Asian Journal of Agriculture and Biology
https://doi.org/10.35495/ajab.2026.047

Original Article

Isolation and characterization of native *Pseudomonas* isolates and their plant growth-promoting potential in tomato under controlled conditions

Zhamila Baimirzayeva[1], Karlygash Korazbekova[2*], Aigul Aitbayeva[2]
[1]Department of Biotechnology, M.Auezov South Kazakhstan Research University, Shymkent, Kazakhstan
[2]Department of Biology, Zhanibekov University, Shymkent, Kazakhstan

**Corresponding author's email:* korazbekova.karlygash@okmpu.kz
Received: 17 February 2026 / Revised: 19 May 2026 / Accepted: 22 May 2026 / Published Online: 11 June 2026

Abstract

Pseudomonas spp. are widely recognized as plant growth-promoting rhizobacteria (PGPR) associated with improved plant performance under greenhouse conditions. This study aimed to isolate and characterize *Pseudomonas* strains from greenhouse soils of the Turkestan region (Kazakhstan) and to evaluate their biochemical traits and effects on tomato (*Solanum lycopersicum*) growth under controlled conditions. Four fluorescent *Pseudomonas* isolates were identified based on morphological characteristics and 16S rRNA gene sequencing as *Pseudomonas baetica* P1-1, *Pseudomonas* sp. N1-2 and N2-2, *Pseudomonas germanica* P4. Seed inoculation assays indicated that the tested strains positively influenced germination and early plant growth parameters, with variation among isolates. The strains also exhibited differences in antioxidant activity, radical scavenging capacity, exopolysaccharide (EPS) production, and indole-related traits. Among them, *P. baetica* P1-1 showed comparatively higher superoxide radical scavenging activity and EPS production, indicating strong functional potential. Compatibility assays revealed no antagonistic interactions among the strains, suggesting their potential use in combined applications. Overall, the results indicate that the studied native *Pseudomonas* strains possess plant growth-promoting and stress-related functional traits that may contribute to their application as bioinoculants in greenhouse tomato production.

Keywords: *Pseudomonas*, PGPR, Tomato, Greenhouse soil, Bacterial compatibility, Gene sequencing

How to cite this article:
Baimirzayeva Z, Korazbekova K and Aitbayeva A. Isolation and characterization of native *Pseudomonas* isolates and their plant growth-promoting potential in tomato under controlled conditions. Asian J. Agric. Biol. 2026: e2026047. DOI: https://doi.org/10.35495/ajab.2026.047

Introduction

Greenhouse tomato production has significant advantages over open-field cultivation in today's challenging climatic conditions. Modern sustainable agricultural requires practicing innovative strategies that enhance crop productivity with minimal environmental impacts, such as the application of plant growth-promoting rhizobacteria (PGPR) (Baimirzayeva et al., 2025; Dashti et al., 2014), the development of climate-smart and stress-resilient crop varieties (Nordstedt et al., 2020; Ansari et al., 2015), the integrated pest management strategies (Romeh, 2018), and the recycling of organic waste into biofertilizers (Bakhov et al., 2021; Korazbekova et al., 2013). They are key approaches that contribute to crop yield improvement and maintain environmental integrity for sustainable agriculture.

Plant growth-promoting rhizobacteria (PGPR) play a crucial role in sustainable agricultural systems by enhancing plant growth, nutrient uptake, and stress tolerance through environmentally friendly mechanisms. PGPR improve plant performance by producing phytohormones such as indole-3-acetic acid (IAA), fixing atmospheric nitrogen, solubilizing phosphates, producing siderophores, and suppressing soil-borne pathogens (Singh et al., 2022; Nerek and Sokołowska, 2022). In greenhouse cultivation systems, where intensive management practices and restricted soil volume often exacerbate biotic and abiotic stresses, the application of PGPR has been shown to enhance plant vigor, increase crop yield, and reduce dependency on chemical fertilizers and pesticides (Romeh, 2018; Nordstedt et al., 2020).

Among PGPR, *Pseudomonas* species are considered one of the most effective and well-studied bacterial groups due to their metabolic diversity, adaptability, and strong rhizosphere colonization ability. *Pseudomonas* spp. are widely distributed in soil and plant-associated environments and include numerous agriculturally important species such as *P. fluorescens*, *P. putida*, *P. aeruginosa*, and *P. syringae* (Gross and Loper, 2009; Dewaliya and Jasodani, 2012; Bhatti et al., 2025). These bacteria promote plant growth through IAA production, nutrient mobilization, siderophore synthesis, and the production of antimicrobial compounds and antibiotics, thereby contributing to biocontrol of phytopathogens and improved plant health (Catara, 2007; Dimkić et al., 2021; Sidorova et al., 2023). They enhance plant tolerance to abiotic stress, inducing systemic resistance, and improving crop productivity under reduced fertilizer inputs, highlighting their significant potential for greenhouse tomato production (Akhtar et al., 2010; Shaikh et al., 2020; Nordstedt et al., 2020; Habbadi et al., 2025).

Previous studies have shown that selected *Pseudomonas* isolates can influence tomato seed germination and early plant growth under controlled conditions (Baimirzayeva et al., 2025). Therefore, the present study aimed to further characterize native greenhouse *Pseudomonas* isolates by evaluating their molecular and biochemical characteristics, antioxidant-associated activities, antibiotic susceptibility, and interstrain compatibility associated with plant growth-promoting potential.

Material and Methods

Soil samples

Soil used in this study was collected from greenhouse facility located in the Turkestan region, Kazakhstan. Prior to experimentation, the elemental composition of the soil was determined using a JSM-6490LV scanning electron microscope (JEOL Ltd., Japan). The analysis was conducted at the Regional Testing Laboratory of Engineering Profile “Structural and Biochemical Materials” at M.Auezov South Kazakhstan Research University, Shymkent, Kazakhstan.

Isolation of *Pseudomonas*

Soil samples were processed for bacterial isolation according to ISO 18400-102:2017 with minor modifications (Baimirzayeva et al., 2025). Soil samples were homogenized and suspended in sterile distilled water (1:9, w/v), followed by shaking for 15 min to facilitate the release of microbial cells from soil aggregates. After short sedimentation, serial tenfold dilutions were prepared, and 0.1 mL aliquots were spread onto nutrient agar plates. The plates were incubated at 37 °C for 48 h. Presumptive *Pseudomonas* isolates were obtained on Pseudomonas Agar (fluorescein) according to Lamichhane and Varvaro (2013). Four morphologically distinct colonies were selected and designated as isolate P1-1, N1-2, N2-2, and P4.

In vitro plant growth assay

In vitro experiments were carried out for assessing the growth-promoting influence of *Pseudomonas* isolates

https://doi.org/10.35495/ajab.2026.047

on tomato *Solanum lycopersicum* following Smirnova et al. (2016) with minor modifications (Baimirzayeva et al., 2025). Experiments were performed at the Research and Production Center for Microbiology and Virology (Almaty, Kazakhstan).

Four *Pseudomonas* isolates (P1-1, N1-2, N2-2, and P4) were cultured on meat peptone agar at 30 °C for 48 h. Cell suspensions were prepared at concentrations of 1×10^4, 1×10^6, and 1×10^8 CFU/mL. Tomato seeds (45 per treatment) were immersed in bacterial suspensions for 3 min, and 15 seeds were placed on Kovrovtsev's medium in Petri dishes. All treatments were performed in triplicate and incubated at 30 °C for 15 days.

Growth nutrient medium

Pseudomonas Agar (Titan Biotech, India), Meat Peptone Agar (MPA) (TM Media, India), and Kovrovtsev's medium were used according to standard formulations. All media were autoclaved at 121 °C for 15 min and poured into Petri dishes after cooling (Baimirzayeva et al., 2025).

Identification of *Pseudomonas* isolates

Molecular identification of the *Pseudomonas* isolates was conducted based on 16S rRNA gene sequencing using universal primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 806R (5′-GGACTACCAGGGTATCTAAT-3′) (Edwards et al., 1989) at the Laboratory of Food Microbiology, Research and Production Center for Microbiology and Virology, Almaty, Kazakhstan.

