

Characterization and Identification of Isolated Rhizobacteria from Different Wheat Field in Vadodara, Gujarat, India

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Abstract: In the present research, wheat rhizosphere soil from Vadodara, Gujarat, was used to extract, characterize, and identify prospective plant growth promoting rhizobacteria (PGPR) with several PGP features (N₂-fixation, P- and K-solubilization, IAA, and siderophore synthesis). Eleven pure colonies in all were isolated, and colonies were chosen for further evaluation. Seven superior PGPR strains were identified by partial sequencing of the 16S rRNA gene based on biochemical, potential PGP characteristics, and growth performance. These strains were identified as *Acinetobacter pittii* DSM 21653, *Shigella sonnei*, *Escherichia fergusonii* ATCC 35469, *Acinetobacter pittii* DSM 21653, *Cellulosimicrobium funkei*, and *Shigella sonnei*. Significant biochemical estimate of the PGP properties has been demonstrated by the chosen bacterial strains. Every strain has superior PGPR features of its own. These bacteria should be utilized more since they have the potential to be employed as biofertilizer.

Keywords: Plant growth promoting rhizobacteria, Wheat rhizosphere, Biofertilizer, Nutrient solubilization, 16S rRNA identification

1. Introduction

The increase and enhancement of agricultural productivity is of highest importance in modern agriculture. The green revolution has made remarkable progress in this area and in the lives of farmers. However, a significant obstacle to sustainable agriculture is the overuse of chemical pesticides and fertilizers in crop fields. This has affected the groundwater, the accessible mineral nutrients, and the condition of the soil. bad circumstances, organic farming could be able to improve things and stop the ecosystem's demise. Therefore, biofertilizer, which is a blend of growth-specific nutrients, is a glimmer of hope for modern growth-oriented agriculture with sustainable crop production while preserving and safeguarding the environment (Sherpa *et al.* 2021). Microbes with favorable characteristics for plants can be found in the rhizosphere. The rhizosphere has a higher microbial population than the rest of the soil because plant roots actively secrete exudate chemicals [Berendsen, Pieterse, and Bakker, 2012]. Because they either directly or indirectly stimulate the growth of their hosts, these bacteria are referred to as plant growth-promoting rhizobacteria (PGPR) (Bhattacharyya and Jha, 20110). According to Lugtenberg and Kamilova (2009) and Sati, Pande, and Samant (2023), rhizospheric bacteria directly promote plant growth by delivering substances that they synthesize or by helping the plant absorb soil nutrients, such as by forming phytohormones and siderophores, dissolving minerals (P, Zn, and K), and lowering ethylene levels in plants.

Recent research has shown the positive and advantageous effects of PGPR on the growth and yield of various crops, particularly wheat, at different environments under variable ecological conditions (Ozturk *et al.*, 2003; Marques *et al.*, 2010; Mehnaz *et al.*, 2010; Zhang *et al.*, 2012). Interest in the beneficial rhizobacteria associated with cereals has

increased. When compared to uninoculated plants, *Pseudomonas fluorescens* inoculation resulted in a notable increase in root weight (19-43%), number of tillers per plant (10-21%), grain yield (15-43%), and straw yield (22-39%) of wheat (Shaharoon *et al.*, 2008). Additionally, 25-30 kg N ha⁻¹ of chemical fertilizer were saved by inoculation with the PGPR strain *Azotobacter* (Narula *et al.*, 2005). In a more recent study, Kumar *et al.* (2014) investigated the impact of PGPRs on wheat growth and yield in both pot and field conditions. They discovered that a triple combination of strains *B. megaterium*, *A. chlorophenolicus*, and *Enterobacter* significantly increased plant height, grain yield, straw yield, and test weight in pot conditions by 17.5, 79.8, 78.6, and 26.7%, and in field conditions by 29.4, 27.5, 29.5, and 17.6%, respectively (Majeed *et al.* 2015).

