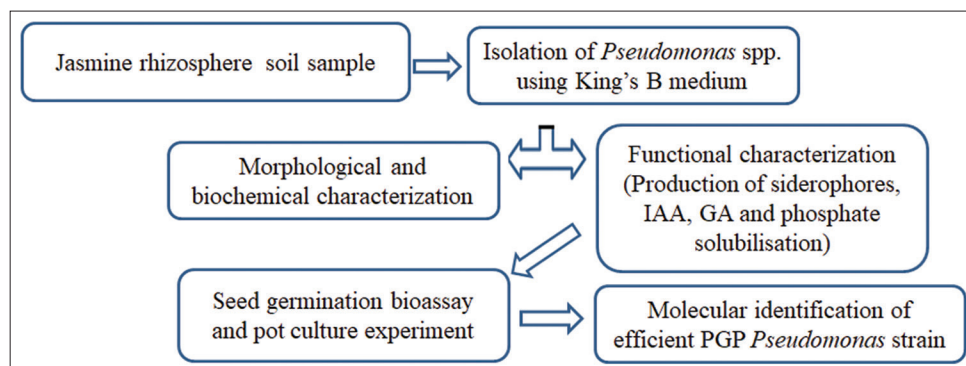


Figure 1: Schematic representation of experimental framework.

2.4. Green Gram Seed Germination Bioassay

The PGP ability of the isolates was evaluated by conducting green gram (*Vigna radiata* [L.] Wilczek) seed germination bioassay [18,19]. Isolated bacteria were grown in King's B broth in culture tubes for 24 h. The cultures were centrifuged at 10,000 rpm for 20 min, and the supernatants were collected. Seeds of uniform size were chosen, washed with 70% (v/v) ethanol for 1 min, and sterilized using 4% (v/v) sodium hypochlorite solution. The seeds were immediately rinsed thrice in sterile water. Sixty uniform-sized sterilized seeds were selected and soaked in the culture supernatant for 12 h. Twenty seeds each were placed aseptically in sterile Petri plates containing wet blotting paper (20 seeds per isolate × 3 replicates = 60 seeds). Uninoculated sterile broth-treated seeds were used as controls. Three replicates were used for each treatment. After 17 h, germination percentage and radicle length were recorded.

2.5. Pot Culture Experiment

To evaluate the PGP potential of the isolates in jasmine, a pot culture experiment was conducted. The experiment comprised seven treatments; each replicated 3 times, and was arranged in a completely randomized design. The treatments included in the study were: T1, Control (uninoculated and treated with sterilized King's B Broth); T2, RP-3; T3, WP-10; T4, NPS-6; T5, NPS-18, and T6, NPS-16. The strains were inoculated into King's B broth and incubated for 7 days at $28 \pm 2^\circ\text{C}$. The cultures thus developed were used to inoculate the rooted jasmine plants. Stem cuttings of jasmine plants of equal length were rooted in a sand-soil mixture (SSM) of 2:1 proportion. Plastic pots of 14.2 cm diameter and 15 cm height were used for this purpose. The propagated materials were maintained in a greenhouse for 2 months. Uniformly rooted cuttings were transplanted into pots containing 200 g of sterilized SSM. The liquid culture was added to each pot. The plants were irrigated regularly with sterile water. Growth indicators, such as height, number of leaves, and internodes, were recorded 180 days after treatment.

2.6 Molecular Identification

The best-performing isolate obtained from the pot culture study was processed for 16S *rRNA* gene sequencing at Yaazh Xenomics, Coimbatore, Chennai, India. Genomic DNA was extracted from the isolate using an EXpure Microbial DNA Isolation Kit (Bogar Biobee Stores Pvt. Ltd., Coimbatore, India). Primers (27 F and 1492 R) were used to amplify the 16S *rRNA* genes. The nucleotide sequences of 27 F and 1492 R were used with 5' AGAGTTTGATCCTGGCTCAG 3' and 5' TACGGTACCTTGTTACGACTT 3', respectively.