PCR amplification was performed in a Mastercycler proS thermal cycler (Eppendorf) using a 25 µL reaction mixture containing 12.5 µL DreamTaq Hot Start PCR Master Mix (2X, Thermo Fisher), 1 µM of each primer, 1 µg of DNA, and nuclease-free water. Thermal cycling conditions included initial denaturation at 95 °C for 7 min, 30 cycles of 95 °C for 30 s, 55 °C for 40 s, and 72 °C for 1 min, followed by final elongation at 72 °C for 10 min. PCR products were visualized on 1.2% agarose gels stained with ethidium bromide and purified using the ExoSAP-IT kit (Applied Biosystems, USA). Sequencing was performed with the BigDye Terminator v3.1 kit (Applied Biosystems, USA), and products were purified using the BigDye XTerminator kit. Capillary electrophoresis of the 16S rRNA gene fragments was carried out on a 3500 DNA Analyzer (Applied Biosystems, USA).

The obtained sequences were edited and assembled using SeqA software and compared with reference sequences available in the NCBI GenBank database using the BLAST algorithm. Multiple sequence alignment was conducted with ClustalW. Phylogenetic analysis was performed using the Maximum Likelihood method with 100 bootstrap replicates in MEGA11, and the resulting phylogenetic tree was visualized using iTOL (https://itol.embl.de/). And the resulting sequences were deposited in GenBank under accession numbers PZ154326, PZ154327, PZ154328 and PZ154329.

Biochemical tests

Biochemical tests for identified *Pseudomonas* strains (*P. baetica* P1-1, *Pseudomonas* sp. N2-2, *P. germanica* P4) were performed at the Laboratory of Microbiology, Gazi University (Ankara, Turkey) using Digilab Hitachi U-1800 spectrophotometer (Jencons PLS, UK). Antioxidant activity was assessed using DPPH, hydroxyl, and superoxide radical scavenging assays according to Zhou et al. (2020) with minor modifications.

DPPH radical scavenging activity

DPPH radical scavenging activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH). One milliliter of bacterial culture was mixed with 1 mL of DPPH solution. The reaction mixture was incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a spectrophotometer. A mixture of DPPH solution and deionized water served as the control. Antioxidant activity (%) was calculated relative to the control absorbance using the following equation (1):

$$\textbf{Antioxidant activity}\ (\%) = \left[1 - \left(\frac{B_{517}}{C_{517}}\right)\right] \times 100 \quad \textbf{(1)}$$

where B_{517} is the absorbance of the sample and C_{517} is the absorbance of the control (DPPH + deionized water).

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was determined using a reaction system containing Coomassie Brilliant Blue, $FeSO_4$, and H_2O_2. Specifically, 1 mL of bacterial suspension was added to 1 mL Coomassie Brilliant Blue (0.435 mM), 2 mL $FeSO_4$ (0.5 mM), and 1.5 mL H_2O_2 (3%, w/v). The mixture was incubated at

https://doi.org/10.35495/ajab.2026.047

37 °C for 1 h. Absorbance was recorded at 624 nm. A reaction mixture without bacterial culture served as the control, while a blank without the Fenton reaction system was used for baseline correction. Hydroxyl radical scavenging activity (%) was calculated accordingly as (2):

$$\%\ \textbf{Hydroxyl radical scavenging activity} = \left(\frac{A_0 - A_1}{A - A_1}\right) \times 100 \quad \textbf{(2)}$$

where A_0 is the absorbance of the reaction mixture containing the sample, A_1 is the absorbance of the control (Coomassie Brilliant Blue + $FeSO_4$ + H_2O_2 + deionized water, without sample), and A is the absorbance of the blank (deionized water only, without sample and Fenton system).

Superoxide anion scavenging activity

Superoxide anion scavenging activity was measured using pyrogallol (3 mM). One milliliter of bacterial culture was mixed with 0.2 mL pyrogallol solution and incubated at room temperature for 5 min. Absorbance was measured at 325 nm. A reaction mixture containing pyrogallol without bacterial culture served as the control. Superoxide scavenging activity (%) was calculated based on absorbance differences between sample and control as given in equation (3):

$$\%\ \textbf{Superoxide anion scavenging activity} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100 \quad \textbf{(3)}$$

where A_1 is the absorbance of the sample, and A_0 is the absorbance of the control solution (pyrogallol + deionized water, without sample).

Indole Test

Indole production was assessed using tryptophan-supplemented liquid medium incubated at 37 °C for 24 h. After incubation, 0.5 mL of Kovac's reagent was added to the culture and gently mixed. The formation of a red ring at the surface within 1–2 min indicated a positive reaction, while a yellow coloration indicated a negative result (MacWilliams, 2009; Alghanmi et al., 2025).

Exopolysaccharide (EPS) production assay

EPS production was evaluated according to Celik et al. (2007) with modifications. Bacterial cultures were grown in liquid medium and heat-treated by boiling for 15 min. After cooling to room temperature, 1.7 mL of trichloroacetic acid (TCA, 10% w/v) was added to precipitate proteins. The mixture was centrifuged at 25.000 × g for 25 min at room temperature. The resulting supernatant was then mixed with an equal volume (1 mL) of 95% ethanol to precipitate exopolysaccharides and centrifuged again at 25.000 × g for 15 min at room temperature. This precipitation–washing process was repeated twice. The final pellet was dissolved in distilled water, and EPS content was quantified using the phenol–sulfuric acid method. Absorbance was measured at 490 nm. A glucose standard curve was constructed using known glucose concentrations, and absorbance was measured at 490 nm. The calibration curve showed a linear relationship between glucose concentration and absorbance, described by the equation (4):

$$\textbf{y = 0.0045x + 0.1105 } (R^2 = 0.9962) \quad \textbf{(4)}$$

where y represents absorbance at 490 nm and x represents glucose concentration (mg/L). EPS concentrations in bacterial samples were calculated by substituting absorbance values into the regression equation, and results were expressed as glucose equivalents (mg/mL).

Antibiotic susceptibility testing of *Pseudomonas* strains

Antibiotic susceptibility of selected *P. baetica* P1-1, *Pseudomonas sp.* N2-2, and *P. germanica* P4 was determined by disk diffusion method (DDM), measuring the zones of bacterial growth inhibition around antibiotic-impregnated disks on agar plates. Bacterial suspensions were standardized to 0.5 McFarland (~1–2 × 10^8 CFU/mL) (McFarland, 1907) and cultured on standard MPA. Ten antibiotics such as streptomycin, tetracycline, ampicillin, chloramphenicol, gentamicin, kanamycin, ciprofloxacin, erythromycin, cefazolin, and nalidixic acid were tested (Table 5). Antibiotic-impregnated disks were placed on the agar surface at minimum distance of 24 mm between centers. Agar plates were incubated at 35 ± 1°C for 24–48 hours under aerobic conditions. The diameters of inhibition zones were measured in mm after incubation. Results were interpreted according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2024) and the Clinical and Laboratory Standards Institute (CLSI, 2024).

https://doi.org/10.35495/ajab.2026.047

Compatibility testing of *Pseudomonas* strains

Biocompatibility of selected *P. baetica* P1-1, *Pseudomonas* sp. N2-2 and *P. germanica* P4 was evaluated using the drop plate method on solid MPA (Di Salvo et al., 2021). Bacterial cultures were grown until the lag phase stabilized, approximately 4–4.5 hours after inoculation. 6-hour-old cultures were combined in 1:1 ratio to test multi-strain consortia. Overnight cultures were standardized to approximately 1×10^7 CFU/mL for inoculation. The distance between the droplets of the test strains was 1–2 mm from the edge of the first drop to ensure partial overlap. Plates were incubated at 37°C under aerobic conditions. The interaction between strains was recorded after 24 and 48 hours. Overlapping area of the drops was observed. Compatibility was detected by complete merging of the droplets or enhancing growth, mutualism, or synergistic interactions. Antagonistic interactions were indicated by the inhibition of one strain by another. Droplet lateral areas served as controls.

Statistical analysis

All measurements were performed in triplicate, and the results are presented as mean ± standard deviation (SD). Statistical analysis was conducted using IBM SPSS Statistics software. One-way analysis of variance (ANOVA) was applied to determine significant differences among treatment means. Post hoc comparisons were performed using Tukey's Honestly Significant Difference (HSD) test. Differences were considered statistically significant at $p < 0.05$. Significant differences among means are indicated using different letters in the tables.

