Identification and characterization of *Pseudomonas* spp. isolated from durian growing soil in the Central Highlands, Vietnam

Van T. Tran[1*], Thu L. Vo[2], Nhi L. T. Nguyen[2], Khang N. Nguyen[2], Toan Q. Dinh[2], & Don D. Le[2]

[1]Research Institute for Biotechnology and Environment, Nong Lam University, Ho Chi Minh City, Vietnam

[2]Faculty of Biological Sciences, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: June 08, 2023
Revised: October 16, 2024
Accepted: October 23, 2024

Keywords

Biological control
Central Highlands
Durian
PGPR
Pseudomonas spp.

***Corresponding author**

Tran Thi Van
Email:
van.tranthi@hcmuaf.edu.vn

ABSTRACT

Durian cultivation is being developed and expanded in the Central Highlands because of its high economic value. The farmers commonly apply pesticides and chemical fertilizers to control diseases and help durian plants growth. However, an alternate approach, using biological products from microorganisms is a sustainable direction for durian cultivation. In particular, plant growth promoting rhizobacteria (PGPR) is widely used in many countries to support crops and increase productivity. This study aimed to isolate native *Pseudomonas* strains from durian orchards in the Central Highlands and investigate some of their characteristics affecting the growth of durian trees. As a result, four bacterial isolates were identified by morphology, biochemical analysis, and 16S rRNA sequence analysis, including TD-Dak Lak, DN-Lam Dong, T1-Dak Nong, and DT-Gia Lai belonging to *P. fluorescens, P. mosselii, P. monteilii,* and *P. putida*. The survey of nitrogen fixation, indole-3-acetic acid (IAA) production, siderophore, and phosphorus solubilization abilities of the four strains showed that all of them were capable of N2 fixation (3.03 to 3.69 mol/mL), IAA production (11.63 to 54.88 µg/mL), solubilizing phosphate (1.43 to 2.26 cm halo ring), and siderophore production. Three strains of *P. putida, P. fluorescens*, and *P. monteilii* increased the germination length of durian seeds by 13.6%, 12.2%, and 9.2%, respectively. All four *Pseudomonas* strains increased plant height from 4.25 to 5.66 cm, and the average number of leaves varied from 2.4 to 3.2 leaves, compared to 1.5 leaves in the control at 90 days. The results indicate that four *P. fluorescens* (TD-Dak Lak), *P. putida* (DT-Gia Lai), *P. mosselii* (DN-Lam Dong), and *P. monteilii* (T1-Dak Nong) were beneficial biological agents that can promote the growth and development of durian trees.

Cited as: Tran, V. T., Vo, T. L., Nguyen, N. L. T., Nguyen, K. N., Dinh, T. Q., & Le, D. D. (2025). Identification and characterization of *Pseudomonas* spp. isolated from durian growing soil in the Central Highlands, Vietnam. *The Journal of Agriculture and Development* 24(3), 78-94.

1. Introduction

Plant growth-promoting rhizobacteria (PGPR) directly stimulate the growth of plants by the nitrogen fixation process (Han et al., 2005), the production of plant growth-regulating hormones, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme production (Correa et al., 2005). Besides, PGPG indirectly inhibits phytopathogenic fungi through the production of chitinases, b-1,3-glucanases, antibiotics, fluorescent pigments, and hydrogen cyanide (HCN) (Pal et al., 2001). *Pseudomonas* spp. is a special species among PGPR genera in terms of root colonization, nitrogen fixation, production of exopolysaccharides, siderophores, HCN, and phytohormones, solubilization of phosphorus, potassium, and zinc, biofilm formation, activity, antioxidant capacity, stress adaptation, and positive interactions with other microbial communities. *Pseudomonas* strains are indeed rhizosphere endophytes promoting good growth of sunflower (*Helianthus annus*) (Pandey et al., 2013). Ngo et al. (2024) determined the level of nitrogen fertilizer replacement of *Pseudomonas* sp. BT1 and BT2 with high-yielding rice plants grown in pots; replacing 50% N with *Pseudomonas* due to the rice yield in potting increased by 23.9% compared to the control. Chu et al. (2020) found that *Pseudomonas* three strains could stimulate the growth of peanuts under saline conditions both *in vitro* and in greenhouse. *P. fluorescens* was reported to manage several diseases caused by soil-borne pathogens (Wei et al., 1996). *Pseudomonas* spp. is widely studied in disease management strategies due to biological control mechanisms. The most common compounds secreted by *Pseudomonas* include phenazine-1-carboxamide, lipopeptide, 2, 4-diacetylphloroglucinol (DAPG), pyrrolnitrin, pyoluteorin, and phenazine-1-carboxylic acid (PCA) (Dimkić et al., 2022). *Pseudomonas* strains survive well under stressful conditions and control certain fungal plant diseases such as okra root rot caused by *Fusarium solani* (35), foliage blight disease caused by *Phytophthora nicotianae* (37), root rot caused by *Rhizoctonia solani* (36) and damping-off disease caused by *Pythium* spp. (34).

Root zone management is a method of effectively enhancing soil nutrients to promote plant growth and productivity (Zia et al., 2021). *Pseudomonas* is an environmentally acceptable alternative to chemical fertilizers because of its active growth-promoting activities. They also support plant growth by promoting resistance to biotic and abiotic stress and providing nutritional support to the host plant. However, to achieve this goal, users must first find and research a source of indigenous *Pseudomonas* strains to fully exploit the potential of this bacterial strain on durian trees and in agricultural applications.

2. Materials and Methods

2.1. Isolation of *Pseudomonas* spp from durian orchard soils and roots

Soil and root samples were collected from durian-growing areas in the Central Highlands provinces, including Gia Lai, Dak Lak, Đak Nong, and Lam Dong. Samples were taken from durian gardens with trees older than three years. To collect soil, the top 10 cm of soil was first removed, and then soil was collected from a depth of 10 - 20 cm using a metal spoon. Approximately 2 - 3 tablespoons of soil were collected at each point, with a total of around 500 g per sample taken from multiple points around the roots. In each garden (or farm), five samples were collected: four from diagonal points and one from the center of the garden. Root samples, weighing about 50 - 100 g, were collected simultaneously with the soil samples and placed in plastic bags. A total of seventy soil and root samples were collected from the durian farm.

