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Isolation and characterization of fluorescent *Pseudomonas* endophyte from lowland creeping-sensitive plant, and its effect on several plant pathogens and plant growth

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Abstract. *Soesanto L, Azkiyah A, Primayuri D, Sundari D, Mugiastuti E. 2025. Isolation and characterization of fluorescent* Pseudomonas *endophyte from lowland creeping-sensitive plant, and its effect on several plant pathogens and plant growth. Biodiversitas 26: 723-730.* Despite declining effectiveness and the risk of pathogen resistance, farmers still prefer synthetic fungicides to control plant disease. A safe alternative to synthetic fungicides is toxins produced by biocontrol agents. This research aimed to isolate the endophytic bacteria, fluorescent *Pseudomonas*, from lowland creeping-sensitive plants (*Mimosa* sp.), analyze its morphological and biochemical characteristics, and assess its impact on pathogens and plant growth promoters. The experiment was conducted at the Plant Protection Laboratory, Faculty of Agriculture, Jenderal Soedirman University, from June to September 2024. Samples of creeping-sensitive plant roots were collected from several marginal soils in the lowlands of Banyumas and Cilacap Regencies. The result showed that a total of 15 isolates of endophytic fluorescent *Pseudomonas* were isolated from the samples. It was also noted that 80% of fluorescent *Pseudomonas* isolates were able to inhibit the growth of several plant pathogens. Fluorescent *Pseudomonas* isolates demonstrate significant variability in their ability to inhibit pathogenic fungi and bacteria, with PE13 and PE14 showing the most effective inhibition and enzyme production capabilities and several isolates had a positive effect on plant growth parameters. Fluorescent *Pseudomonas* exhibited both abilities through the production of several hydrolysis enzymes (lipase, cellulase, protease, and chitinase), HCN, siderophores, and phosphate solubilizing compounds.

Keywords: Antagonistic bacteria, endophyte, fluorescent *Pseudomonas*, *Mimosa*, plant pathogens

INTRODUCTION

Plant diseases pose a significant challenge in crop cultivation that leading to yield reductions up to 40% (Nazarov et al. 2020; Richard et al. 2022). Plant disease control plays an important role in ensuring food security, economic stability, and environmental sustainability (Rizqon and Wahyuni 2021). Reducing crop losses can contribute to addressing global food insecurity, which impacted an estimated 927.6 million people in 2022 (Richard et al. 2022).

Farmers still use synthetic fungicides widely to control plant disease despite their diminishing effectiveness and the risks of pathogen resistance. A prolonged use of a certain type of synthetic fungicide can make pathogen resistant or form a resting structure (Thind 2022), even triggering the emergence of new pathogen strains that are more resistant to chemicals (Qiu et al. 2022). By extension, the unwise use of synthetic pesticides can negatively impact non-target organisms (Barathi et al. 2024) and the environment (Kaur et al. 2024). Additionally, chemical residues can affect soil microbial life, harm the environment (Riyaz et al. 2021), and cause residues in food products (Ahmad et al. 2024).

The search for new control alternatives that are safe, effective, environmentally sound, and supportive of sustainable agriculture is one of the priorities in disease management nowadays. Biological control may protect plants throughout their life cycle using toxic compounds (Ayaz et al. 2023; Haq et al. 2024) and offers long-term sustainability as the agent can reproduce and persist in the field (Bonaterra et al. 2022; Tyagi et al. 2024). Some biological control agents, especially antagonistic microbes, promote plant growth, enhance nutrient uptake, and induce resistance (Köhl et al. 2019; Bonaterra et al. 2022).

Endophytic microbes, known for their antagonistic ability and stability in controlling plant pathogens, are the most researched microbial groups for biological control (Rana et al. 2020). Mycolytic enzymes, siderophores, antibiotics, or volatile compounds are some of the microbes that inhibit pathogens from growing (Dimkić et al. 2022; Amoo et al. 2023) by inhibiting pathogen germination and sporulation, competing for nutrients, or causing parasitism or mycophagy (Köhl et al. 2019; Vanegas et al. 2020; Caballero-Flores et al. 2022). Bacteria can induce systemic responses in plants by expressing enzymes such as peroxidases, phenyl ammonia, endoglucanases, and chitinases throughout plant tissues, thereby enabling the plant to defend against pathogens (Kour et al. 2024).

Endophytic microbes play a role as plant growth promoters by colonizing roots, increasing root branching and root number, and increasing growth through direct or indirect mechanisms (Rana et al. 2020; Adeleke et al. 2021). The microbes can also produce phytohormones, increase nitrogen fixation and phosphate solubilization, modify root function, improve plant nutrition, and affect

whole plant physiology (Afzal et al. 2019; Rana et al. 2020).