Results

Pseudomonas isolation

Greenhouse soil composition is given in Table 1. JSM-6490LV SEM analysis revealed oxygen (52.08%) and silicon (20.22%) as the dominant elements. They are followed by calcium (6.41%), aluminum (6.28%) and iron (5.11%), with minor amounts of carbon, magnesium, potassium, sodium, and titanium.

Table-1. Greenhouse Soil characteristics (JSM-6490LV SEM).

Elements	C	O	Na	Mg	Al	Si	K	Ca	Ti	Fe
Weight, %	4.48	52.08	0.76	1.75	6.28	20.22	2.42	6.41	0.48	5.11

From this soil samples were isolated and selected *Pseudomonas* strains based on fluorescein production, which became visible at the fifth dilution, confirming their genus identity (Figure 1). *Pseudomonas* colonies were purified and cultured on selective agar. Microscopic examination (10×100, immersion oil) showed rod-shaped, motile cells without Gram staining, capsule, or endospore formation, consistent with *Pseudomonas* characteristics.

https://doi.org/10.35495/ajab.2026.047

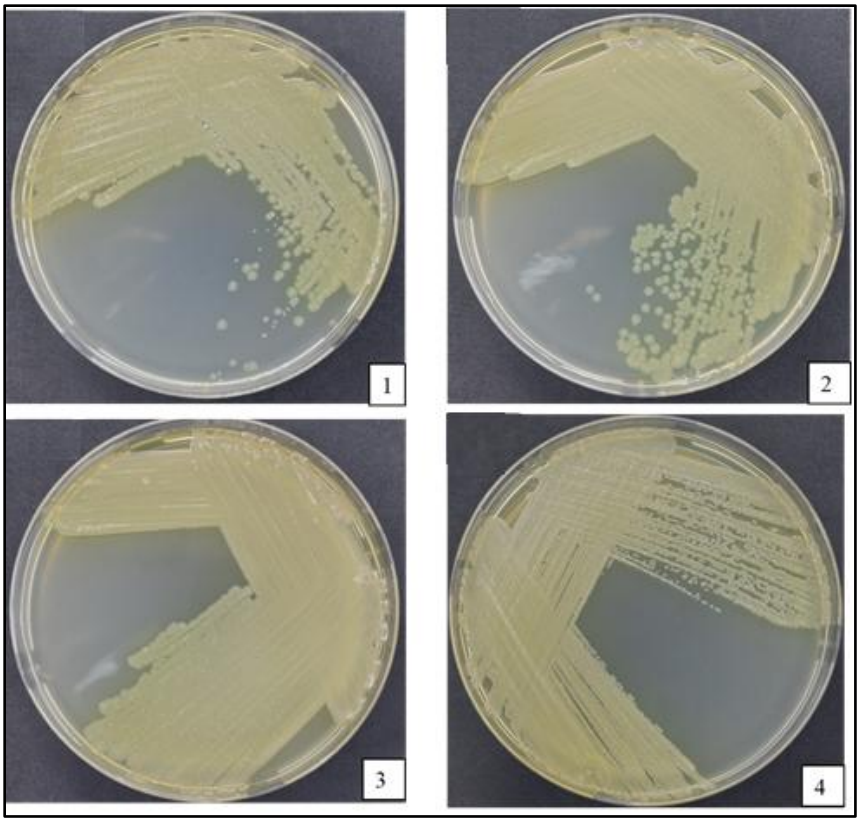

Figure-1. Isolation of *Pseudomonas* strains from soil samples and detection of fluorescein production. Isolates are indicated as follows: 1 – isolate P1-1; 2 – isolate N1-2; 3 – isolate N2-2; 4 – isolate P4.

Growth promotion

Growth effects of different *Pseudomonas* isolates on tomato *Solanum lycopersicum* seed germination, plant survival, and early growth parameters is fully evaluated and reported in detail by Baimirzayeva et al. (2025). Briefly, inoculation with the tested *Pseudomonas* isolates resulted in concentration-dependent effects on tomato germination, survival, and early growth parameters according to Table 2. In the control treatment, germination reached 71% with 72% seedling survival. Treatment with isolate P1-1 increased germination to 84% and 86.6% at 1×10^4 and 1×10^6 CFU/mL, respectively. Although higher concentration in 1×10^8 CFU/mL reduced survival to 61%. Isolate N1-2 showed relatively stable germination in 75.5–84.0% and high survival rates, reaching 92% at 1×10^8 CFU/mL. Isolate N2-2 demonstrated the highest germination (89%) and survival to 93% and 97% at 1×10^6 and 1×10^8 CFU/mL, respectively. In contrast, isolate P4 exhibited variable responses, with reduced germination and survival at 1×10^6 CFU/mL (60% and 52%, respectively), while 1×10^8 CFU/mL resulted in improved values comparable to other treatments. Stem height and root length varied among strains and concentrations. The greatest stem elongation was observed in plants treated with isolate P4, equal to 36.2 mm at 1×10^4 CFU/mL, whereas the longest roots were recorded following inoculation with isolate N1-2 at 1×10^6 CFU/mL, equal to 20.8 mm. Overall, the results indicate strain- and dose-dependent variability in the response of tomato seedlings under the tested conditions.

https://doi.org/10.35495/ajab.2026.047

Table-2. Growth Effect of Pseudomonas isolates on tomato *Solanum lycopersicum* over 15 days.

Isolate	Treatment	Germination		Survival		Growth parameters	
		pieces (out of 45)	%	Number of plants	%	Stem height, *mm*	Root length, *mm*
Control		32	71.0	23	72	32.6±1.6^{b}	13.4±1.0^{c}
P1-1	1x10^{4}	38	84.0	31	82	32.0±1.1^{b}	13.0±0.9^{c}
	1x10^{6}	39	86.6	31	79	33.4±1.4^{b}	11.9±0.8^{c}
	1x10^{8}	33	73.3	20	61	30.6±1.1^{c}	11.1±0.7^{c}
N1-2	1x10^{4}	34	75.5	31	91	32.6±1.5^{b}	14.5±0.8^{c}
	1x10^{6}	35	78.0	28	80	34.4±1.6^{a}	20.8±1.4^{a}
	1x10^{8}	38	84.0	35	92	33.8±1.4^{a}	16.5±1.0^{b}
N2-2	1x10^{4}	31	69.0	28	90	27.8±1.7^{d}	13.9±1.0^{c}
	1x10^{6}	35	78.0	34	97	33.3±1.7^{a}	16.5±1.0^{b}
	1x10^{8}	40	89.0	37	93	31.2±1.7^{b}	17.9±1.2^{b}
P4	1x10^{4}	30	66.6	27	90	36.2±1.6^{a}	12.8±0.9^{c}
	1x10^{6}	27	60.0	14	52	28.7±1.4^{c}	13.5±0.9^{c}
	1x10^{8}	38	84.0	32	84	35.9±1.7^{a}	13.3±0.7^{c}

Notes: Values are expressed as mean ± standard deviation (SD).
Different superscript letters within the same column indicate statistically significant differences among treatments according to one-way ANOVA followed by Tukey's HSD post hoc test at $p < 0.05$.

Comparative analysis of 16S rRNA gene sequences

The obtained 16S rRNA gene sequences of the isolated strains were deposited in the GenBank database under accession numbers PZ154326, PZ154327, PZ154328, and PZ154329. Comparative sequence analysis using the BLAST algorithm demonstrated that all isolates exhibited high similarity to representatives of the genus *Pseudomonas*. The phylogenetic trees constructed from 16S rRNA gene sequences consistently placed the studied isolates within the *Pseudomonas* clade, confirming their taxonomic affiliation (Figures 2–5).

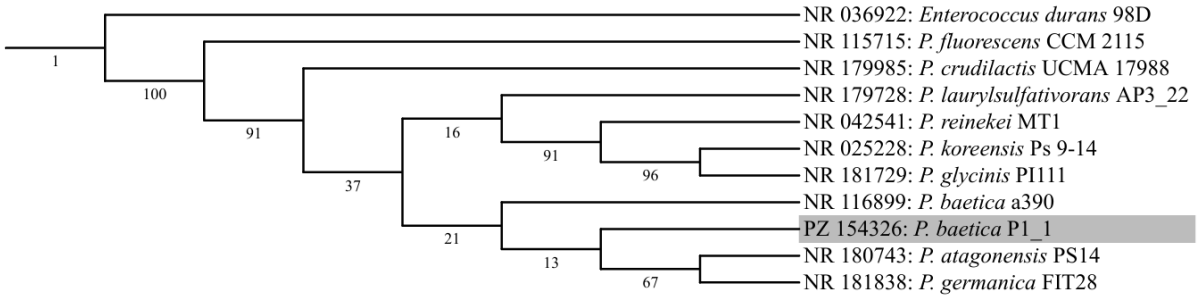

Figure-2. Phylogenetic tree of isolated bacteria (isolate P1-1) *P. baetica* P1-1.