Table 1. The soil and root samples collected from durian farms

No.	Host	Location	GPS	Samples
1	Durian 3 years; covered grass	ChưPrông, Gia Lai	13°50'38.5"N 107°56'16.0"E	10
2	Durian years; inter-cropping coffee	Đăk Đoa, Gia Lai	13°54'02.7"N 108°02'34.7"E	8
3	Durian 8 years; covered grass	Đạ Huoai, Lâm Đồng	11°22'46.3"N 107°32'51.6"E	10
4	Durian garden of many ages	Bảo Lộc, Lâm Đồng	11°30'36.0"N 107°53'48.1"E	10
5	Durian 6 years; inter-cropping coffee	Cư M'gar, ĐăkLăk	12°46'09.4"N 108°08'15.7"E	10
6	Durian 8 years; covered grass	Buôn Ma Thuột, ĐăkLăk	12°57'35.0"N 108°07'09.6"E	8
7	Durian garden of many ages, covered grass	Gia Nghĩa, Đắk Nông	12°01'14.1"N 107°40'03.9"E	14
Total				70

From the rhizosphere and root samples: Ten grams of rhizosphere soil were added to 90 ml of sterile physiological water and shaken at 120 rpm for 20 min. Then 0.1 mL of dilution at concentrations of 10^{-3} - 10^{-5} was spread onto King's B (Mew & Rosales, 1986).

From the rhizoplane samples: 10 g of root segments were added to 90 mL of sterile physiological water and shaken for 2 h at 120 rpm. Then, 0.1 mL of the dilution was spread onto King's B medium (Mew & Rosales, 1986).

From the endorhizosphere samples: The collected durian roots were washed under running water treated with a 2.5% sodium hypochlorite solution, for 3 min, and rinsed three times with sterile distilled water (Mew & Rosales, 1986). Ten grams of these roots were then added to 90 mL of sterile physiological water and shaken for 2 h at 120 rpm. Afterward, 0.1 mL of each dilution was spread onto King's B medium and incubated at room temperature (30 ± 2°C) for 24 - 48 h. Bacterial strains growing on the agar plates were purified and stored in Yeast Dextrose Carbonate (YDC) medium at -80°C with 40% glycerol (Parke et al., 1986).

2.2. Physiological and biochemical characterization of *Pseudomonas* strains

Biochemical tests: According to Cappucino & Sherma (1992), *Pseudomonas* spp. was classified based on catabolic reactions and biochemical, physiological, and nutritional characteristics. Bacterial strains were characterized on King's B plates at 37°C. Biochemical tests were performed on all isolates, including the Gram test, oxidase test, catalase test, mannitol utilization, carbon source utilization, glucose fermentation, motility, nitrate reduction, arginine-dihydrolase, gelatin liquefaction, siderophore production, and IMViC test (Cappucino & Sherman, 1992).

Indole acetic acid (IAA) phytohormone production test: The IAA production test was performed according to Bric et al. (1991). Sterilized LB medium (Sigma, pH 7.5) supplemented with five mM L-tryptophan was poured into 90 mm diameter agar plates. Bacterial strains were inoculated into the central of the agar plates, which were then covered with Whatman paper (82 mm diameter, No. 2). After 48 h of incubation, 5 mL of Salkowski reagent (2% $FeCl_3$ in 35% perchloric acid) was added. The presence of IAA was indicated by the formation of a characteristic red halo around colonies with a diameter of 2 mm. Each isolate was tested in triplicate.

The bacteria were grown in TSB medium supplemented with 0.1 g/L tryptophan, shaken at 150 rpm and 30 ± 2°C in the dark for four days. One milliliter of the inoculum was then centrifuged to remove biomass. The supernatant was mixed with an equal volume of improved Salkowski reagent (1:2 ratio of bacteria to reagent) and incubated in the dark for 1 h (Glick, 1995). The presence of IAA was indicated by the development of a pink-red color, and the absorbance was measured at 535 nm. To quantify IAA, a standard curve was created using IAA concentrations ranging from 0 to 50 µg/mL with Salkowski reagent on a spectrophotometer at 530 nm. The calibration curve was generated with the form $y = ax + b$ ($R^2 > 0.95$). Measurements from the samples were compared to this standard curve to determine IAA concentration. Each strain was tested in triplicate.

IAA Quantification: Bacteria were grown in TSB medium supplemented with 0.1 g/L tryptophan, shaken at 150 rpm and 30 ± 2°C in the dark for four days. Afterward, 1 mL of the inoculum was centrifuged to remove biomass. The supernatant was mixed with an equal volume of improved Salkowski reagent (1:2 ratio of bacteria to reagent) and incubated in the dark for 1 h (Glick, 1995). The IAA presence was indicated by a pink-red color, and absorbance was measured at 535 nm. Standard IAA isolations, with concentrations ranging from 0 to 50 µg/mL, were prepared and measured with Salkowski reagent using a spectrophotometer at 530 nm A calibration curve ($y = ax + b$, with $R^2 > 0.95$) was generated to standardize the measurements. Each strain was tested in triplicate.

Biological nitrogen fixation: The biological nitrogen fixation capability of *Pseudomonas* spp. was assessed by growing bacterial cultures in peptone broth with Nessler's reagent. The negative control was performed with sterile peptone broth without bacterial inoculation. The positive bacterial strain for biological nitrogen fixation was identified by a color change in the culture medium from brown to yellow after 3 - 7 days of incubation. Subsequently, the optical density of the culture medium was measured at 450 nm with a UV-Vis spectrophotometer. Ammonia concentration was estimated using a standard curve of ammonium sulfate, with concentrations ranging from 0.1 to 1 µmol/mL.

Phosphate solubilization: Phosphate solubilization by the isolated *Pseudomonas* spp. was assessed using Pikovskaya agar medium. Bacteria were inoculated in the center of the plates (90 mm in diameter) (Pikovskaya, 1948). Each isolate was tested in triplicate. After nine days of incubation at 30°C, the diameter of the solubilization halo (DSH) was measured using the appropriate formula as follow:

DSH = THD − CD (1), in which, THD is the total halo diameter, CD is the colony diameter.

In addtion to the qualitative test, a quantitative phosphate solubilization test in broth using 250 mL flasks containing 100 mL of NBRIP medium (Nautiyal, 1999). One milliliters of a homogeneous bacterial suspension grown for two days was added into the flask. The samples were incubated on a rotary shaker at 130 rpm at 30 ± 2°C for nine days under the laboratory condition. After incubation, the samples were centrifuged at 12000 rpm for 5 min, and the supernatant was filtered. An aliquot of 5 mL from the supernatant was used to measure the amount of dissolved phosphate. The phosphate concentration in the culture supernatant was estimated using the method described by Olsen & Sommers (1982). Each bacterial isolate tested in triplicate and the control treatment was conducted under the same conditions without bacterial inoculation.

Siderophore production: The ability to produce siderophores was determined using improved CAS (Chrome Azurol S) agar medium (Milagres et al., 1999). Bacteria were cultured on a synthetic siderophore medium. Siderophore production was indicated by a color change around the colonies to lemon yellow or dark yellow. The CAS medium was prepared with the following components: 60.5 mg CAS, 72.9 mg hexadecyltrimethylammonium bromide (HDTMA), 30.24 g piperazine-1,4-bis (2-ethanesulfonic acid) (PIPES), 1 mM $FeCl_3 \cdot 6H_2O$ in 10 mL 10 mM HCl, and 0.9% (w/v) agar.