The polymerase chain reaction products were sequenced on an ABI 3730xl sequencer (Applied Biosystems). A basic local alignment search tool (BLAST) in GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used to compare the obtained sequences. Multiple sequence alignment of sequences was performed using the program MUSCLE 3.7 (http://phylogeny.lirmm.fr/phylo.cgi/one_task.cgi?task_type=muscle). Divergent and poorly aligned regions were removed using the program Gblocks 0.91b (http://phylogeny.lirmm.fr/phylo.cgi/one_task.cgi?task_type=gblocks). Phylogenetic analysis was carried out using the PhyML 3.0 aLRT program (<http://www.atgc-montpellier.fr/phyml/>). Tree Dyn 198.3 (http://phylogeny.lirmm.fr/phylo.cgi/one_task.cgi?task_type=treedyn) was used for tree rendering [20].

2.7. Statistical Analysis

All experiments were conducted in triplicate ($n = 3$), and the data obtained are expressed as mean $\pm$ standard deviation (SD). Data were processed using analysis of variance (ANOVA) with a significance threshold set at $P \leq 0.05$. When ANOVA was significant at $P < 0.05$, Tukey's test was performed to separate group mean values. The online agricultural statistics tool, a general R-based analysis platform for experimental statistics 1.0.0, was used in the statistical analysis [21].

3. RESULTS AND DISCUSSION

3.1. Isolation of Representative *Pseudomonas* spp.

Six representative *Pseudomonas* strains were successfully obtained from the rhizosphere soils. All plants displayed PGP traits, as evidenced by the production of IAA, GA, and siderophores. NPS-18 notably exhibited phosphate solubilization ability with all other PGP traits [Figure 2], highlighting its potential as a robust bioinoculant.

3.2. Morphological and Biochemical Characterization

The isolates were subjected to morphological and biochemical characterization [Table 2]. Sah *et al.* and Gutierrez-Albanchez *et al.* [22,23], reported that members of the genus *Pseudomonas* are Gram-negative and rod-shaped with polar flagella. Studies have also confirmed that these bacteria are typically catalase and oxidase positive [24,25], and some species of *Pseudomonas* also exhibit phosphate solubilization properties [26-28]. This evidence reinforces the consistency of our results with those of the previously reported studies.

3.3. IAA and GA Biosynthesis

One of the key mechanisms by which PGPR enhances plant growth is the synthesis of IAA and GA [29-31]. All the strains produced IAA and GA [Table 3]. Among the strains, maximum IAA production was observed in WP-10 ($38.06 \pm 1.47 \mu\text{g/mL}$), followed by NPS-18 ($37.87 \pm 0.56 \mu\text{g/mL}$), while RP-3 recorded the lowest ($24.62 \pm 0.18 \mu\text{g/mL}$). Notably, many species of *Pseudomonas* have been reported to produce higher levels of IAA than other PGPR [32]. In the current study, the highest GA production was observed in strain RP-3 ($278.63 \pm 2.89 \mu\text{g/mL}$), followed by WP-8 ($278 \pm 3.96 \mu\text{g/mL}$),



Figure 2: Isolate NPS-18 showing clear zone when grown on modified Sperber's medium. This is an evidence for phosphate solubilizing property of the isolate.

Table 2: Morphological and biochemical properties of representative *Pseudomonas* isolates obtained from *Jasminum sambac* (L.) Aiton.

Characters	<i>Pseudomonas</i> isolates					
	RP-3	WP-8	WP-10	NPS-6	NPS-18	NPS-16
Colony characteristics	Small, Round white, Opaque raised	Medium, irregular, yellowish green, convex	Small, round, cream, flat, opaque	Small, round, yellowish green, convex	Medium, irregular, translucent, light-green, flat	Small, round, cream, flat, opaque
Cell shape	Rod	Rod	Rod	Rod	Rod	Rod
Gram reaction	—	—	—	—	—	—
Catalase	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+
Starch hydrolysis	—	—	—	—	—	+
Gelatin liquefaction	—	—	—	+	+	—
Siderophore production	+	+	+	+	+	+
Endospore formation	—	—	—	—	—	—
Motility	+	+	+	+	+	+
Phosphate solubilization	—	—	—	—	+	—

+: Positive result, — : Negative result.