The isolate P1-1 clustered together with *Pseudomonas baetica* strain a390, supporting the high sequence similarity (99.72%) obtained by BLAST analysis. The phylogenetic topology further supported the close relationship between the isolate and *P. baetica*, as both strains clustered within the same branch. Based on these molecular characteristics, the isolate was designated as *Pseudomonas baetica* P1-1 (PZ154326). Phylogenetic analysis of strain N1-2 revealed its affiliation with the genus *Pseudomonas*. The isolate clustered together with *Pseudomonas monteilii* CIP 104883 and *Pseudomonas putida* IAM 1236, indicating close evolutionary relatedness to these taxa. Several neighboring species, including *P. reinekei*, *P. glycinis*, and *P. koreensis*, were also positioned within the same phylogenetic lineage, supporting the placement of strain N1-2 within the *Pseudomonas* group (Figure 3).

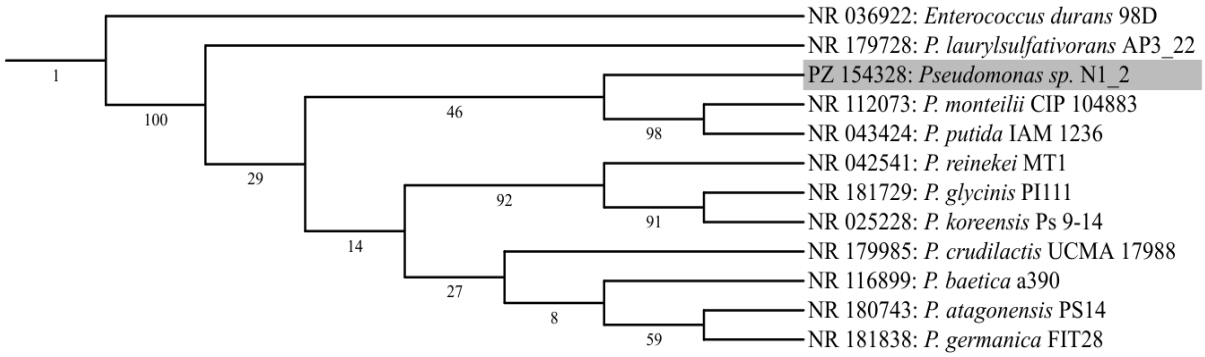

Figure-3. Phylogenetic tree of isolated bacteria (isolate N1-2) *Pseudomonas sp.* N1-2.

Similarly, strain N2_2 formed a distinct cluster with *Pseudomonas glycinis* PI111 and *Pseudomonas koreensis* Ps 9-14. Despite the moderate bootstrap support observed for the nearest branch, the overall phylogenetic structure consistently assigned the isolate to the genus *Pseudomonas*. The clustering pattern also demonstrated its relationship with several ecologically important *Pseudomonas*, including *P. reinekei*, *P. baetica*, and *P. putida*.

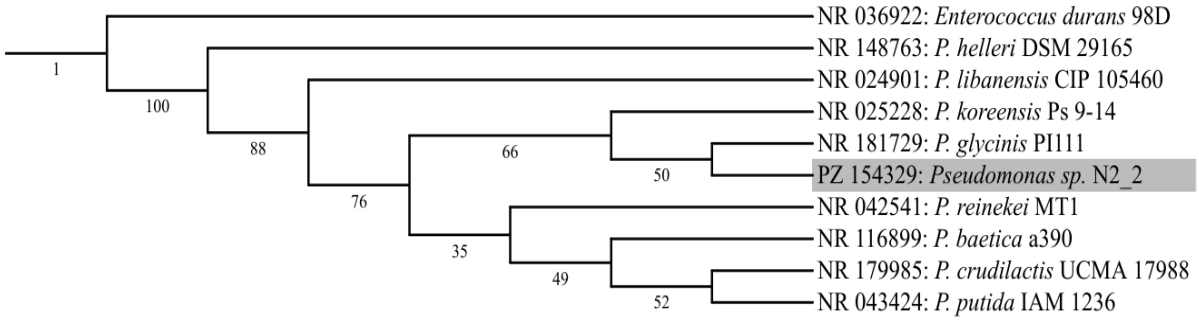

Figure-4. Phylogenetic tree of isolated bacteria (isolate N2-2) *Pseudomonas sp.* N2-2.

Isolate P4 was closely related to P. germanica FIT28 (NR181838) based on 16S rRNA gene analysis, sharing 99.43% sequence identity (Figure 5). In the phylogenetic tree, the isolate grouped together with *P. germanica* and related *Pseudomonas* species, including *P. crudilactis*, *P. laurylsulfatovorans*, *P. putida*, and *P. reinekei*. Although some internal nodes displayed relatively low bootstrap values, the overall tree topology clearly supported the affiliation of isolate P4 with the genus *Pseudomonas* (Figure 5).

The phylogenetic analysis based on 16S rRNA gene sequences demonstrated that isolate P4 belongs to the genus *Pseudomonas*. The strain clustered closely with *Pseudomonas germanica* FIT28 (NR181838), sharing 99.43% sequence identity, which indicates a high level of evolutionary relatedness (Figure 5). In addition, isolate P4 grouped within the same phylogenetic lineage as *P. crudilactis*, *P. laurylsulfatovorans*, *P. putida*, and *P. reinekei*, confirming its affiliation with the *Pseudomonas* clade. Although several internal branches showed relatively low bootstrap support, the overall tree topology consistently positioned isolate P4 among closely related *Pseudomonas* species.

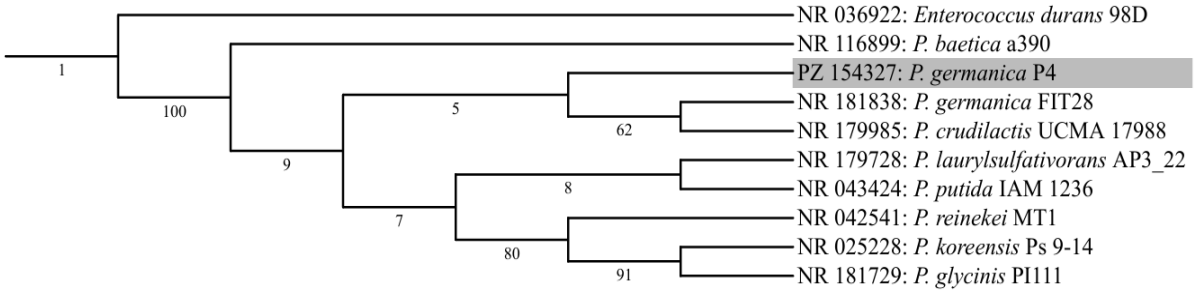

Figure-5. Phylogenetic tree of isolated bacteria (isolate P4) *P. germanica* P4.

Biochemical properties

All *Pseudomonas* strains exhibited antioxidant capacity, radical scavenging activity, and exopolysaccharide (EPS) production, as summarized in Table 3. Significant differences were observed among the tested strains. Among the isolates, *P. baetica* P1-1 demonstrated the highest overall antioxidant potential, showing significantly higher DPPH radical scavenging activity (24.00 ± 0.01%), which was statistically similar to *Pseudomonas sp.* N2-2 (21.00 ± 0.01%), but significantly higher than *P. germanica* P4 (8.00 ± 0.01%). In addition, *P. baetica* P1-1 exhibited strong superoxide anion scavenging activity (61.00 ± 0.01%), which did not differ significantly from *Pseudomonas sp.* N2-2 (55.00 ± 0.01%), but was significantly higher than *P. germanica* P4 (11.00 ± 0.02%).