The goal of the study was to separate the local strains from the rhizosphere and endo-rhizosphere of wheat cultivated on various soils in Vadodara, Gujarat. 16S rRNA sequence analysis was used to identify representative isolates after these bacteria were described and screened for PGP potentials *in vitro*.

2. Materials and Methods

2.1 Collection of Sample

Soil samples were collected from various Wheat farms in the outskirts of Vadodara. The rhizospheric soil was collected from different farm at the location: Location A- 22.276040° N, 73.118372° E; Location B 22.258121° N, 73.129928° E and Location C: 22.274869° N, 73.250255° E. Figure 1 shows the geological locations of the sample collection site.



Figure 1: Geographical locations of sampling sites

2.2 Isolation of Rhizobacteria:

Following processing, all soil samples were serially diluted up to 10^{-6} . Aliquots (0.1 mL) of dilutions ranging from 10^{-6} to 10^{-8} were spread-plated on Pikovskaya's agar and cultured for seven days at $28 \pm 2^{\circ}\text{C}$. From the initial isolates on Pikovskaya's agar plates, certain colonies exhibiting unique morphology—such as mucoid texture, coloration, or solubilization halos—were separated and purified. N agar plates were used to keep the isolates.

2.3 Biochemical Characterization of Rhizobacteria and Identification:

According to Somasegaran and Hoben (1994), colony morphology, size, color, shape, gum production, and growth pattern were noted after 24 hours of development on LB agar plates at $28 \pm 2^{\circ}\text{C}$. A total of eleven chosen isolates underwent additional secondary screening. Secondary screening for phosphate solubilization, siderophore generation, and Indole Acetic Acid (IAA) synthesis was performed on each of the eleven isolates (Figure 2).

2.4 Biochemical Characterization

Nitrate reduction test: Each colony was injected with a loopful of nitrate broth and incubated for 24 hours at $28 \pm 2^{\circ}\text{C}$. Sulfanilic acid and α -naphthylamine reagents were added after the incubation period. The reduction of nitrate to nitrite was shown by the development of a red color. Zinc dust was added to verify the reaction if no color appeared.

Indole production: A loopful of each culture was

inoculated into tryptone broth and incubated under standard conditions to determine the synthesis of indole. Kovac's reagent was added to the broth culture following incubation. The color development at the medium's surface layer was used to record the reaction.

MR-VP Test: MR-VP broth was used for the Voges-Proskauer and methyl red tests. A part of the broth culture was utilized for the methyl red test after incubation, which involved adding methyl red indicator and watching for a color shift. α -naphthol and potassium hydroxide were added to the incubated broth, thoroughly mixed, and left to stand for color development in order to perform the Voges-Proskauer test.

Citrate utilization: Simmons citrate agar slants were used to measure citrate utilization. Every culture was streaked in a zigzag pattern across the slant's surface and incubated for 24 to 48 hours at $28 \pm 2^{\circ}\text{C}$, with longer incubation times if needed. During interpretation, growth on the slant and changes in the medium color were taken into account.

Urease production: Christensen's urea agar was used to measure urease production. After being inoculated onto the medium's surface, the isolates were cultured under carefully monitored circumstances. Urease activity was interpreted using the development of an alkaline reaction in the media, which was shown by a change in color.

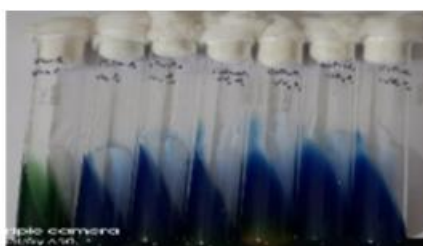
Oxidase activity: Freshly produced colonies and oxidase reagent were used to measure oxidase activity. The reagent was applied to a little amount of bacterial growth, and the color development was monitored right away.