Table 3: Phytohormone producing efficiency of *Pseudomonas* spp.

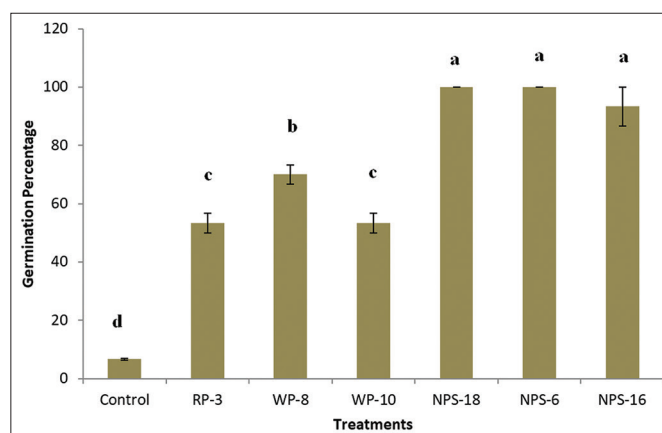
Isolate name	IAA ($\mu\text{g/mL}$)	GA ($\mu\text{g/mL}$)
NPS-16	30.21 $\pm$ 0.98 ^b	225.70 $\pm$ 1.64 ^c
NPS-6	30.30 $\pm$ 1.28 ^b	243.10 $\pm$ 2.25 ^b
NPS-18	37.87 $\pm$ 0.56 ^a	245.60 $\pm$ 1.55 ^b
RP-3	24.62 $\pm$ 0.18 ^{c*}	278.63 $\pm$ 2.89 ^a
WP-10	38.06 $\pm$ 1.47 ^a	276.80 $\pm$ 8.79 ^a
WP-8	24.73 $\pm$ 1.11 ^c	278.00 $\pm$ 3.96 ^a

*Letters designates significant differences among values ($P \leq 0.05$). Treatment with the same letters is not significantly different. Values are means $\pm$ SD ($n=3$). IAA: Indole acetic acid, GA: Gibberellic acid

WP-10 ($276.80 \pm 8.79 \mu\text{g/mL}$), and NPS-18 ($245.60 \pm 1.55 \mu\text{g/mL}$) [Table 3]. Sharma *et al.*, [33] observed a range of GA production in *Pseudomonas* from 419.2 to 485.8 $\mu\text{g/mL}$. As evidenced by several studies, IAA and GA are well known for their roles in promoting overall plant growth [34,35]. Seed germination bioassays and pot culture experiments were conducted to evaluate the influence of these phytohormones on early plant development.

3.4. Seed Germination Bioassay

Pseudomonas strains obtained in the current investigation significantly improved seed germination and radicle elongation in *V. radiata* (L.) R. Wilczek when compared to the uninoculated control [Figure 3]. Among the various treatments, NPS-18 and NPS-6 demonstrated remarkable performance, exhibiting 100% germination after 17 h of incubation. Similarly, seed inoculation with NPS-16 was 93.33% within the same duration and was statistically at par with NPS-18 and NPS-6. In contrast, the uninoculated control exhibited the lowest germination percentage ($6.66 \pm 0.28\%$), indicating the positive influence of bacterial inoculation on early seedling vigor. The enhanced germination observed in the inoculated treatments is likely associated with the increased activity of hydrolytic enzymes, such as α -amylases, which are crucial for breaking down endospermic starch into soluble sugars that fuel embryonic growth. The production of such enzymes is often regulated by the microbial production of phytohormones [36], particularly IAA and GA, which are known to stimulate enzyme

**Figure 3:** Effect of *Pseudomonas* spp. on green gram seed germination.

Letters designates significant differences among values ($P \leq 0.05$). Treatment with the same letters is not significantly different. Values are means $\pm$ SD ($n = 3$).

activity, cell division, and tissue differentiation. Several studies have documented the role of these hormones in promoting seed germination, root initiation, elongation, and overall plant development [37-39].