In contrast, hydroxyl radical scavenging activity varied significantly among strains, with *P. germanica* P4 showing the highest activity (15.00 ± 0.0133%), followed by *Pseudomonas sp.* N2-2 (9.00 ± 0.0128%) and *P. baetica* P1-1 (4.00 ± 0.011%), all of which differed significantly from each other ($p < 0.05$).

Regarding EPS production, *P. baetica* P1-1 produced the highest yield (32.33 ± 0.005 mg/L), followed by *P. germanica* P4 (18.55 ± 0.001 mg/L), while *Pseudomonas sp.* N2-2 exhibited the lowest production (9.44 ± 0.007 mg/L), with all differences being statistically significant according to Tukey's HSD test ($p < 0.05$).

Table-3. Antioxidant and EPS activity of *Pseudomonas* strains.

Bacteria strains	***P. baetica* P1-1**	***Pseudomonas sp.*N2-2**	***P.germanica* P4**
DPPH, %	24.00±0.010[a]	21.00±0.010[a]	8.00±0.0100[b]
Hydroxyl radical scavenging, %	4.00±0.011[c]	9.00±0.0128[b]	15.00±0.0133[a]
Superoxide anion scavenging, %	61.00±0.010[a]	55.00±0.010[a]	11.00±0.0200[b]
EPS production, mg/L	32.33±0.005[a]	9.44±0.007[c]	18.55±0.0010[b]

Notes: Values are expressed as mean ± standard deviation (SD).
Different superscript letters within the same column indicate statistically significant differences according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).

Notably, this strain produced the lowest amount of EPS, reaching 9.44±0.007 mg/L. In comparison, *P. germanica* P4 exhibited the weakest antioxidant potential (8±0.01%) and the lowest superoxide anion scavenging activity, equal to 11±0.02%. However, this strain showed the strongest hydroxyl radical scavenging capacity among all isolates (15±0.0133%). EPS synthesis by *P. germanica* P4 was intermediate, amounting to 18.55±0.0010 mg/L. All identified *Pseudomonas* strains yielded positive results in the indole test, resulting in rapid formation of a clear red ring at the culture surface within 1–2 minutes after addition of Kovac's reagent. Absence of yellow coloration in all samples further confirmed positive reactions.

Antibiotic susceptibility

All tested *Pseudomonas* strains exhibited differential sensitivity to the antibiotics evaluated (Table 4). Significant differences were observed among strains depending on the antibiotic type ($p < 0.05$). *P. baetica* P1-1 showed reduced susceptibility compared to the

other strains, exhibiting resistance to cefazolin and intermediate sensitivity to ampicillin and erythromycin. In contrast, *Pseudomonas* sp. N2-2 and *P. germanica* P4 were generally more susceptible, showing high inhibition zones across most tested antibiotics.

Gentamicin, kanamycin, ciprofloxacin, and nalidixic acid demonstrated the strongest inhibitory effects against all strains, indicating broad-spectrum activity. The observed resistance of *P. baetica* P1-1 to cefazolin may be associated with the presence of β-lactamase-mediated resistance mechanisms, which are commonly reported in environmental *Pseudomonas* isolates.

Table-4. Antibiotic susceptibility of *Pseudomonas* strains.

Antibiotic	***P. baetica* P1-1**		***Pseudomonas sp.* N2-2**		***P. germanica* P4**	
	Zone (mm)	Sensitivity	Zone (mm)	Sensitivity	Zone (mm)	Sensitivity
Streptomycin (10 µg)	20.0±0.1^{c}	S	34.0±1.0^{b}	S	45.5±2.5^{a}	S
Tetracycline (30 µg)	25.0±0.2^{b}	S	22.5±2.5^{c}	S	30.0±5.0^{a}	S
Ampicillin (10 µg)	15.0±0.0^{c}	I	17.5±2.5^{b}	S	37.5±2.5^{a}	S
Chloramphenicol (30 µg)	25.0±5.0^{c}	S	40.0±0.1^{b}	S	52.5±2.5^{a}	S
Gentamicin (10 µg)	27.0±0.1^{c}	S	45.0±0.1^{b}	S	50.0±0.0^{a}	S
Kanamycin (30 µg)	20.0±0.1^{c}	S	50.0±0.0^{a}	S	39.0±4.0^{b}	S
Ciprofloxacin (5 µg)	30.0±0.0^{c}	S	50.0±0.0^{a}	S	47.5±2.5^{b}	S
Erythromycin (15 µg)	20.0±0.1^{c}	I	34.0±1.0^{a}	S	27.5±2.5^{b}	S
Cefazolin (30 µg)	15.0±0.1^{c}	R	25.0±0.0^{b}	S	35.0±0.0^{a}	S
Nalidixic acid (10 µg)	20.0±0.1^{c}	S	27.5±2.5^{b}	S	45.0±5.0^{a}	S

Notes: Values are expressed as mean ± standard deviation (SD).
Antibiotic susceptibility was interpreted according to CLSI guidelines (S = sensitive, I = intermediate, R = resistant).
Differences among means for each antibiotic were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test at $p < 0.05$.
Different superscript letters within the same row indicate statistically significant differences among bacterial strains.

Biocompatibility of *Pseudomonas* strains

The results of the biocompatibility assessment demonstrated that the selected *Pseudomonas* strains can be successfully co-cultivated in paired and mixed combinations, indicating their suitability for the development of multicomponent bacterial consortia. Comparative analysis of growth characteristics showed that the lag phase of the tested strains was highly synchronized, beginning approximately 4–4.5 hours after inoculation. Based on this observation, mixed cultures were prepared using 6-hour-old cultures combined at a 1:1 ratio. All three investigated strains, *P. baetica* P1-1, *P. germanica* P4 and *Pseudomonas* sp. N 2-2, exhibited complete biocompatibility, as evidenced by enhanced growth responses and the frequent merging of growth spots in the zone of co-cultivation (Table 5). No signs of growth inhibition or antagonistic interactions were observed. As illustrated in Table 5, *Pseudomonas* strains belonging to different species positively influenced each other and stimulated growth during co-cultivation. The nature of interstrain interactions among the tested isolates was therefore classified as compatible, with no detectable antagonism between individual strains.

Table-5. Biocompatibility of selected *Pseudomonas* strains.

Treatment	Name of culture	Results	Biocompatibility
1+1	*P. baetica* P1-1 *P. baetica* P1-1	Merging	+
2+2	*Pseudomonas sp.* N2-2 *Pseudomonas sp.* N2-2	Merging	+
3+3	*P. germanica* P4 *P. germanica* P4	Merging	+
1+2	*P. baetica* P1-1 *Pseudomonas sp.* N2-2	Merging	+
1+3	*P. baetica* P1-1 *P. germanica* P4	Merging	+
2+3	*Pseudomonas sp.* N2-2 *P. germanica* P4	Merging	+

Notes: (+) – biocompatibility (complete fusion of droplets during growth).
(–) – antagonism (overlap of one culture with another or growth suppression).
1 – *P. baetica P*1-1; **2** – *Pseudomonas sp.* N2-2; **3** - *P. germanica P*4.

Discussion

Pseudomonas spp. were isolated from greenhouse soil characterized by elevated concentrations of key mineral elements, including Si, Al, Ca, Fe, and K. Such a mineral composition indicates a relatively stable nutrient matrix, which may be attributed to greenhouse management practices that reduce nutrient leaching and limit soil weathering processes. The elemental profile of the experimental soil (Table 1), comprising naturally occurring Si, Ca, Al, Fe, K, and Mg, corresponds well with the typical mineral composition of agricultural soils described by Aghnatios et al. (2014). All isolates from this analyzed greenhouse soil samples, later classified as *P. baetica* P1-1*, P. germanica* P4*,* and *Pseudomonas sp. N1-2* and N2-2, demonstrate fluorescein production, providing initial confirmation of their affiliation with the genus *Pseudomonas*. Fluorescein production has been widely reported as a reliable phenotypic marker for preliminary identification of *Pseudomonas* species (Imtiaz and Saleem, 2025; Meliani et al., 2017; Pal et al., 2016). Fluorescent pigment production has been most frequently associated with species such as *P. putida, P. fluorescens, P. aeruginos* (Imtiaz and Saleem, 2025; Meliani et al., 2017). Microscopic examination revealed consistent morphological features across all isolates. Bacterial cells were Gram-negative, motile, and rod-shaped, which corresponds well with the characteristic traits of *Pseudomonas spp.* described by Urgancı et al. (2022).