Catalase activity: A drop of 3% hydrogen peroxide was added to fresh bacterial growth on a clean slide to measure catalase activity. Effervescence was then monitored. Starch agar medium was used to measure amylase activity. After spot-inoculation, the isolates were cultured for 48 to 72 hours. Following incubation, the plates were inundated with iodine solution, and the creation of a clear zone surrounding the colony was used to assess starch hydrolysis.

Carbohydrate utilization: By inoculating each culture with a variety of carbohydrates, such as glucose, sucrose, fructose, lactose, arabinose, galactose, and mannitol, the cultures' capacity to use carbohydrates was assessed. The utilization was assessed based on the culture's ability to develop and use these sugars. The preliminary phenotypic characterisation of the bacterial isolates was based on the observations from the cultural, morphological, and biochemical tests, all of which were conducted under aseptic circumstances.



Nitrate Reductase



Citrate Utilization



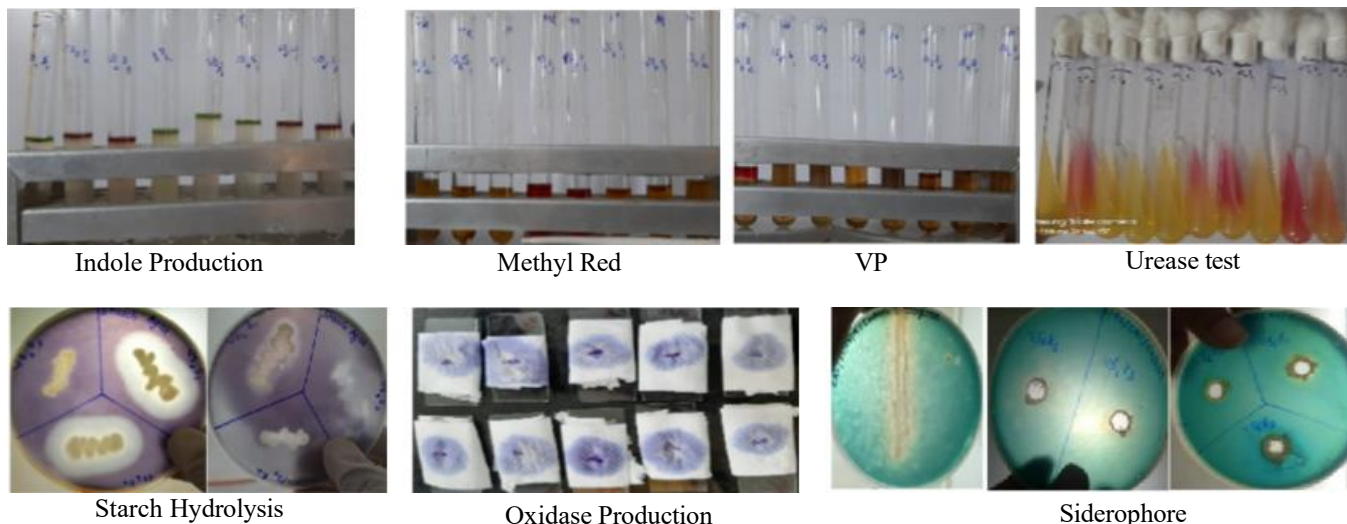


Figure 2: Biochemical Characterization

2.5 Quantitative Determination of PGP Traits:

Quantitative determination of indole-3-acetic acid (IAA) production

According to Khalid *et al.* (2004), IAA generation by the isolates was measured using a colorimetric technique based on the Salkowski reagent. For 48-72 hours, each isolate was cultivated in L tryptophan-supplemented nutritional broth (or a suitable medium like King's B broth) at $28 \pm 2^\circ\text{C}$ while being shaken. Following incubation, the culture was centrifuged to extract the bacterial cells, and the cell-free supernatant was gathered for additional examination. The Salkowski reagent (0.5 M FeCl_3 in 35% perchloric acid) was combined with a determined volume of the supernatant and allowed to sit at room temperature for 20 to 30 minutes in the dark. The presence of IAA in the culture filtrate was identified by the formation of a pink to red color. Spectrophotometric measurements of the color's intensity were made at 530 nm. Using known concentrations of pure IAA, a standard curve was created, and the absorbance readings were used to determine the amount of IAA in the culture supernatant. Each isolate's final IAA concentration was reported in $\mu\text{g/mL}$.