Molecular identification and phylogenetic analysis: Pseudomonas spp. was identified using molecular analyses based on 16S rRNA sequences. Three to four pure colonies of each bacterial strain, grown on King's B medium for 24 h were suspended in sterile water. The DNA was extracted using the TopPURE DNA extraction kit (HI - 112, ABT, Vietnam) and checked using gel electrophoresis on a 1% agarose gel. The DNA was stored at -20°C (Yang et al., 2008) and amplified by PCR using the primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') of the 16S rRNA gene, producing a DNA fragment of approximately 1500 bp (Turner, 1999). The PCR reaction was performed in a total volume of 25 µL, including 25 ng of DNA, 2.5 µL of 10 × PCR buffer with 20 mM $MgCl_2$, 2.5 µL of dNTPs (2 mM), 0.75 µL of forward primer (10 µM), 1.5 µL of reverse primer (10 µM), and 0.125 µL of FastStart polymerase (5 U/µL). The PCR conditions included an initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 45°C for 45 sec, and extension at 72°C for 2 min, with a final elongation at 72°C for 7 min. The PCR products were purified. The equipment used included a thermal cycler (Bioer, China), an electrophoresis kit, nuclease-free water, agarose, and a GelDoc It2. The PCR sequences were compared to 16S rDNA sequences in GenBank using the NCBI BLAST program. Bacterial strains were processed with the BioEdit V7.0.9.0 tool. Alignment and clustering were performed using the neighbor-joining method (Tamura, 1992). Phylogenetic trees were constructed using MEGA version 7.0 with the accession number of *Pseudomonas* strains sequence found in Gene Bank in Table 2 (Kumar et al., 2016). Bootstrap analysis with 1000 replicates was conducted to assess the reliability of the phylogenetic tree.

Table 2. Accession number of sixteen sequences in GenBank used for this analysis phylogenetic tree

Pseudomonas species	Accession number
Pseudomonas fluorescens strain CIFRI.BTL.S-10	PP759351.1
Pseudomonas fluorescens strain psf3	MN256389.1
Pseudomonas mosselii strain PtA1	CP024159.1
Pseudomonas mosselii strain BS011	CP023299.1
Pseudomonas mosselii strain PH4	CP104107.1
Pseudomonas plecoglossicida strain MR134	MG674349.1
Pseudomonas putida strain NBRC 12996	AB680362.1
Pseudomonas putida strain GPSD-19 HQ270550.1	
Pseudomonas putida strain HN2010	MT515798.1
Pseudomonas putida strain HN2013	MT515799.1
Pseudomonas monteilii strain EO4	OQ999328.1
Pseudomonas monteilii strain JM13	MN758773.1
Pseudomonas monteilii strain WR9	MN733114.1
Pseudomonas monteilii strain SKPf2	KR422299.1
Pseudomonas aeruginosa PAO1	CP053028.1
Azotobacter vinelandii CA	CP005094.1

2.3. Effects of *Pseudomonas* spp. on durian germination seed ability

The experimental setup included 5 treatments: NT0 (control without bacterial strain), NT1 (*P. monteilii*), NT2 (*P. putida),* NT3 (*P. mosselii*), and NT4 (*P. fluorescens*). Each treatment involved 20 Dona durian seeds and was replicated five times. The seeds were soaked washed, sterilized with 70% alcohol for 30 sec, and then rinsed three times with sterilized distilled water. Subsequently, the seeds were immersed in bacterial cell suspension (10^7 cfu/mL) for 30 min, dried for 10 min and placed in a plastic box (30-10-5 cm) lined with paper soaked in sterile distilled water. The seeds were then covered with a layer of damp paper and kept at 27°C. The percentage of germinated seeds and the length of the durian sprouts were recorded after 7 days.

$$\text{Germination seeds (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \text{ x } 100\%$$

2.4. Plant growth assay for testing the effects of the four *Pseudomonas* isolates on durian plant growth

The germination durian seeds were conducted in pots (25 - 20 - 25 cm) filled a substrate consisting of a soil and burned husks mixture (2:1 v/v). The substrate was inoculated with *Pseudomonas* strain suspensions (10^8 cfu/mL) at 10 mL per pot, following the method described by Amkraz (2010), and this inoculation was repeated monthly. The pots were placed in a greenhouse maintained at 26°C with 75 ± 5% relative humidity and were watered every 2 days. Weekly fertilization was carried out using a commercial nutrient solution (NPK 20-20-20)

(Amkraz, 2010). Control plants, derived from untreated seeds, were transplanted into soil substrates without inoculants. All treatments were replicated ten times and arranged in a completely randomized design. After 30, 60, and 90 days, measurements of plant height (aerial region) and the number of leaves were recorded.

2.5. Data analysis

Data were collected, synthesized, and calculated using Microsoft Excel, analyzed with one-way ANOVA, and graded using MINITAB 16.

3. Results and Discussion

3.1. Isolation of *Pseudomonas* spp.

Bacteria strains were purified and examined for colony morphology and pigmentation through gram staining, as well as described on specialized media. Cultural characteristics of isolates were observed, including shape, size, surface, margin, color, odor, and pigmentation, and were recorded according to Bergey's Handbook of Definitive Bacteria (Bergy & Holt, 1994). Cell shape and gram reaction were documented using the standard procedures outlined by Barthalomew & Mittewar (1950). The results in Table 3 showed that the rhizospheric soil of durians was rich in bacteria with the *Pseudomonas* genus being predominant among the isolates. The bacteria were isolated from three planes: the rhizosphere and roots, the rhizoplane, and the endorhizosphere. Thirteen bacterial strains were isolated from seventy samples collected from various locations on the durian farm: two from the endorhizosphere, six from outside the rhizosphere and roots, and five from the rhizoplane. The *Pseudomonas* spp. group constituted 46.15% of the isolated bacteria, including 7% from the rhizosphere soil, 20% from the rhizoplane, and 0.21% from the endorhizospheric component. Differences in isolation rates were attributed to factors such as crop type, soil type, plant age, sampling season, and cultivation practices. These findings are consistent with those reported by Qessaoui et al. (2019), who identified 19 fluorescent *Pseudomonas* isolates from soil, fruits, and vegetable samples, including 11 isolates from the rhizoplane, five isolates from the endorhizosphere, and three isolates from the rhizospheric soil.