Furthermore, radicle growth from the germinated seeds (after 17 h of incubation) was significantly influenced by the bacterial treatments [Figure 4]. Seeds inoculated with NPS-6 exhibited the highest radicle length ($63.63 \pm 2.31 \text{ mm}$), which was significantly superior to all other treatments. The strains NPS-16 and NPS-18 resulted in the radicle lengths of $45.30 \pm 0.60 \text{ mm}$ and $43.30 \pm 1.57 \text{ mm}$, respectively, and were statistically at par with each other, ranking next best to NPS-6. The uninoculated control recorded the minimum radicle length ($3.60 \pm 0.26 \text{ mm}$) compared to all the other treatments. The increased radicle length in these treatments may be attributed to the synthesis and release of phytohormones by *Pseudomonas* strains, which enhance root elongation through cell expansion and division [40]. The results of this study align with those of several reports emphasizing the beneficial effects of PGPR on seed germination and root development [41,42].

3.5. Pot Culture Experiment

The effect of *Pseudomonas* isolates on jasmine growth was evaluated using a pot culture experiment. Compared with the uninoculated control, treatment with *Pseudomonas* significantly enhanced plant growth [Table 4 and Figure 5]. NPS-18 demonstrated outstanding improvement in plant growth, recording a significantly greater plant height (61.33 ± 4.16 cm) and number of internodes (28.00 ± 2.64) compared to all the other treatments, including control. This accounted for an increase of 95.8% and 13.94% compared to plant height and number of internodes, respectively, relative to the control [Table 4]. Treatments with NPS-6 and NPS-18 recorded the highest number of leaves of 24.66 ± 3.78 and 24.33 , respectively, which are significantly higher than the uninoculated control (20.00 ± 2.00).

Previous studies suggested that the PGP effect mediated by *Pseudomonas* is due to the secretion of phytohormones [43,44], production of siderophores, and increased bioavailability of nutrients [45]. In the current study, the production of phytohormones (IAA and GA) and siderophores in all isolates was observed. Moreover, NPS-18 demonstrated phosphate-solubilizing activity, thereby strengthening its role in plant development. Our previous studies documented the importance of phytohormone secretion and phosphate-solubilizing ability of PGP organisms in stimulating plant

Table 4: Effect of inoculation of *Pseudomonas* spp, on plant height, number of leaves and number of internodes of *Jasminum sambac* (L.) Aiton after 180 days after inoculation.

Treatment	Plant height (cm)	Number of leaves	Number of internodes
Uninoculated control	31.33±2.51 ^{e*}	20.00±2.00 ^b	24.66±0.57 ^b
NPS-16	47.66±3.21 ^c	20.33±2.30 ^b	20.33±1.15 ^c
NPS-6	55.33±2.08 ^b	24.66±3.78 ^a	25.66±0.57 ^{ab}
NPS-18	61.33±4.16 ^a	24.33±1.15 ^a	28.00±2.64 ^a
RP-3	33.00±3.46 ^d	20.00±1.00 ^b	21.00±1.00 ^c
WP-10	34.33±0.57 ^d	18.66±2.30 ^b	20.66±1.52 ^c
WP-8	36.66±0.57 ^d	20.33±1.52 ^b	21.66±1.15 ^c

*Data are represented as mean±standard deviation of three replicates. Different letters designates significant differences determined based on one-way ANOVA with Tukey’s test ($P<0.05$). ANOVA: Analysis of variance

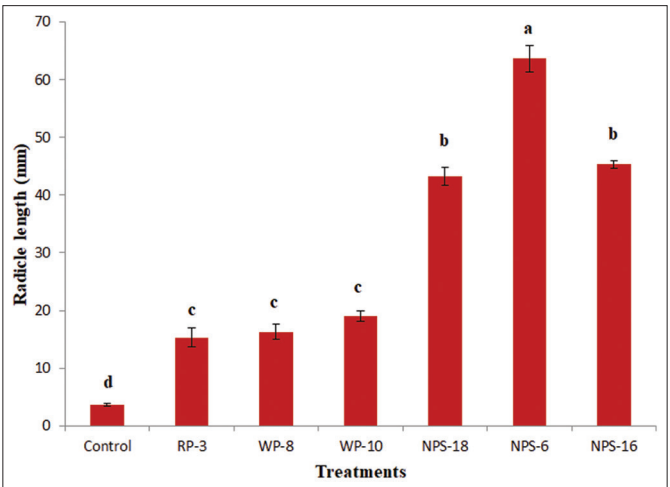


Figure 4: Effect of *Pseudomonas* spp. on green gram radicle growth. Letters designates significant differences among values ($P \leq 0.05$). Treatment with the same letters is not significantly different. Values are means $\pm$ SD ($n = 3$).