By measuring out 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.8, and 1 mL of IAA standard stock solution into test tubes, a standard curve was created. One or two drops of orthophosphoric acid, two milliliters of Salkowski reagent, and three milliliters of nutritional broth were added to the IAA solutions. One or two drops of ortho-phosphoric acid were added to one milliliter of nutritional broth to create a blank. Next, two milliliters of Salkowski reagent were added to the test tube without the IAA stock solution. After 30 minutes of dark incubation at room temperature, the absorbance of both solutions was measured at 535 nm. Plotting the IAA solution concentration on the X-axis against the absorbance at 535 nm on the Y-axis produced a standard curve.

Quantitative estimation of phosphate solubilization efficiency

According to Mehta & Nautiyal (2001), a tricalcium phosphate (TCP) based approach was used to assess the isolates' phosphate solubilization potential. For seven to ten days, the isolates were shaken and incubated at $28 \pm 2^\circ\text{C}$ in

flasks filled with sterile phosphate solubilizing media. Following incubation, the culture broth was centrifuged, and the amount of soluble phosphate released into the medium was estimated using the cell-free supernatant. According to the laboratory protocol, the amount of soluble phosphate in the supernatant was calculated calorimetrically using the ammonium molybdate ascorbic acid method. The phosphate concentration was determined using a phosphate standard curve and the absorbance of the generated color was measured at a suitable wavelength (about 650-700 nm). Each isolate's phosphate solubilization efficiency was measured in $\mu\text{g/mL}$ to indicate how much phosphate was solubilized under the specified circumstances.

All assays were carried out under aseptic conditions, and each treatment was replicated at least three times to ensure reproducibility of the data.

Qualitative detection of siderophore production

The Chrome Azurol S (CAS) agar method, as published by Schwyn and Neilands (1987), was used to qualitatively evaluate siderophore production. On CAS agar plates, fresh bacterial cultures were spot-inoculated and incubated for the necessary amount of time at $28 \pm 2^\circ\text{C}$. After that, the plates were examined to see if the media around the colony had changed color. The emergence of an orange or yellowish-orange halo surrounding the colony against the blue backdrop of the CAS medium suggested the production of siderophore. The bacterial isolate's produced siderophores chelate iron from the chrome azurol S dye complex, causing a noticeable decolorization around the colony. The tube method was also used to determine the siderophore production, and the following formula was used to calculate the percentage of siderophore units.

$$\text{Siderophore unit} = (1 - \text{As}/\text{Ar}) \times 100\%$$

Where, Ar is the absorbance of reference and As is the absorbance of sample.

The most effective PGPR isolates were chosen for the pot-based wheat experiment using the quantitative results from these secondary screening tests in addition to the core biochemical traits.

2.6 Molecular Characterization

The phenol-chloroform-isoamyl alcohol approach, which was modified from conventional protocols, was used to extract genomic DNA from pure cultures of each PGPR isolate (Sambrook *et al.*, 1989). After 24 hours of bacterial cell growth in Luria-Bertani (LB) broth, the cultures were harvested by centrifugation at 10,000 × g for 10 minutes at 4°C. To weaken cell walls, the pellet was resuspended in 500 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) with 20 mg/mL lysozyme and incubated for 30 minutes at 37°C. After adding 10% SDS (50 µL) and proteinase K (100 µg/mL), the mixture was incubated for one hour at 56°C. After adding an equal proportion of phenol:chloroform: isoamyl alcohol (25:24:1) and gently mixing, the mixture was centrifuged for 15 minutes at 12,000 × g. DNA was precipitated overnight at -20°C using 0.1 volume 3 M sodium acetate (pH 5.2) and 2.5 volumes ice-cold absolute ethanol after the aqueous phase was moved to a new tube. After centrifuging the DNA pellet at 12,000 × g for 15 minutes at 4°C, it was cleaned with 70% ethanol, allowed to air dry, and then resuspended in 50 µL of nuclease-free water. NanoDrop was used to measure purity using the A260/A280 ratio (1.8-2.0), and 0.8% agarose gel electrophoresis was used to verify integrity.