Table 3. Bacterial strains were isolated from position of durian in the Central Highlands, Vietnam

No.	Name of isolates	Origin	Location	Bacterial genus
1	DT-Gia Lai	Rhizoplane	Gia Lai	*Pseudomonas* spp.
2	ĐTe-Lam Dong 1	Rhizoplane	Lam Dong	*Enterobacter* sp.
3	Đte-Lam Dong 2	Rhizoplane	Lam Dong	*Bacillus* sp.
4	TD-DaK Lak	Rhizoplane	Dak Lak	*Pseudomonas* spp.
5	DN-Lam Dong	Rhizoplane	Lam Dong	*Pseudomonas* spp.
6	CS-Gia Lai	Rhizosphere and roots	Gia Lai	*Pseudomonas* spp.
7	GN-Dak Nong 1	Rhizosphere and roots	Dak Nong	*Bacillus* sp.
8	PL-Gia Lai 1	Rhizosphere and roots	Gia Lai	*Enterobacter* sp.
9	PL-Gia Lai 2	Rhizosphere and roots	Gia Lai	*Pseudomonas* spp.
10	PL-Gia Lai 3	Rhizosphere and roots	Gia Lai	*Burkholderia* sp.
11	T1-Dak Nong	Rhizosphere and roots	Dak Nong	*Pseudomonas* spp.
12	BMT-Dak Lak	Endorhizosphere	Dak Lak	*Bacillus* sp.
13	PT-Gia Lai	Endorhizosphere	Gia Lai	*Rhizobium* sp.

3.2. Morphology and biochemical characterization of *Pseudomonas* strains

The genus *Pseudomonas* consists of Gram-negative, aerobic, non-spore-forming, rod-shaped bacteria. They are a physiologically diverse group, with the potential to form extracellular slime. The diameter of *Pseudomonas* cells is typically less than 1 μm (Lysenko, 1961). In this research, we focused on the biochemical and molecular characteristics of *Pseudomonas* spp. All six *Pseudomonas* spp. were characterized based on colony morphology on King's B agar medium, cell morphology, and Gram reaction (Table 4). Colonies developed into small to medium-sized, smooth, glistening colonies (Figure 1A). Under the microscope, these isolates appeared as Gram-negative, small, single rods without sporulation. Colonies developed into small to medium-sized, smooth, and glistening colonies (Figure 1A).

Table 4. Growth characteristics of colonies and cells on King's B of six *Pseudomonas* spp.

Isolates	Colonies on King's B agar	Cells
DT-Gia Lai	Colonies are circular, with characteristics that can be flat, raised, convex, or umbonate. They have entire, smooth edges, transparent, and odorless.	Gram-negative rods with polar flagella, motile, and forming extracellular slime irregularly.
T1-Dak Nong PL-Gia Lai 2	Colonies on King' B agar medium are circular and non-pigmented. They are also non-haemolytic on blood agar.	Gram-negative, cells are circular and smooth.
DN-Lam Dong CS-Gia Lai	Colonies are circular and non-pigmented when grown on nutrient agar, measuring 1.8 - 2.0 mm in diameter after 24 h. The bacteria are non-spore-forming rods with polar flagella and are motile.	Gram-negative, with cells that are circular and smooth.
TD-Dak Lak	Colonies are circular, flat, raised, or convex, with entire, smooth edges, transparent appearance, and no odor. They are non-pigmented.	Gram-negative rods that do not form spores. They are motile by polar flagella and may form irregular capsules or extracellular slime.

The isolated bacteria were identified through biochemical characterization according to Bergey's Manual of Systematic Bacteriology (Bergey & Holt, 1994; Boone et al., 2001). Six *Pseudomonas* spp. isolates were tested for various biochemical properties including IMVIC, oxidase, catalase, carbohydrate fermentation, denitrification, H_2S production, starch hydrolysis, gelatin liquefaction, etc. All six isolates tested positive for fluorescence (Figure 1B), catalase test, oxidase test, gelatine liquefaction, glucose, and citrate utilization. In contrast, they were negative for pyocyanin, starch hydrolysis, ethanol, Voges Prausker's, methyl red, and indole test (Table 5).

Table 5. Biochemical characterization of the six *Pseudomonas* isolates

Test	DT-Gia Lai	DN-Lam Dong CS-Gia Lai	T1-Dak Nong PL-Gia Lai 2	TD-Dak Lak
Gram reaction	-	-	-	-
Fluorescin	+	+	+	+
Pyocyanin	-	-	-	-
Catalase	+	+	+	+
KOH (3%)	+	+	+	+
Gelatine	+	+	+	+
Starch hydrolysis	-	-	-	-
Oxidase	+	+	+	+
Ethanol	-	-	-	-
Glucose	+	+	+	+
Mannitol	-	+	-	-
Maltose	+	+	-	+
H_2S	-	+	+	-
Indole	-	-	-	-
MR	-	-	-	-
VP	-	-	-	-
Citrate utilization	+	+	+	+

+: Positive; -: Negative; MR: -Methyl red; VP: – Voges Praskaur's test.

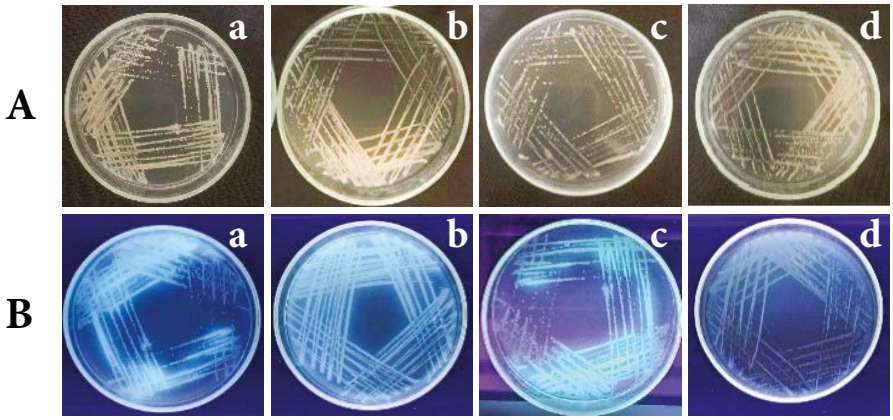

Figure 1. Morphology of 4 *Pseudomonas* spp. on King' s B agar at 24 h, 37 °C (A) and morphology of 4 *Pseudomonas* spp on King' s B agar at 24 h, 37 °C for fluorescens test under UV light (B). a. DT-Gia Lai; b. DN-Lam Dong; c. TD-Dak Lak; d. T1-Dak Nong.