Pseudomonas inoculation can positively influence plant growth parameters in range between 10^4 and 10^8 CFU/mL (Baimirzayeva et al., 2025). Variability in germination, survival, and seedling growth metrics may be associated with differences in metabolic traits, including antioxidant capacity, indole production, and exopolysaccharide synthesis, identified in this study. For example, *P. putida* FA-56 was reported to produce 23.02 μg mL^{-1} IAA, leading to significant increases in plant height, stem thickness, and fruit yield as mentioned in Hernández-Montiel et al. (2017). *Pseudomonas spp.* has been shown to support reduced fertilizer input without compromising productivity. Yield of *Solanum lycopersicum* increased for 54% and 73% in inoculation with *P. putida* ACJ14 and *Pseudomonas sp.* C14, respectively, even under conditions of 50% reduced fertilization, accompanied by elevated lycopene levels exceeding 130 mg/kg (Torres-Solórzano et al., 2025). Moreover, inoculation with *Pseudomonas* strains has been associated with improvements in fruit nutritional quality, including increased concentrations of tannins, lycopene, and carotenoids, as well as enhanced antioxidant activity (Issifu et al., 2023). Collectively, these findings reinforce the role of *Pseudomonas* spp. as environmentally friendly alternatives to chemical fertilizers in sustainable greenhouse tomato production.

In this study, the affiliation of the isolated strains with plant growth-promoting rhizobacteria is consistent with our findings and supported by previous reports on

the growth-enhancing potential of *P.baetica* in different plant systems. *P. baetica* JS-16 significantly increased biomass and key growth parameters in tea, tobacco, and chili pepper under greenhouse conditions according to Zhang et al. (2024). *P. baetica* P1-1 identified in present work exhibited plant growth-promoting traits, including the highest superoxide anion scavenging activity equal to 61.00±0.010%, EPS production in average 32.33±0.005 mg/L, and positive indole formation, that are known to contribute to improved plant performance. Neutralization of reactive oxygen species (ROS), particularly superoxide radicals, is a key mechanism by which PGPB alleviate oxidative stress in plants, especially under abiotic stress conditions such as drought, salinity, and temperature extremes (Backer et al. 2018; Vurukonda et al. 2016). Antioxidant activity in rhizobacteria has been linked to improved plant antioxidant defense systems, leading to better growth and stress tolerance (Kumar et al., 2020). In addition, the reported ability of *P. baetica* to stimulate plant growth under heavy metal stress by González et al. (2021) supports the robustness of this species and aligns with the functional characteristics observed in *P. baetica* P1-1. Therefore, the strong antioxidant capacity observed in *P. baetica* P1-1 determines a significant contribution to plant stress mitigation and growth promotion.

Plant growth promotion by *P. germanica* remains limited. Isolated *P. germanica* P4 displays several biochemical properties associated with beneficial rhizobacteria, including positive indole production and hydroxyl radical scavenging activity equal to 15.00±0.0133% together with moderate EPS production on average 18.55±0.0010 mg/L. Hydroxyl radicals are considered among the most reactive and damaging ROS, and their targeted neutralization results in protecting plant root tissues from oxidative injury according to Mhamdi and Van Breusegem (2018). The moderate EPS production observed in *P. germanica* P4 may further enhance plant growth metrics by promoting biofilm formation, improving soil aggregation, and providing physical protection to both bacterial cells and plant roots under environmental stress conditions (Naseem and Bano, 2014). This observation is consistent with previous findings that *P. germanica*, which is present in a rhizobacterial consortium (Dobrzyński et al., 2025), and isolated from *Iris germanica* roots (Atanasov et al., 2022), improves plant growth and stress tolerance, especially when acting as part of a broader microbial community.

Pseudomonas spp. are among the most investigated PGPR, due to their high metabolic flexibility, strong rhizosphere colonization ability, and capacity to establish multiple beneficial interactions with host plants (Parray et al., 2016). In the present study, *Pseudomonas sp.* N2-2 possesses key functional attributes associated with plant growth promotion and rhizosphere competence. They demonstrate moderate biochemical activities, including superoxide anion seavenging in the range 55.00±0.010%, DPPH activity equal to 21.00±0.010%, and positive indole formation, which are known to influence root development and plant vigor. Although EPS production by *Pseudomonas sp.* N2-2 was relatively low on average 9.44±0.007 mg/L, the presence of stable radical scavenging activity suggests a complementary plant growth-promoting strategy focused on mitigating oxidative stress in the rhizosphere rather than extensive biofilm formation. EPS synthesis has been shown to support bacterial adhesion to root surfaces and ensure persistence in the rhizosphere. These abilities lead to facilitating long-term plant–microbe interactions (Costa et al., 2018; Naseem et al., 2018). Parray et al. (2016) report that such characteristics are typical of efficient root-colonizing bacteria that contributes to nutrient mobilization and hormone-mediated stimulation of plant growth.

These findings are in agreement with previous reports demonstrating that *Pseudomonas spp.* promote plant growth through multiple direct and indirect mechanisms, including phosphate solubilization, siderophore production, and phytohormone synthesis, particularly IAA (Backer et al., 2018; Bhatti et al., 2025; Habbadi et al., 2025; Singh et al., 2022), suppression of phytopathogens, and enhancement of plant stress tolerance (Alghanmi et al., 2025; Pandey and Gupta, 2020). All tested *Pseudomonas* species exhibited a positive indole reaction. It shows their ability to metabolize tryptophan and produce indole or indole-derived compounds. This trait is closely linked to the biosynthesis of indole-3-acetic acid (IAA), a key phytohormone regulating root development and plant growth (Nerek and Sokołowska, 2022; Sadarahalli et al., 2022; Singh et al., 2022). The universal presence of indole production among the identified *P. baetica* P1-2, *P. germanica* P4 and *Pseudomonas sp.* N2-2 indicates that auxin-mediated growth stimulation represents a shared mechanism contributing to the observed enhancement of tomato growth parameters.

All tested *Pseudomonas* strains demonstrated high sensitivity to aminoglycosides and fluoroquinolones, which is consistent with susceptibility patterns previously reported for environmental *Pseudomonas* isolates. For example, Meng et al. (2020) reported susceptibility of environmental *Pseudomonas* spp. to gentamicin and ciprofloxacin.

The resistance of *P. baetica* P1-1 to cefazolin may be associated with intrinsic or acquired β-lactamase-mediated resistance mechanisms, which are commonly documented in *Pseudomonas* species, including resistance to cephalosporins and other β-lactam antibiotics. Nevertheless, the overall susceptibility profile observed in *P. baetica* P1-1, *P. germanica* P4, and *Pseudomonas* sp. N2-2 suggests a relatively low level of antibiotic resistance compared with multidrug-resistant environmental and clinical *Pseudomonas* strains described by Odumosu et al. (2016).

Assessment of antibiotic susceptibility is important in PGPR characterization because it contributes to biosafety evaluation and helps estimate the potential risk of dissemination of antibiotic resistance determinants in agricultural environments. Therefore, the overall susceptibility patterns of the tested strains support their potential suitability for agricultural and PGPR applications, in agreement with observations reported by Dimkić et al. (2021).

According to biocompatibility tests, selected *Pseudomonas* strains can coexist and function cooperatively within the rhizosphere, enhancing their persistence and functional stability. Multi-strain PGPR consortia often outperform single isolates by providing complementary mechanisms of action, including improved nutrient mobilization, phytohormone production, and disease suppression (Naqvi et al., 2026; Trivedi et al., 2017). The positive interstrain interactions in the present study suggest synergistic effects that may result in more consistent plant growth promotion under greenhouse conditions (Naqvi et al., 2026). Together with reported growth-promoting, biochemical and biocontrol traits, the antibiotic sensitivity profile reinforces the suitability of the studied *P. baetica* P1-1, *P. germanica* P4 and *Pseudomonas sp*. N2-2 for sustainable agriculture.