Using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), the 16S rRNA gene was amplified by PCR with a target size of approximately 1500 bp (Lane, 1991). 50 ng of template DNA, 0.4 µM of each primer, 200 µM dNTPs, 2.5 mM MgCl₂, 1 U Taq DNA polymerase (Thermo Fisher), and 1× PCR buffer were all included in each 25 µL reaction. The thermal cycling

conditions were as follows: 5 minutes of initial denaturation at 95°C; 35 cycles of 95°C/30 s, 55°C/45 s, and 72°C/90 s; and 10 minutes of final extension at 72°C. PCR products were measured, purified using the QIAquick PCR Purification Kit (Qiagen), and confirmed using 1.2% agarose gel electrophoresis.

Using the same primers, purified amplicons were sequenced in both directions using Sanger sequencing (Applied Biosystems 3730xl). BioEdit v7.2 was used to construct consensus sequences, which were then manually trimmed for quality (Phred score >30). According to Kumar *et al.* (2018), sequences exhibiting ≥98% identity to type strains were ascribed taxonomic identity, deposited in GenBank (accession numbers:), and phylogenetic trees were built using MEGA X via neighbor-joining method with 1000 bootstrap iterations.

The NCBI BLAST at <http://www.ncbi.nlm.nih.gov/blast/Blast.cgi> was used to compare the acquired gene sequences with those in the Gen Bank databases. Accession numbers were obtained by submitting sequences to the NCBI GenBank database.

3. Results and Discussion

Isolation and Morphological characteristics: Based on colony characteristics obtained from the soil samples from the wheat rhizosphere, a total of 11 bacterial morphotypes were identified. Eight isolates were determined to be Gram negative and three to be Gram positive based on the results of the Gram staining (Table 1).

Table 1: Colony Morphology of Selected Isolates

Isolate code	Colony size and shape	Colony color	Elevation	Margin	Gram reaction
VS2S1	Small, Pin-point	White	Fused	Entire	Gram negative
VS2S2	Small, Pin-point	Brown	Fused	Raised	Gram negative
VS2S3	Large, Myceloid	Red	Raised	Entire	Gram negative
VS3S3	Small, Pin-point	Yellow	Raised	Entire	Gram Positive
VS4S1	Small, Conglomerate	Milky White	Raised	Undulate	Gram positive
VS4S2	Large, Myceloid	Golden Yellow	Fused	Irregular	Gram negative
VS6S1	Large, Irregular	Off-white	Raised	Entire	Gram negative
VS6S2	Small, Round	Off -white	Fused	Undulate	Gram negative
VS6K2	Small, Round	White	Raised	Undulate	Gram negative
VS6K3	Small, Round	White	Raised	Entire	Gram Positive
VS6A1	Large, Round	Off white	Raised	Entire	Gram negative

Biochemical Characterization of Rhizobacteria and Identification:

The biochemical tests used to identify isolates phenotypically include the Catalase, Oxidase, Nitrate Reduction, Citrate Utilization, Urease, Gelatinase, and Carbohydrate Utilization tests (Holt *et al.*, 1994). For biochemical activity, all of the results were reported in Table 2.