3.3. 16S rRNA gene sequencing and phylogenetic tree

Sequencing of the 16S rRNA gene is a standard method for classifying bacteria into new species or genera. This approach is particularly useful for identifying particularly useful for identifying new species that have never been successfully cultured (Weisburg et al., 1991). *Pseudomonas* sp. can be isolated from a variety of plant species (Rodríguez et al., 2006). Phylogenetic analysis based on 16S rRNA

gene sequences is a fundamental technique for bacterial identification, as these genes are considered basic to bacterial classification (Zeng et al., 2016). The 16S rRNA sequence of the DT-Gia Lai strain showed 100% similarity with *P. putida* on GenBank. To determine its phylogeny, a phylogenetic tree was constructed and compared with sequences of available *P. putida* strains and closely related species using BLAST sequencing and the software Mega X. The neighbor-joining tree created from the aligned sequences revealed that DT-Gia Lai clustered with *P. putida* HN2013 and *P. putida* HN2010, with a bootstrap value of 65 (Figure 2). The gene sequence analysis of the 16S rRNA region indicated that the DN-Lam Dong strain showed 100% similarity with *P. mosselii*. Figure 2 shows that DN-Lam Dong shares the same branch as *P. mosselii* PtA1, with a bootstrap value of 89. Similarly, the 16S rRNA gene sequence analysis of the T1-Dak Nong strain revealed a 100% sequence similarity with *P. monteilii* on GenBank. T1-Dak Nong aligns with *P. monteilii* SKPf2 and *P. monteilii* EO4 on the same branch, with a bootstrap value of 85. *P. fluorescens* and *P. putida*, both containing ACC-deaminase have been shown to improve wheat growth even with low doses of nitrogen (Naveed et al., 2008). Similarly, Kavino et al. (2010) found a significant impact of *P. fluorescens* on the leaf nutrient content and yield of bananas. Rakh et al. (2019) reported that, *Pseudomonas* cf. *monteilii* (isolate 29) inhibited *Fusarium oxysporum* the causative agent of *Fusarium* wilt disease in groundnuts, by up to 60.75% in in vitro studies. Additionally, *P. mosselii* BS011 isolated from the rhizosphere soil of rice plants, exhibited strong inhibitory activity against the rice blast fungus *Magnaporthe oryzae*. The gene cluster c-xtl was identified as essential for this inhibitory activity, and the crude extract from the BS011 fermentation sample significantly inhibited the development of *M. oryzae* at a concentration of 10 µg/mL (Wu et al., 2018). These findings suggest that PGPR strains could serve as an effective alternative to high-cost fertilizers, supporting sustainable agriculture.

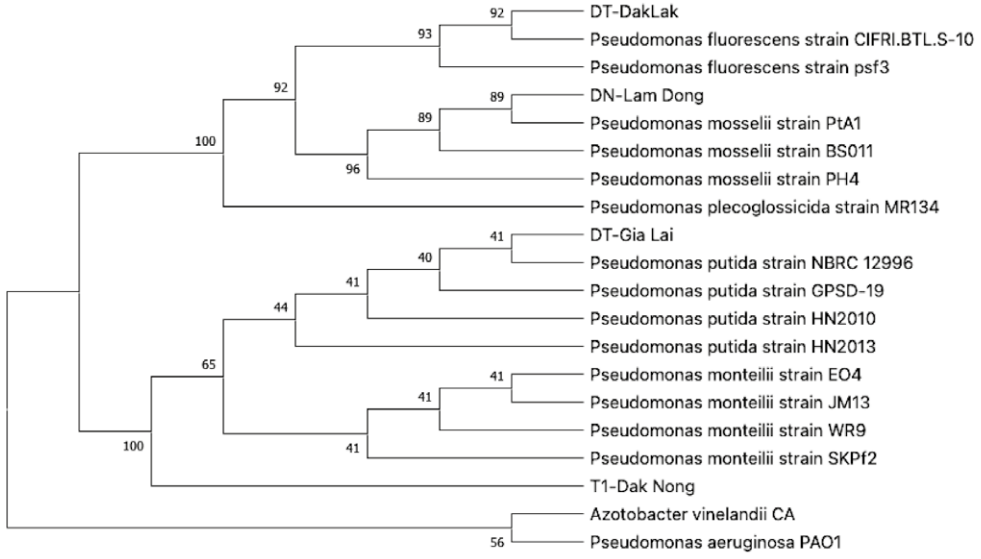

Figure 2. Neighbor-joining phylogenetic analysis of Pseudomonas spp. was conducted with similar species from GenBank, based on 16S rRNA gene sequence data. The numbers on each branch represent bootstrap values, which are percentages derived from an analysis of 100 replicates.

The PGPR influence plant growth through several mechanisms, including increasing mineral nutrient solubility, fixing nitrogen, providing nutrients to plants, inhibiting soil-borne pathogens (by producing hydrogen cyanide, siderophores, antibiotics, enzymes such as chitinase, and competing nutrients), enhancing plant tolerance to drought, salinity, and metal toxicity, and producing phytohormones such as indole-3-acetic acid (IAA) (Gupta et al., 2000). Results shown in Table 6 showed that four *Pseudomonas sp.* isolates can produce indole-3-acetic acid, ammonia, solubilized phosphate, and siderophores. The ability to solubilize phosphate among these four *Pseudomonas* strains was qualitatively assessing, halo ring diameters ranging from 1.43 cm to 2.26 cm. Phosphorus (P) is a macronutrient crucial for plant growth and development (Soetan et al., 2010). However, the content of soluble phosphates in the soil is low due to its strong absorption by soil mineral and particles. Li et al. (2019) reported that *Pseudomonas* species isolated from the soil and rhizomes of various plant are capable of phosphorus solubilization. Additionally, treatment with *Pseudomonas* spp. has been shown to increase biomass yield and phosphorus uptake in *Triticum aestivum* (Ma et al., 2011).

Table 6. Producing indole-3-acetic acid, ammonia, and siderophores and solubilize phosphate of *Pseudomonas* spp.

Isolates	IAA		Nitrogen fixation		Phosphate solubillization		Siderophore
	Q	µg/mL	Q	Mol/mL	Q	HD (cm)	Q
DT-Gia Lai (*P. putida*)	+	54.88^{a} 1.00	+	3.25^{b}	+	1.43^{c} 0.25	+
DN-Lam Dong (*P. mosselii*)	+	12.07^{c} 0.93	+	$3,14^{b}$	+	2.04^{a} 0.40	+
T1-Dak Nong (*P. monteilii*)	+	19.09^{b} 0.91	+	$3,03^{c}$ 0.55	+	1.72^{b} 0.28	+
TD-Dak Lak (*P. fluorescens)*	+	11.63^{c} 1.17	+	$3,69^{a}$	+	2.26^{a} 0.35	+

HD: halo ring diameter, Q: qualitative (+: yes –: no). Values indicate mean values (± SD); different letters indicate significant differences within a row or column at P < 0.01.