Conclusion

In conclusion, Pseudomonas isolates obtained from greenhouse soil, assigned as *P. baetica* P1-1, *P. germanica* P4, and two *Pseudomonas* sp. N1-2 and N2-2, demonstrated characteristics commonly associated with plant growth-promoting bacteria, including antioxidant activity, indole production, EPS synthesis, and antibiotic susceptibility profiles. In vitro seed inoculation experiments showed isolate- and concentration-dependent effects on tomato (*Solanum lycopersicum*) germination and early growth parameters, such as stem height and root length. Among the tested isolates, *P. baetica* P1-1 exhibited comparatively stronger effects under the experimental conditions. Overall, the findings support the potential of selected *Pseudomonas* isolates as candidates for further investigation in sustainable tomato production systems.

Acknowledgement

The authors sincerely acknowledge the Research and Production Center for Microbiology and Virology (Almaty, Kazakhstan) and the Laboratory of Microbiology, Gazi University (Ankara, Turkey) for providing laboratory facilities and technical support essential for this study.

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: None.

Contribution of Authors

Baimirzayeva Z: Contributed to conceptualization, developed the methodology, performed experimental work, collected data, conducted formal analysis, and prepared the original draft of the manuscript.

Korazbekova K: Conceptualized the study, provided supervision, performed validation, interpreted data, and contributed to writing – review and editing.

Aitbayev A: Supported methodology, collected data, performed statistical analysis, prepared visualizations, and contributed to manuscript editing.

All authors read, revised and approved the final draft of the manuscript.

References

Aghnatios C, Losno R and Dulac F, 2014. A fine fraction of soil used as an aerosol analogue during the DUNE experiment: sequential solubility in water, decreasing pH step-by-step. Biogeosciences. 11: 4627–4633. https://doi.org/10.5194/bg-11-4627-2014

Akhtar MS, Shakeel U and Siddiqui ZA, 2010. Biocontrol of Fusarium Wilt by *Bacillus Pumilus*, *Pseudomonas Alcaligenes* and *Rhizobium Sp.* on Lentil. Turk. J. Biol. 34(1): 1-7. https://doi.org/10.3906/biy-0809-12

Alghanmi L, Elhady A, Parween S and Hirt H, 2025. Plant growth promotion and pathogen protection by the desert endophyte *Pseudomonas grandensis* R4-79. BMC Microbiology. 25(1): 690. https://doi.org/10.1186/s12866-025-04431-4

Ansari M, Shaheen T, Bukhari SA and Husnain T, 2015. Genetic improvement of rice for biotic and abiotic stress tolerance. Turk. J. Bot. 39: 911–919. https://doi.org/10.3906/bot-1503-47

Atanasov KE, Galbis DM, Gallego J, Serpico A, Bosch M, Altabella T and Ferrer A, 2022. *Pseudomonas germanica* sp. nov., isolated from *Iris germanica* rhizomes. Int. J. Syst. Evol. Microbiol. 72(3): 005268. https://doi.org/10.1099/ijsem.0.005268

Bakhov ZK, Sultangalieva KU, Zhumadilova NB, Drevin VE and Gizzatova GL, 2021. Solution of environmental problems of livestock breeding through intensification of waste treatment processes, 012008. In Proceedings, International Conference on Engineering Studies and Cooperation in Global Agricultural Production, 27-28 August 2020, IOP Conference Series Earth and Environmental Science, 659(1), Rostov Region, Russian Federation. https://doi.org/10.1088/1755-1315/659/1/012008

Baimirzayeva Z, Korazbekova K, Yüksekdağ Z and Akhanov U, 2025. Growth effect of *Pseudomonas* isolates on tomato in greenhouse production. Pak. J. Biol. Sci. 28(4): 236–245. https://doi.org/10.3923/pjbs.2025.236.245

Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Smith DL and Smith J, 2018. Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front. Plant Sci. 9: 1473. https://doi.org/10.3389/fpls.2018.01473

Bhatti FA, Naveed M, Asghar HN, Basra SMA and Ishaque W, 2025. Insights into cross-host colonization and nitrogen fixation in wheat by *Populus euphratica*-associated endophytic diazotrophs. Asian J. Agric. Biol. e2025230. https://doi.org/10.35495/ajab.2025.230

Catara V, 2007. *Pseudomonas corrugata*: plant pathogen and/or biological resource? Mol. Plant Pathol. 8(3): 233-244. DOI:10.1111/j.1364-3703.2007.00391.x

Celik GY, Aslim B and Beyatli Y, 2007. Characterization and production of the exopolysaccharide (EPS) from *Pseudomonas aeruginosa* G1 and *Pseudomonas putida* G12 strains. Carbohydr. Polym.73(1): 178–182. https://doi.org/10.1016/j.carbpol.2007.11.021

CLSI (Clinical and Laboratory Standards Institute), 2024. Performance Standards for Antimicrobial Susceptibility Testing. CLSI, Wayne, PA.

Costa OYA, Raaijmakers JM and Kuramae EE, 2018. Microbial extracellular polymeric substances: ecological function and impact on soil aggregation. Front. Microbiol. 9: 1636. https://doi.org/10.3389/fmicb.2018.01636

Dashti N, Montasser MS, Ali N and Cherian VM, 2014. Influence of plant growth promoting rhizobacteria on fruit yield, pomological characteristics and chemical contents in cucumber mosaic virus-infected tomato plants. Kuwait J. Sci. 41(2): 205-220

Dewaliya V and Jasodani R, 2012. Isolation and Identification of soil isolates of *Pseudomonas* species via FAME analysis. Int. J. Sci. Res. 2: 557-560

Dimkić I, Janakiev T, Petrović M, Degrassi G and Fira D, 2021. Plant-associated *Bacillus* and *Pseudomonas* antimicrobial activities in plant disease suppression via biological control mechanisms - A review. Physiol. Mol. Plant Pathol. 117: 101754. https://doi.org/10.1016/j.pmpp.2021.101754

Di Salvo LP, García JE, Puente ML, Amigo J, Anríquez A, Barlocco C, Benintende S, Bochatay T, Bortolato M, Cassán F, Castaño C, Catafesta M, Coniglio A, Díaz M, Galián LR, Gallace E, García P, De Salamone IEG, Landa M and Vallejo D, 2021. The drop plate

method as an alternative for *Azospirillum spp.* viable cell enumeration within the consensus protocol of the REDCAI network. Rev. Argent. Microbiol. 54(2): 152–157. https://doi.org/10.1016/j.ram.2021.05.002

Dobrzyński J, Kulkova I, Jakubowska Z and Wróbel B, 2025. Non-native PGPB consortium consisting of *Pseudomonas sp.* G31 and *Azotobacter sp.* PBC2 promoted winter wheat growth and slightly altered the native bacterial community. Sci. Rep. 15(1): 3248. https://doi.org/10.1038/s41598-025-86820-3

EUCAST (European Committee on Antimicrobial Susceptibility Testing), 2024. Breakpoint tables for interpretation of MICs and zone diameters. EUCAST, Växjöб Sweden.

Edwards U, Rogall T, Blocker H, Emde M and Bottger EC, 1989. Isolation and direct complete nucleotide determination of entire genes: characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res. 17(19): 7843–7853. https://doi.org/10.1093/nar/17.19.7843

Habbadi K, Idrissi MGE, Houssaini SEIE, Maafa I, Aoujil F, Benbouazza A, Achbani EH and Ferrahi M, 2025. Assessing the impact of PGPR and water retention agents on bread wheat growth: an experimental study. Asian J. Agric. Biol. 2025(1): 2024072. https://doi.org/10.35495/ajab.2024.072

Hernández-Montiel LG, Contreras CJC, Amador BM, Hernández LV, Aguilar EEQ and Contreras RGC, 2017. Efficiency of two inoculation methods of *Pseudomonas putida* on growth and yield of tomato plants. J. Soil Sci. Plant Nutr. 17(4): 1003–1012. https://doi.org/10.4067/s0718-95162017000400012

Imtiaz S and Saleem M, 2025. Fluorescence Spectroscopy Based Identification of *Pseudomonas Aeruginosa* and *Escherichia Coli* Suspensions. J. Fluoresc. 35: 1381–1391. https://doi.org/10.1007/s10895-024-03608-w

Issifu M, Naitchede LH, Ateka EM, Onguso JM and Ngumi VW, 2023. Synergistic effects of substrate inoculation with *Pseudomonas* strains on tomato phenology, yield, and selected human health-related phytochemical compounds. Agrosyst. Geosci. Environ. 6: e20435. https://doi.org/10.1002/agg2.20435