Table 2: Biochemical Characterization

Sr. No	Characteristics	Isolate Code										
		VS2S1	VS2S2	VS2S3	VS3S3	VS4S1	VS4S2	VS6A1	VS6S1	VS6S2	VS6K2	VS6K3
1	Grams Reaction	Gram Negative	Gram Negative	Gram Negative	Gram Positive	Gram Positive	Gram Negative	Gram Negative	Gram Negative	Gram Negative	Gram Negative	Gram Positive
2	Indole 3 Acetic Acid	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
3	Phosphate	Negative	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Positive	Positive
4	Siderophore	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Positive
5	ACC	Negative	Positive	Positive	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Positive
6	Glucose	A/-	A/G	A/-	A/G	A/G	-/-	-/-	A/-	A/-	A/-	A/-

7	Sucrose	A/-	A/G	A/-	-/-	A/-	A/G	-/-	A/-	A/-	-/-	-/-
8	Fructose	A/-	A/G	A/-	A/G	A/-	A/G	-/-	A/-	A/-	-/-	-/-
9	Lactose	A/G	-/-	A/-	A/G	-/-	-/-	-/-	-/-	-/-	-/-	-/-
10	Arabinose	A/-	A/G	A/-	A/G	-/-	A/G	A/-	A/-	A/-	-/-	A/-
11	Galactose	A/-	A/G	A/-	-/-	A/-	A/G	-/-	A/-	A/-	A/-	A/-
12	Mannitol	A/-	A/G	A/-	A/G	-/-	A/G	-/-	A/-	A/-	-/-	-/-
13	Oxidase	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Positive	Negative	Negative
14	Catalase	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
15	Amylase	Negative	Negative	Positive	Negative	Positive	Negative	Negative	Positive	Positive	Negative	Negative
16	Citrate	Negative	Positive	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive
17	Urease	Negative	Positive	Negative	Negative	Negative	Positive	Negative	Positive	Positive	Positive	Positive
18	Nitrate	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Negative	Negative	Negative	Negative
19	Indole	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Positive	Positive	Negative	Negative
20	VP	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
21	Methyl Red	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative

Interpretation

A-Yellow colour acid, G-Gas Bubble, A/- - indicates only Acid is present, A/G - indicates both Acid and Gas is Present, -/- Both acid and gas are absent

Quantitative Determination of PGP Traits:

Table 3: Quantitative Determination of PGP Traits

ISOLATE	IAA(µg/ml)	PHOSPHATE(µg/ml)	SIDEROPHORE %	ACC OD600nm
VS1S1	6.428			
VS1S2	36.78			
VS2S1	65.35			0.41(OD=0.5)
VS2S2	4.65	160	35	
VS2S3	26.15			
VS3S1	27.85			0.674(OD=0.75)
VS3S2	38.58	-	-	-
VS3S3	42.14			
VS4S1	29.64	211		
VS4S2	92.28			0.651(OD=0.75+0.5)
VS6A1	30.04			
VS6S1	13.51		80	
VS6S2	38.57		75	
VS6K2	15.35	122.64	50	
VS6K3	24.05	126.23	72	

It is well known that plant growth-promoting rhizobacteria (PGPR) enhance plant nutrition, alter root development architecture, and simultaneously improve plant responses to external stressors (Salem et al., 2007; Glick, 1995). Numerous bacterial genera have been identified as viable candidates for promoting plant growth, including Bacillus, Pseudomonas, Azospirillum, Azotobacter, Bradyrhizobium, and Rhizobium. The morphological characteristics and biochemical activity of the isolated bacteria in the current study (Tables 1 and 2) show that they share characteristics with the genera Enterobacter, Bacillus, Pseudomonas, Serratia marscecens, etc. Of the eleven isolates, four exhibited the ability for phosphate solubilization, five were siderophores, and four were positive for the synthesis of indole (Table 2). Isolates VS2S2, VS6S1, VS6S2, VS6K2, and VS6K3 which are positive for multiple PGP Traits. Numerous characteristics that promote plant growth are known to be present in rhizospheric bacteria (Upadhyay and Srivastava, 2010; Kumar et al., 2012; Saini et al., 2016). Rhizobacteria stimulate plant growth in a variety of ways, either directly through the synthesis of hormones and phosphate solubilization or indirectly through the creation of