Pseudomonas spp. satisfied the ion needs by consuming siderophores produced by other rhizosphere microorganisms. As soil copper concentrations increase, bacterial strains capable of producing high levels of siderophores experience less inhibition. Bacterial siderophores play a crucial role in host plants by influencing iron homeostasis and immune function (Hesse et al., 2018). Under conditions of iron deficiency, siderophore-producing bacteria assist plants in improving iron absorption. The results showed that *Pseudomonas* strains produced siderophores on CAS medium (Table 4). The production of the phytohormone IAA (indole-3-acetic acid) by the four *Pseudomonas* strains was notably high, ranging from 11.63 to 54.88 µg/mL. Fluorescent *Pseudomonads*, such as *P. chlororaphis*, *P. putida*, *P. aeruginosa*, *P. monteilii*, *P. plecoglossicida*, *P. fluorescens*, *P. fulva*, and *P. mosselii* are known for their phosphate solubilizing ability (Maheshwari, 2011). Ravindra et al. (2008) reported that the up to 41% of fluorescent *Pseudomonad* bacteria

isolated from banana roots were phosphate-solubilizers. Additionally, some fluorescent Pseudomonad species produce various phytohormones including auxin, gibberellin, cytokinin, and abscisic acid, with IAA being particularly important for plant growth (Patten & Glick, 2002). IAA production by *Pseudomonas* spp. enhances the density and length of root hairs, improving the plant's ability to uptake water and nutrients, thereby stimulating plant growth (Kumar et al., 2005). Certain *Pseudomonas* species are also capable of nitrogen fixation, positively impacting plant growth and productivity (Nicole et al., 2003). Andrade et al. (1998) reported that *P. fluorescens* enhanced rhizobacterial symbiosis in peas through the *Nif* gene. The four *Pseudomonas* strains tested were able to fix nitrogen, with ammonia concentrations reaching 3.03-3.69 mol/mL.

The PGPR plant growth, yield, and stress tolerance by improving nutrient uptake from the soil through various mechanisms such as phosphate solubilization, siderophore production, immobilization of proteins, phytohormone production, antifungal activity, and induction of systemic drug resistance. Due to these benefits, PGPR, particularly strains from the genus *Pseudomonas*, are used as biological alternatives to agrochemical products like fertilizers and pesticides. This approach reduces soil pollution and improves the quality of fruits and vegetables.

3.4. Effects of *Pseudomonas* spp. on germination seed ability and growth of durian plants

Results regarding the effects of *Pseudomonas* strains on germination of durian seeds showed that germination rates were 100% for both the control and the *Pseudomonas*-treated seeds. While germ length in the control and *P. mosselii* strain (DN-LamDong) did not differ significantly, *P. fluorescens* (TD-DakLak), *P. putida* (DT-GiaLai), and *P. monteilii* (T1-DakNong) exhibited significant differences compared to the control. The highest germ lengths were observed in seeds treated with *P. putida, P. fluorescens*, and *P. monteilii* measuring 6.98 cm, 6.84 cm, and 6.37 cm, respectively, compared to 5.31 cm in the uninoculated control (Figure 3). Pseudomonas strains have been shown to enhance seed germination, seedling vigor, and tomato plant height to 50.66 cm and 48 cm, respectively, compared to 30 cm in untreated plants.

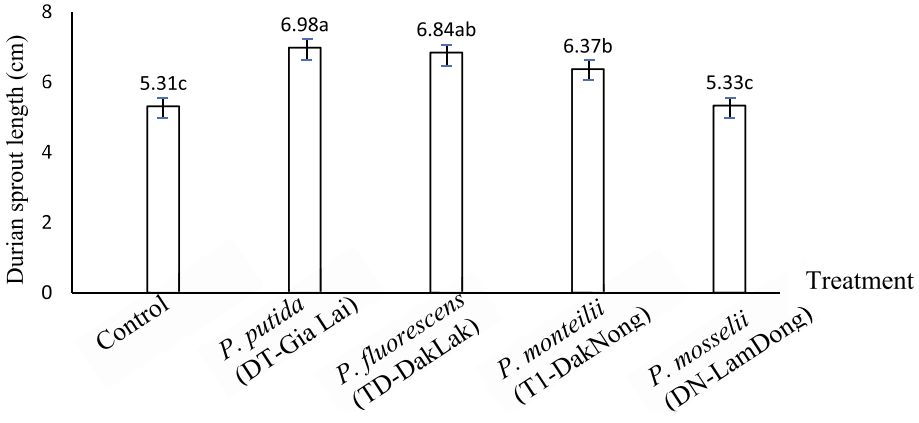

Figure 3. Effect of *Pseudomonas* isolates on durian seedling growth. Bars with the different letters indicate significantly differences at $P < 0.01$.

Table 7. Effect of seedling treatment with *Pseudomonas* strains on plant height, leaf number in different days

Treatments	Plant height (cm)			Leaf number (leaves)		
	30 days	60 days	90 days	30 days	60 days	90 days
DT-Gia Lai (*P. putida*)	21.64^{a} 0.65	36.92^{a} 0.50	43.16^{a} 0.37	3.0^{a} 0.4	15.1^{b} 0.5	18.3^{a} 0.4
TD-Dak Lak (*P. fluorescens)*	21.71^{a} 0.51	36.00^{ab} 0.98	42.38^{ab} 0.50	2.9^{b} 0.3	14.6^{c} 0.7	18.1^{ab} 0.3
T1-Dak Nong (*P. monteilii*)	20.66^{b} 0.54	33.52^{bc} 1.30	41.75^{b} 1.05	2.9^{b} 0.4	14.9^{c} 0.6	18.2^{ab} 0.4
DN- Lam Dong (*P. mosselii*)	19.54^{bc} 0.56	34.35^{b} 1.18	42.09^{ab} 0.56	2.8^{c} 0.5	15.9^{a} 0.8	17.5^{b} 0.5
Control	18.86^{c} 0.44	31.08^{c} 0.62	37.50^{c} 0.67	2.7^{d}	12.0^{d} 0.4	15.1^{c} 0.4

Different letters indicate significant differences within a column at P < 0.01.

The recorded data showed a significant increase in plant height of durians plants treated with different *Pseudomonas* strains compared to the untreated control (Table 7). All four *Pseudomonas* strains effectively improved plant height across all treatments compared to the uninoculated control. Specifically, the height of durian trees treated with *P. fluorescens* increased from 4.25 to 5.66 cm over 90 days. Among the strains, *P. putida* (DT-Gia Lai) was found to be most effective in enhancing plant height. Additionally, the number of durians leaves increased by an average of 2.4 to 3.2 leaves per plant in the Pseudomonas-treated groups compared to the control after 90 days. These results underscore the effectiveness of *Pseudomonas* strains as PGPR that can significantly enhance plant growth. Supporting this, Sarma & Saikia (2014) reported that *P. fluorescens* and *P. putida* increased rice plant height by 16.2% and 15.6%, respectively, compared to the uninoculated control after 30 days. Similarly, Qessaoui et al. (2019) showed that fluorescent *Pseudomonas* isolates from the rhizosphere significantly increased tomato plant height. Notably, the *Pseudomonas* strain Q13B increased plant height by 31.5% and collar diameter by 48.57% compared to the control. Additionally, *Pseudomonas* sp. (Q1B) significantly enhanced the number of leaves by 83.33% 20 days after transplantation.