González D, Blanco C, Probanza A, Jiménez PA and Robas M, 2021. Evaluation of the PGPR Capacity of Four Bacterial Strains and Their Mixtures, Tested on *Lupinus albus* var. Dorado Seedlings, for the Bioremediation of Mercury-Polluted Soils. Processes. 9(8): 1293. https://doi.org/10.3390/pr9081293

Gross H and Loper JE, 2009. Genomics of secondary metabolite production by Pseudomonas spp. Nat. Prod. Rep. 26(11): 1408-1446. DOI:10.1039/b817075b

Kumar A, Singh S, Gaurav AK, Srivastava S and Verma JP, 2020. Plant growth-promoting bacteria: biological tools for the mitigation of salinity stress in plants. Front. Microbiol. 11: 1216.https://doi.org/10.3389/fmicb.2020.01216

Korazbekova KU, Bakhov ZhK and Saparbekova AA, 2013. Dry Fermentation of Agricultural Waste in the Modified Leach-bed Reactor with Immobilization of Microorganisms. Biotechnology. 12: 236-244. https://doi.org/10.3923/biotech.2013.236.244

Lamichhane JR and Varvaro L, 2013. A new medium for the detection of fluorescent pigment production by *Pseudomonads*. Plant Pathol. 62: 624-632. https://doi.org/10.1111/j.1365-3059.2012.02670.x

MacWilliams MP, 2009. Indole Test Protocol (pp. 1-9). American Society for Microbiology. https://asm.org/protocols/indole-test-protocol

Meliani A, Bensoltane A, Benidire L and Oufdou K, 2017. Plant Growth-Promotion and IAA Secretion with *Pseudomonas fluorescens* and *Pseudomonas putida*. RRJBS. 6(2): 16-24.

Meng L, Liu H, Lan T, Dong L, Hu H, Zhao S, Zhang Y, Zheng N and Wang J, 2020. Antibiotic Resistance Patterns of *Pseudomonas spp.* Isolated from Raw Milk Revealed by Whole Genome Sequencing. Front. Microbiol. 11: 1005. https://doi.org/10.3389/fmicb.2020.01005

McFarland J, 1907. Nephelometric standards for estimating the number of bacteria in suspensions. JAMA. 49, 1176–1178

Mhamdi A and Van Breusegem F, 2018. Reactive oxygen species in plant

development. Development. 145(15): dev16 4376. https://doi.org/10.1242/dev.164376

Naseem H and Bano A, 2014. Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance of maize. J. Plant Interact. 9: 689–701. https://doi.org/10.1080/17429145.2014.902125

Naseem H, Ahsan M, Shahid MA and Khan N, 2018. Exopolysaccharides producing rhizobacteria and their role in plant growth and drought tolerance. J. Basic Microbiol. 58(12): 1009–1022. https://doi.org/10.1002/jobm.201800309

Naqvi SAH, Umar UUD and Rehman AU, 2026. Host-specific multi-strain PGPR consortium delivers superior bacterial leaf blight control and yield enhancement in rice through integrated seed priming and foliar applications. Eur. J. Plant Pathol. 174(5). https://doi.org/10.1007/s10658-026-03212-7

Nerek E and Sokołowska B, 2022. *Pseudomonas spp.* in biological plant protection and growth promotion. AIMS Environ. Sci. 9(4): 493-504. https://doi.org/10.3934/environsci.2022029

Nordstedt NP, Chapin LJ, Taylor CG and Jones ML, 2020. Identification of Pseudomonas Spp. That Increase Ornamental Crop Quality During Abiotic Stress. Front. Plant Sci. 10: 1754. https://doi.org/10.3389/fpls.2019.01754

Odumosu BT, Ajetunmobi O, Dada-Adegbola H and Odutayo I, 2016. Antibiotic susceptibility pattern and analysis of plasmid profiles of *Pseudomonas aeruginosa* from human, animal and plant sources. SpringerPlus. 5: 1381. https://doi.org/10.1186/s40064-016-3073-9

Pal D, Kotesthane A and Dey U, 2016. Screening for Plant Growth Promoting Activity (PGPA) of fluorescent *Pseudomonas spp*. Int. J. Pure Appl. Biosci. 4(2): 156–162. https://doi.org/10.18782/2320-7051.2270

Pandey S and Gupta S, 2020. Evaluation of *Pseudomonas sp.* for its multifarious plant growth promoting potential and its ability to alleviate biotic and abiotic stress in tomato (*Solanum lycopersicum*) plants. Sci. Rep. 10: 20951. https://doi.org/10.1038/s41598-020-77850-0

Parray JA, Jan S, Kamili AN, Qadri RA, Egamberdieva D and Ahmad P, 2016. Current Perspectives on Plant Growth-Promoting Rhizobacteria. J. Plant Growth Regul. 35(3): 877–902. https://doi.org/10.1007/s00344-016-9583-4

Romeh AA, 2018. Integrated Pest Management for Sustainable Agriculture, pp. 215-234. In A. Negm and M. Abu-hashim (eds), Sustainability of Agricultural Environment in Egypt: Part II. The Handbook of Environmental Chemistry, vol. 77. Springer, Cham. https://doi.org/10.1007/698_2018_267

Sadarahalli UP, Manjunatha GN, Kuttappa TC, Sheshagiri A and Kumbar M, 2022. Application of *Pseudomonas* Strains for Biocontrol of Commercial Crops Susceptible to Plant Pathogens: A Review. Agric. Rev. 45(4): 600-608. https://doi.org/10.18805/ag.R-2451

Shaikh S, Yadav N and Markande AR, 2020. Interactive potential of *Pseudomonas* species with plants. JABB. 8(6): 101-111. https://doi.org/10.7324/JABB.2020.80616

Sidorova TM, Allahverdyan VV and Asaturova AM, 2023. Role of *Pseudomonas* Bacteria and Their Metabolites in the Biocontrol of Phytopathogenic Microorganisms. Agrochemistry. 5: 83-93. https://doi.org/10.31857/S0002188123050071

Singh P, Singh RK, Zhou Y, Wang J, Jiang Y, Shen N, Wang Y, Yang L and Jiang M, 2022. Unlocking the strength of plant growth promoting Pseudomonas in improving crop productivity in normal and challenging environments: a review. J. Plant Interact.17(1): 220-238. https://doi.org/10.1080/17429145.2022.2029963

Smirnova IE, Sadanov AK and Galimbaeva RSh, 2016. Biological method for improving germinating and productivity of melilot, pp: 21-28. In Proceedings, Recent trends in PGPR research for sustainable crop productivity. 4th Asian PGPR Conference Proceedings, 3-6 May 2015, Asian PGPR Society, Hanoi, Vietnam.

Vurukonda SSKP, Vardharajula S, Shrivastava M and SkZA, 2016. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiol. Res. 184: 13–24. https://doi.org/10.1016/j.micres.2015.12.003

Torres-Solórzano P, La Cruz HR, Altamirano-Hernández J, Macías-Rodríguez L, Campos-García J and Luna-Cruz A, 2025. Inoculation with *Pseudomonas spp.* in *Solanum lycopersicum* increases yield and fruit quality under nutrient shortage conditions. PeerJ. 13: e19796. https://doi.org/10.7717/peerj.19796

Trivedi P, Schenk PM, Wallenstein MD and Singh BK, 2017. Tiny Microbes, Big Yields: enhancing food crop production with biological solutions. Microb. Biotechnol. 10(5): 999–1003. https://doi.org/10.1111/1751-7915.12804

Urgancı NN, Yılmaz N, Alaşalvar GK and Yıldırım Z, 2022. *Pseudomonas aeruginosa* and Its Pathogenicity. TURJAF. 10(4): 726–738. https://doi.org/10.24925/turjaf.v10i4.726-738.4986

Zhang T, Jian Q, Yao X, Guan L, Li L, Liu F, Zhang C, LiD, Tang H and Lu L, 2024. Plant growth-promoting rhizobacteria (PGPR) improve the growth and quality of several crops. Heliyon. 10(10): e31553. https://doi.org/10.1016/j.heliyon.2024.e31553

Zhou J, Yang Q, Zhu X, Lin T, Hao D and Xu J, 2020. Antioxidant activities of Clerodendrum cyrtophyllum Turcz leaf extracts and their major components. PLoS ONE. 15(6): e0234435. https://doi.org/10.1371/journal.pone.0234435