siderophores (Lugtenberg et al., 2009). Additionally, it is well known that rhizobacteria isolated from the rhizosphere of wheat exhibited a variety of characteristics that promote plant growth (Rana et al., 2011). According to the current study's findings, seven of the eleven isolates tested positive for one or more multiple plant growth-promoting characteristics, making them useful PGPRs (Table 2). The synthesis of bacterial extracellular enzymes has significant commercial potential and is crucial for controlling plant diseases (Geetha et al., 2014). According to Sinsabaugh et al. (1991), soil enzymes such as cellulase, chitinase, protease, and phosphatase are essential for preserving soil fertility, ecology, and health. All 11 of the PGPR isolates were examined for the synthesis of extracellular enzymes, including as pectinase, protease, cellulase, amylase, xylanase, and chitinase, due to the potential commercial uses of these enzymes. Every isolate in our investigation tested positive for one or more extracellular enzymatic activities (Table 2). Therefore, it can be said that the isolates that were assessed for a variety of characteristics could be a viable option for promoting plant growth.

All 11 isolated bacteria were quantitatively analyzed for

IAA, phosphate, siderophore, and ACC. Table 3 presents a tabulation of the results. Table 3 shows that all 11 isolates in our study were capable of producing IAA while growing in medium. Compared to other isolates, isolate VS4S2 produced the most IAA (92.28 µg l-1). Isolate VS2S2 produced the least quantity of IAA (4.65 µg l-1).

The KCl standard curve was used to calculate the quantity of soluble K produced by the bacterial isolates (Table 3). According to Table 3, the strain that produced the highest concentration of soluble K at 6 days of inoculation was VS4S1 (211 µg·mL-1), followed by VS2S2 (160 µg·mL-1) and VS6K2 (122.64 µg·mL-1).

In the Aleksandrov broth medium, Culture VS6S1 generated the maximum siderophores unit (80.0%) in the estimation test of siderophores production (Table 3).

VS6K3 (72%) and VS6S2 (75%) came next.

Molecular identification of isolated PGPR:

Using the 27F and 1492R primers, molecular identification by partial sequencing of the 16S rRNA gene produced roughly 1500 bp 16S rRNA fragments that were effectively amplified. The isolated bacterial resemblance to known plant-associated bacteria was revealed by BLAST comparison searches against the NCBI nucleotide database from the NCBI GenBank and the phylogenetic tree created (Figure 1). According to the results, VS2S1 is identified as *Acinetobacter pittii* DSM 21653, VS2S2 as *Shigella sonnei*, VS2S3 as *Escherichia fergusonii* ATCC 35469, VS4S2 as *Acinetobacter pittii* DSM 21653, VS6K2 as *Cellulosimicrobium funkei*, VS6K3 as *Shigella sonnei*, and VS4S1 as *Acinetobacter pittii* (Table 3).