4. Conclusions

Four *Pseudomonas* spp. were isolated from the rhizoplane and rhizosphere durians trees in the Central Highlands, Vietnam. Four bacterial isolates were identified by morphology, biochemical analysis, and 16S rRNA sequence analysis as *P. fluorescens* (TD-Dak Lak), *P. mosselii* (DN-Lam Dong), *P. monteilii* (T1-Dak Nong), and *P. putida* (DT-Gia Lai) siderophore and solubilizing phosphate. They also demonstrated biological nitrogen fixation capacities ranging from 3.03 to 3.69 mol/mL and IAA production levels between 11.63 and 54.88 μg/mL. Inoculation with these *Pseudomonas* strains significantly improved plant height compared to the uninoculated control. Specifically, durian plants inoculated with *P. fluorescens* showed an increase in height from 4.25 cm to 5.66 cm

over 90 days. Among the strains, *P. putida* (DT-Gia Lai) was the most effective in enhancing plant height. Additionally, the number of durian leaves increased by an average of 2.4 to 3.2 leaves per plant in the *Pseudomonas* treated groups compared to the control after 90 days. These findings indicate that *P. fluorescens, P. putida, P. mosselii, and P. monteilii* are beneficial biological agents that promote the growth and development of durian trees. Therefore, these indigenous *Pseudomonas* strains have the potential to be used as biofertilizers or bioproduct to enhance durian cultivation in local agricultural conditions.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgements

Nong Lam University, Ho Chi Minh City based research CS-CB23-VIENCNSH-03 is thankfully acknowledge.

References

Amkraz, N., Boudyach, E., Boubaker, H., Bouizgarne, B., & Aoumar, A. A. B. (2010). Screening for *fluorescent pseudomonades*, isolated from the rhizosphere of tomato, for antagonistic activity toward *Clavibacter michiganensis* subsp. *michiganensis. World Journal of Microbiology and Biotechnology* 26, 1059-1065. https://doi.org/10.1007/s11274-009-0270-5.

Andrade, G., Mihara, K. L., Linderman, R. G., & Bethlenfalvay, G. J. (1997). Bacteria from rhizosphere and hyphosphere soils of differentarbuscular-mycorrhizal fungi. *Plant and Soil* 192(1), 71-79. https://doi.org/10.1023/A:1004249629643.

Barthalomew, J., & Mittewer, T. (1950). A simplified bacterial strain. *Stain Technology* 25(3), 153-156. https://doi.org/10.3109/10520295009110979.

Bergey, D. H., & Holt, J. G. (1994). *Bergey's manual of determinative bacteriology* (9th ed.). Maryland, USA: Williams and Wilkins.

Boone, D. R., Castenholz, R. W., Garrity, G. M., Brenner, D. J., Staley, J.T., & Krieg, N. R. (2001). *Bergey's manual of systematic bacteriology* (2nd ed.). New York, USA: Springer.

Bric, J. M., Bostock, R. M., & Silverstone, S. E. (1991). Rapid *in Situ* assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. *Applied and Environmental Microbiology* 57(2), 535-538. https://doi.org/10.1128/aem.57.2.535-538.1991.

Cappucino, J., & Sherman N. (1992). *Microbiology: A laboratory manual* (3rd ed.). San Francisco, USA: Benjamin-Cummings Publishing Company.

Chu, T. N., Bui, L. V., & Hoang, M. T. T. (2020). *Pseudomonas* PS01 isolated from maize rhizosphere alters root system architecture and promotes plant growth. *Microorganisms 8*(4), 471. https://doi.org/10.3390/microorganisms8040471.

Correa, J. D., Barrios, M. L., & Galdona, R. P. (2005). Screening for plant growth-promoting rhizobacteria in *Chamaecytisus proliferus* (tagasaste), a forage tree-shrub legume endemic to the CanaryIslands. *Plant and Soil* 266, 261-272. https://doi.org/10.1007/s11104-005-0754-5.

Dimkić, I., Janakiev, T., Petrović, M., Degrassi, G., & Fira, D. (2022). Plant-associated *Bacillus* and *Pseudomonas* antimicrobial activities in plant disease suppression via biological control mechanisms - A review. *Physiological and Molecular Plant Pathology* 117, 101754. https://doi.org/10.1016/j.pmpp.2021.101754.

Glick, B. R. (1995). The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* 41(2), 109-117. https://doi.org/10.1139/m95-015.

Gupta, A., Gopal, M., & Tilak, K. V. B. R. (2000). Mechanism of plant growth promotion by rhizobacteria. *Indian Journal of Experimental Biology* 38(9), 856-862.

Han, J., Sun L., Dong, X., Cai, Z., Yang, H., Wang, Y., & Song, W. (2005). Characterization of a novel plant growth promoting bacteria *Delftia tsuruhatensis* HR4 both as a diazotroph and apotential biocontrol agent against various pathogens. *Systematic and Applied Microbiology* 28(1), 66-76. https://doi.org/10.1016/j.syapm.2004.09.003.

Hesse, E., O'Brien, S., Lujan, A. M., & Veen, E. M. (2018). Ecological selection of siderophore producing microbial taxa in response to heavy metal contamination. *Ecology Letters* 21(1), 117-127. https://doi.org/10.1111/ele.12878.

Kavino, M., Harish, S., Kumar, N., Kumar, S., & Samiyappan, R. (2010). Effect of chitinolytic PGPR on growth, yield and physiological attributes of banana (*Musa* spp.) under field conditions. *Applied Soil Ecology* 45(2), 71-77.

Kumar, R., Ayyadurai, N., Pandiaraja, P., Reddy A.V., Venkateswarlu, Y., Prakash, O., & Sakthivel, N. (2005). Characterization of antifungal metabolite produced by a new strain *Pseudomonas aeruginosa* PUPa3 that exhibits broad-spectrum antifungal activity and biofertilizing traits. *Journal of Applied Microbiology* 98(1), 145-154. https://doi.org/10.1111/j.1365-2672.2004.02435.x.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology* and *Evolution* 33(7), 1870-1874. https://doi.org/10.1093/molbev/msw054.

Li, X., Jousset, A., de Boer, W., Carri'on, V. J., Zhang, T., & Wang, X. (2019). Legacy of land use history determines reprogramming of plant physiology by soil microbiome. *Journal of the International Society for Microbial Ecology* 13(3), 738-751. https://doi.org/10.1038/s41396-018-0300-0.

Lysenko, O. (1961). *Pseudomonas-An* attempt at a general classification. *Journal of General Microbiology* 25(3), 379-408. https://doi.org/10.1099/00221287-25-3-379.

Maheshwari, D. K. (2011). *Bacteria in agrobiology: Crop ecosystems. Heidelberg, Germany: Springer. https://doi.org/10.1007/978-3-642-18357-7.*

Ma, Y., Rajkumar, M., Luo, Y., & Freitas, H. (2011). Inoculuation of endophytic bacteria on host and non-host plants effects on plant growth and Ni uptake. *Journal of Hazardous Materials* 195, 230-237. https://doi.org/10.1016/j.jhazmat.2011.08.034.

Mew, T., & Rosales, A. (1986). Bacterization of rice plants for control of sheath blight caused by *Rhizoctonias solani*. *Phytopathology* 76, 1260-1264. DOI:10.1094/Phyto-76-1260.