growth [46-50]. Therefore, the enhanced growth observed in plants treated with *Pseudomonas* is likely due to synergistic effects from its multiple beneficial traits.

3.6. Molecular Characterization of Efficient PGPR

Based on all PGP traits, the most efficient strain identified in the present study was NPS-18. Therefore, this strain was subjected to molecular characterization by 16S rRNA sequencing [Figures 6 and 7]. Sequence analysis of NPS-18 revealed 100% homology with *Pseudomonas aeruginosa* as depicted in the phylogenetic tree [Figure 8]. The 16S rRNA gene sequence of isolate NPS-18 was submitted to the NCBI GenBank (accession number PV862270). This result is consistent with a study by Chopra *et al.* [51], who isolated *P. aeruginosa* from the tea rhizosphere and demonstrated its attributes, including phytohormone synthesis, phosphate solubilization, and siderophore production. Several independent studies have documented using this bacterium



Figure 5: Effect of *Pseudomonas* spp. on growth of *J. sambac* (L.) Aiton.

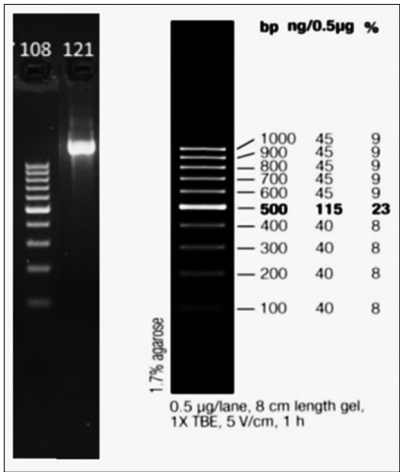
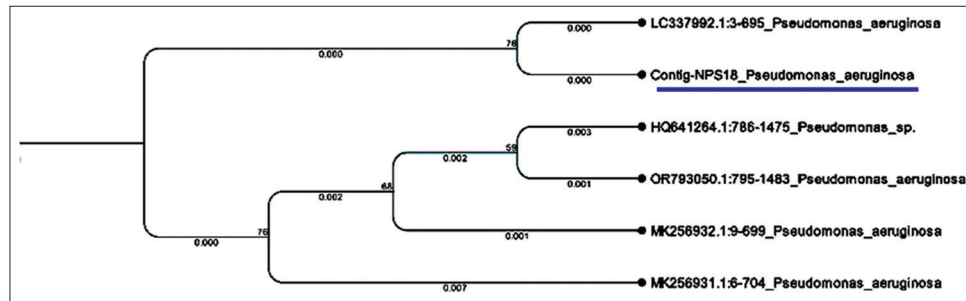


Figure 6: Agarose gel electrophoresis of polymerase chain reaction amplified 16S rRNA gene region. Lane 108: 1kb DNA ladder used as molecular weight marker, Lane 121: Amplified 16S rRNA gene product (approximately 1000 bp) from isolate NPS-18.