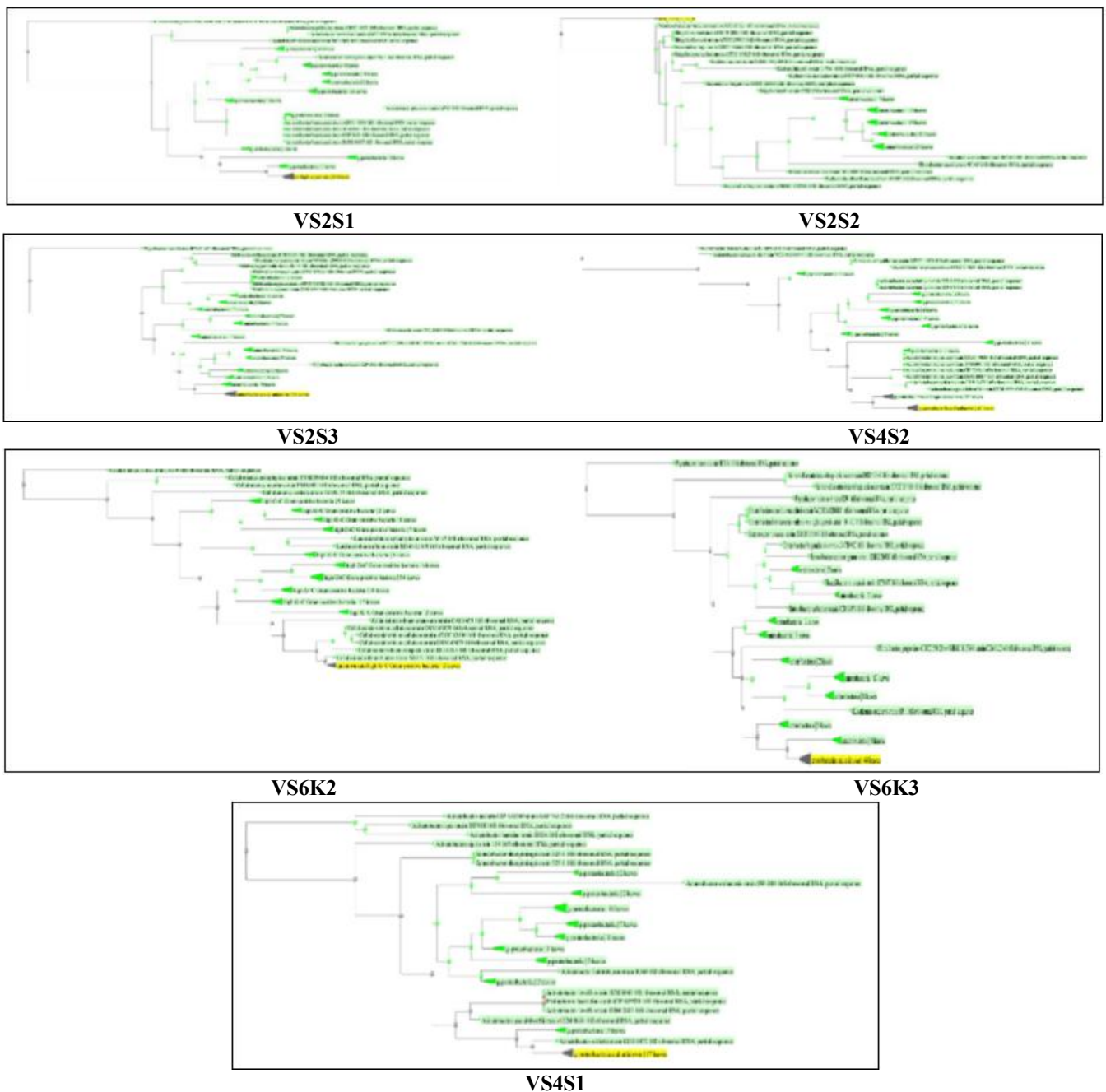


Figure 3: Phylogenetic tree VS2S1, VS2S2, VS2S3, VS4S2, VS6K2, VS6K3, VS4S1 based on the 16S rRNA sequences of the selected strains and related bacteria.

Table 4: Molecular bacterial identification using 16S rRNA gene sequences

Isolates	16S rRNA fragment length (bp)	Closest relatives in NCBI	NCBI accession number	Similarity (%)
VS2S1	672	Acinetobacter pittii DSM 21653	NR 117621.1	99.40
VS2S2	516	Shigella sonnei	NR 104826.1	97.87
VS2S3	733	Escherichia fergusonii ATCC 35469	NR 027549.1	98.35
VS4S2	436	Acinetobacter pittii DSM 21653	NR 117621.1	100
VS6K2	808	Cellulosimicrobium funkei	NR 042937.1	97.77
VS6K3	756	Shigella sonnei	NR 104826.1	98.28
VS4S1	822	Acinetobacter pittii	NR 117930.1	99.75

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