Milagres, A.M., Machuca, A., & Napoleão, D. (1999). Detection of siderophore production from several fungi and bacteria by a modification of chrome azurol S (CAS) agar plate assay. *Journal of Microbiology Methods* 37(1), 1-6. https://doi.org/10.1016/S0167-7012(99)00028-7.

Nautiyal, C. S. A. (1999). Efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol Letters* 170(1), 265-270. https://doi.org/10.1111/j.1574-6968.1999.tb13383.x.

Naveed, M., Zahir, Z. A., Khalid, M., Asghar, H. N., Akhtar, M. J., & Arshad, M. (2008). Rhizobacteria cointaining ACC-Deaminase for improving growth and yield of wheat under fertilized conditions. *Pakistan Journal of Botany* 40, 1231-1241.

Ngo, H. L., Huynh, T. Q., Nguyen, N. H. B., Tran, N. B. V., & Nguyen, T. T. H. (2024). Effects of ceftazidime exposure on phenotypic characteristics of *pseudomonas aeruginosa* ATCC 9027. In Vo, V. T., Nguyen, T. H., Vong, B. L., Le, N. B., & Nguyen, T. Q. (Eds.), *IFMBE Proceedings of The 9th International Conference on the Development of Biomedical Engineering in Vietnam* (95). https://doi.org/10.1007/978-3-031-44630-6_87.

Nicole, D., Lin, M., Guo, X., Ma, L., Carreño-Lopez, R., & Elmerich, C. (2003). Nitrogen fixation genetics and regulation in a *Pseudomonas stutzeri* strain associated with rice. *Microbiology* 149(8), 2251-2262. https://doi.org/10.1099/mic.0.26270-0.

Olsen, S. R, & Sommers, L. E. (1982). Phosphorus. In Page, A. L. (Ed.). *Methods of soil analysis: Part 2 Chemical and microbiological properties* (403-430). Wisconsin, USA: American Society of Agronomy.

Pal, K. K., Tilak, K. V. B. R., Saxena, A. K., Dey, R., & Singh, C. S. (2001). Suppression of maize root diseases caused by *Macrophomina phaseolina, Fusarium moniliformae and Fusarium graminearumby* plant growth promoting rhizobacteria. *Microbiology Research* 156(3), 209-223. https://doi.org/10.1078/0944-5013-00103.

Pandey, R., Chavan, P. N., Walokar, N. M., Sharma, N., Tripathi, V., & Khetmalas, M. B. (2013). *Pseudomonas stutzeri* RP1: A versatile plant growth promoting endorhizospheric bacteria inhabiting sunflower (*Helianthus annus*). *Research Journal of Biotechnology* 8(7), 48-55.

Parke, J., Moen, R., Rovira, A., & Bowen, G. (1986). Soil water flow affects the rhizosphere distribution of a seed-borne biological control agent *Pseudomonas fluorescens*. *Soil Biology Biochemistry* 18(6), 583-588. https://doi.org/10.1016/0038-0717(86)90079-9.

Patten, C. L., & Glick, B. R. (2002). Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Applied and Environmental Microbiology* 68(8), 3795-3801. https://doi.org/10.1128/aem.68.8.3795-3801.2002.

Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Mikrobiologiya* 17, 362-370.

Qessaoui, R., Bouharroud, R., Furze, J. N., Aalaoui, M. el, Akroud, H., Amarraque, A., Vaerenbergh, J. Van, Tahzima, R., Mayad, E. H., & Chebli, B. (2019). Applications of new Rhizobacteria *Pseudomonas* isolates in Agroecology via fundamental processes complementing plant growth. *Scientific Reports* of *Nature* 9(1), 12832. https://doi.org/10.1038/s41598-019-49216-8.

Rakh, R. R., Raut, L.S., Atnoorkar, A. A., & Dalvi, S. M. (2019). In vitro antagonism of *Pseudomonas* cf. *monteilii* 9 against *Fusarium oxysporum*, a causative agent of Fusarium wilt disease of groundnut. *Review of Research* 1(1), 37-47.

Ravindra, N. P., Raman, G., Badri, N. K., & Sakthivel, N. (2008). Assessment of genetic and functional diversity of phosphate solubilizing fluorescent pseudomonads isolated from rhizospheric soil. *BMC Microbiology* 8, 230. https://doi.org/10.1186/1471-2180-8-230.

Rodríguez, H., Fraga, R., Gonzalez, T., & Bashan, Y. (2006). Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant Soil* 287, 15-21. https://doi.org/10.1007/s11104-006-9056-9.

Sarma, R. K., & Saikia, R. (2014). Alleviation of drought stress in mung bean by strain *Pseudomonas aeruginosa* Ggrj21. *Plant Soil* 377, 111-126. https://doi.org/10.1007/s11104-013-1981-9.

Soetan, K. O., Olaiya, C. O., & Oyewole, O. E. (2010). The importance of mineral elements for

humans, domestic animals and plants: a review. *African Journal of Food Science* 4(5), 200-222.

Tamura, K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. *Molecular Biology* and *Evolution* 9(4), 678-687. https://doi.org/10.1093/oxfordjournals.molbev.a040752.

Turner, S., Pryer, K. M., Miao, V. P., & Palmer, J. D. (1999). Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *Journal* of *Eukaryotic Microbiology* 46(4), 327-328. https://doi.org/10.1111/j.1550-7408.1999.tb04612.x.

Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology* 173(2), 697-703. https://doi.org/10.1128/jb.173.2.697-703.1991.

Wu, L., Xiao, W., Chen, G., Song, D., Khaskheli, M. A., Li, P., Zhang, S., & Feng, G. (2018). Identification of *Pseudomonas mosselii* BS011 gene clusters required for suppression of Rice Blast Fungus *Magnaporthe oryzae*. *Journal of Biotechnology* 282, 1-9. https://doi.org/10.1016/j.jbiotec.2018.04.016.

Yang, L., Barken, K. B., Skindersoe, M. E., Christensen, A. B., Givskov, M., & Tolker-Nielsen, T. (2007). Effects of iron on DNA release and biofilm development by *Pseudomonas aeruginosa*. *Microbiology* 153(5), 1318-1328. https://doi.org/10.1099/mic.0.2006/004911-0.

Zeng, Q., Wu, X., & Wen, X. (2016). Effects of soluble phosphate on phosphate-solubilizing characteristics and expression of GCD Gene in *Pseudomonas frederiksbergensis* Jw-Sd2. *Current Microbiology* 72(2), 198-206. https://doi.org/10.1007/s00284-015-0938-z.

Zia, M. A., Riaz, R., Batool, A., Yasmin, H., Nosheen, A., Naz, R., & Hassan, M. N. (2021). Glucanolytic rhizobacteria associated with wheat-maize cropping system suppress the *Fusarium* wilt of tomato (*Lycopersicum esculentum* L.). *Scientia Horticulturae* 287, 110275. https://doi.org/10.1016/j.scienta.2021.110275.