AGGACGGGGAGATGTGCAGTACGCCGTTTGGGATCCTTGAGATCTATTAGTGGCGACGCTAACG
CGATAAGTCGACCCGCTGGGAGATACGCCCGCAAGGTTAAAACTCAATGAATTGACGG
GAGGCGGCACAAACGGCTGGGACATGTGGTTTAATTGAAGAACCGCGAAGAACGCTTACC
TGCCCTTGACATCGTGAAGAATTTCGAGAGATCGATGTTGGTGCTTCGGGAAGCTCAGACAC
AGGTGCTGCATGGCTGTCTGCAGCTCGTGTGCTGAGATGTTGGGTGAAGTCCCGTAGCA
GCGCAACCCCTTGTCTTAGTTACCAAGCAGCTCGGGTGGGCACTCTAAGGAGACTGCCGGT
GCACAAACCGGAGAAAGGTGGGGATGACGCTCAAGTCAATCATGCCCTTACGGCCAGGGCT
ACACACGCTGTACAATGGTCTGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCC
CATAAAACCGATCGTAGTCGGGATCGCAGTCTGCAACTCGACTCGGTGAAGTCGGAATC
CGTAGTAATCGTAGCAAGAATGTCAACGGTGAATACGTTCCCGGGCCTTGATACACACCGC
CGCTCACACATGGGAGTGGGTGCTCCAGAAGTAAGTACTAACCACAAGGGGACG
GTTACACCGGAGTGATTCATGATGGGGTGAAGTCTAAAGGGGGGTCCCCAAATTTTGTCT
CCCCGCCTTTGCGCACTCAATGTAAGTATCATCCGAGCGGTCACTTCGCCACTGGG
TTCTTCTCTATATCTACTACTTTCACCGCTACACGGAGATTCCACCACCTCTACCGTATC
TCTATCTCAGTAGTTTGGATCGAGCTCCCACTGTAGCCGGCGGATTTACATCAACACT
GCTGGAAACCACTACACGCGCGGCTGTACGTCCTAGTAATTTCCGATTAAACGCTTGACCC
CTTCGTATTAAACCGCGGCTGCTGGCTACGAAAGTTAGCCGGGTGCTTATTTCTGTGGTTA
ACGGTCAAAAACAAGCAAGGATATTAGCTTTACTTGCCCTTTCTCCCAACTTAA
TGTGCTTTTAACGATTCCAAAGACCATTAATTTCCCTCCCCCGGCTTGGGGGGGG
GTTAAGGGGTTTTTCCCCCCCCTTTGGCCCAAATAATTTCCCCCCCCCTGTTCCCTCC
CCCGCAAGGAATTTTGGAAACGGGGCTCTCAATTCGGTTTGTGTAATTGGACGCCTCC
CTTTCCCCAACCCCTTTTACAAGGGAATCG

as a potential bioinoculant to promote plant growth and control plant diseases [52-55]. In addition, the Directorate of Groundnut Research (ICAR), Junagadh, developed a microbial consortium, NutBoost, that includes *P. aeruginosa* BM 6 (PGPR4) as one of its components and is recommended for groundnut cultivation [56]. However, recent studies have underscored the inherently pathogenic nature of certain *P. aeruginosa* strains, which possess a sophisticated arsenal of virulence determinants, such as quorum-sensing circuits, biofilm formation, secretion systems, and antibiotic resistance that enable them to colonize immunocompromised hosts [57-60]. Furthermore, Vasco *et al.* [61] highlighted the need to distinguish naturally occurring environmental isolates from clinical isolates, observing that clinical isolates often show traits such as greater motility, higher stress tolerance, and longer persistence in host-associated environments that are usually absent in strains from natural environments. The isolate *P. aeruginosa* (NPS-18), obtained in this study, was from the rhizosphere soil of jasmine with no clinical background and therefore, may be used as a bioinoculant. Nevertheless, we also acknowledge



The diagram illustrates the hormonal regulation of plant growth and development, specifically focusing on the auxin (IAA) and gibberellin (GA) pathways and their interaction with the bacterial siderophore BS.

Plant Cell Pathway:

- IAA Pathway:** IAA (Indole-3-acetic acid) is shown as a green cluster. It is converted to IAA Amide (blue oval) by the enzyme GH3 (pink oval). IAA Amide is then converted to IAA (green oval) by the enzyme ABCB (green oval). IAA Amide is also converted to Auxin (purple oval) by the enzyme ARF (yellow oval). Auxin is then converted to ARF (yellow oval) by the enzyme Ubiquitin Degradation (blue oval). ARF is then converted to ARF (yellow oval) by the enzyme ARF (yellow oval). ARF is then converted to ARF (yellow oval) by the enzyme ARF (yellow oval).
- GA Pathway:** GA (Gibberellin) is shown as a green cluster. It is converted to GA (green oval) by the enzyme NPF 3.1 (purple oval). GA is then converted to DELLA (blue oval) by the enzyme DELLA (blue oval). DELLA is then converted to PIFs (purple oval) by the enzyme PIFs (purple oval). PIFs are then converted to GA Response Genes (blue oval) by the enzyme GA Response Genes (blue oval).
- Cell Division & Elongation:** The ARF pathway leads to Cell Division & Elongation (orange oval).
- Tissue Growth & Development:** The GA pathway leads to Tissue Growth & Development (blue oval).

Bacterial Siderophore (BS) Pathway:

- Iron Channel:** The Iron Channel (green funnel) is shown transporting Fe³⁺ (orange spheres) from the Bacterial Siderophore (BS) (blue structure) into the cell.
- Periplasmic Binding Protein (BS-PBP):** The BS-PBP (pink oval) is shown binding Fe³⁺ (orange spheres) and Fe²⁺ (brown spheres) in the periplasm.
- ATP/ADP Cycle:** The BS-PBP is shown using ATP (yellow oval) to transport Fe³⁺ (orange spheres) into the cell, converting it to ADP (yellow oval).
- Enzyme Activation:** The BS-PBP is shown activating the enzyme ABCG37 (orange oval) via Enzyme Activation (yellow arrow).

Figure 9: The probable mode of action of *Pseudomonas* spp. obtained in the current study.

the importance of conducting additional risk assessments, such as genome-based analysis of virulence and resistance genes, before proceeding with field-level deployment.

Finally, we hypothesized that [Figure 9] the *Pseudomonas* strain NPS-18 isolated in this study enhances plant growth by producing siderophores and phytohormones (IAA and GA). IAA produced by the isolate is transported to the root hair cells *via* ATP-binding cassette transporters, resulting in elevated intracellular IAA levels. This may lead to the ubiquitination and subsequent degradation of the Aux/IAA repressor protein [62]. This process activates auxin-responsive genes, prompting growth and development of the plant. In addition, GA produced by the isolates binds to the gibberellin insensitive dwarf (GID 1) receptors located on the root hair cell membrane, forming the GA-GID 1 complex. This complex interacts with the TVHYNP motif of the DELLA protein. As a result, the DELLA protein undergoes ubiquitylation and is consequently degraded *via* the 26S proteasome pathway, activating PIF transcription factors. Furthermore, the process induces GA-responsive genes [63], thereby promoting tissue growth and development. Similarly, siderophores (BSP) produced by bacterial strains chelate Fe³⁺ ions present in the soil. Successively, they are transported to plant root hair cells *via* ABCG37 transporters [64]. The enhanced intracellular availability of iron boosts the activity of iron-dependent enzymes involved in phytohormone biosynthesis, DNA replication, photosynthesis, and respiration, culminating in plant growth.

The findings of the study hold significant promise for green floriculture, particularly for jasmine cultivation. The identified efficient bacterial strains are promising bioinoculants for enhancing flower production in an eco-friendly manner. With this background, the findings of the current study are protected under Indian patent rights with application No. 202541007306 dated February 07, 2025. They are being made available to needy farmers on a non-profit basis, in alignment with the Nation's vision for digital and smart agriculture.

4. CONCLUSION

The study provides insights into PGP *Pseudomonas* spp. associated with rhizosphere soils of GI tagged Udupi jasmine. NPS-18 demonstrated the highest efficiency among the six isolates, exhibiting multifunctional traits that enhance plant growth. Based on 16S *rRNA* gene sequencing, the best-performing isolate was identified as *P. aeruginosa*. These experiments were performed under regulated greenhouse conditions, which may not accurately reflect the complexity of the natural field conditions. Further, expanding the scope of sampling to diverse geographic regions could help identify more effective PGPR strains. Despite these limitations, this study is the first report on the isolation and functional characterization of indigenous *Pseudomonas* spp. from the rhizosphere of Udupi jasmine, laying the groundwork for future applications in sustainable floriculture.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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