The creeping-sensitive plant (*Mimosa* sp.) is reported to contain many microbes, especially bacterial groups that interact with plants either as endophytic or rhizosphere microbes (Sánchez-Cruz et al. 2019; Selangga and Listihani 2021). These microbes can act as plant pathogen antagonists, inducers of plant resistance, plant growth promotors, and bio-remediators (Rizqon and Wahyuni 2021; Abdullahi et al. 2020). Various antagonistic bacteria that interact with the creeping-sensitive plant are *Bacillus* sp., *Pseudomonas* sp., *Rhizobium* sp., Enterobacter, *Serratia* spp., and actinomycetes (Abdullahi et al. 2020; Rizqon and Wahyuni 2021; Nufus et al. 2022).

Fluorescent *Pseudomonas* are widely used as biological control agents for soil-borne and air-borne diseases through control mechanisms, including competition for nutrition and infection sites (Silverio et al. 2022), hyperparasites (Dimkić et al. 2022), production of microbial inhibitory compounds (Dimkić et al. 2022), plant resistance, and promoting plant growth (Sah et al. 2021). Fluorescent *Pseudomonas* was reported to produce antibiotics phenazine-1-carboxylic acid (PCA) and other derivatives, 2,4 diacetyl phloroglucinol (DAPG), pyrrolidine (Prn), and pyoluteorin (Plt) (Zeng et al. 2023; Maurya et al. 2024). The aim this research was to identify the potential fluorescent *Pseudomonas* of the lowland creeping-sensitive plant endophyte roots and determine the mechanism of these microbes as biological control and plant growth promoters. The results of this research may contribute valuable references in the collection of biological agents and, eventually, biofungicide products.

MATERIALS AND METHODS

The research was conducted from July to October 2024 at the Plant Protection Laboratory, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto. A two-part study focused on the isolation and characterization of fluorescent *Pseudomonas*, endophytic bacteria from creeping-sensitive plant roots, also evaluated the its antagonistic activity and ability to control the plant pathogens.

Isolation

The endophytic fluorescent *Pseudomonas* was isolated from creeping-sensitive plant (*Mimosa invisa*) root samples collected through purposive sampling in the lowlands (0-200 m above sea level) from the Banyumas and Cilacap Regencies. For isolation, roots were washed, dried with tissue, and surface sterilized with 70% alcohol (1 min), followed by 20% natrium hypochlorite (5 minutes), and Ringer's thiosulphate solution (5 min). After that, roots were crushed with 90 mL Phospate Buffered-Saline in a sterile mortar, then grown on Kings B media by serial dilution and pour plate technique (Sravani and Patil 2023). The isolated bacteria were purified for further characterization. Characterization of antagonistic bacteria, included shape, edges, and color of colonies; Gram test; catalase test; oxidase test; and the ability to produce endospores.

Antagonism test

The ability of antagonistic microbes to inhibit the plant pathogens was tested using the dual culture method. The pathogens used were, including fungi *Fusarium* sp., *Colletotrichum* sp., *Pythium* sp., and bacteria *Ralstonia solanacearum* and *Xanthomonas* sp. The inhibition of pathogen growth was observed from the formation of clear zone diameter (bacterial pathogens) or growth inhibition power (fungal pathogens). Fungal growth inhibition was calculated using the formula of Wonglom et al. (2019).

$$I = \frac{C - T}{C} \times 100\%$$

Where:

I : Antagonist inhibition rate (%)

C : The radius of the pathogen colony opposite the center of the antagonist colony

T : The radius of the pathogen colony towards the center of the antagonist colony

Hypersensitive test

A hypersensitivity test was conducted on 4-week-old tobacco plant leaves. One mL of bacterial suspensions of 15 isolates, namely PE12, PE13, PE14, PE15, PE21, PE22, PE23, PE24, PE25, PE26, PE27, PE28, PE34, PE36, and PE37 isolates was injected using a syringe into the lower surface of tobacco leaves. The inoculated tobacco plants were incubated for 24-48 hours, followed by the observation of hypersensitive reactions. Bacterial isolates that showed necrosis symptoms on tobacco plants were categorized to be potential plant pathogens (Amaria et al. 2023).

Plant growth-promoting test

A total of 15 isolates of fluorescent *Pseudomonas* were used to analyze the ability of bacteria as plant growth promoters. The experiment was done in completely randomized design. The study consisted of 16 treatments, included 15 treatments using bacterial isolates of fluorescent *Pseudomonas* and one control treatment without bacteria. Each treatment used 10 cucumber seeds. In treatment, cucumber seedlings were soaked in bacterial suspensions of PE12, PE13, PE14, PE15, PE21, PE22, PE23, PE24, PE25, PE26, PE27, PE28, PE34, PE36, and PE37 isolates. The planting seeds were soaked for 2-4 hours. In the control, seeds were only soaked in sterile water. Plant height, fresh weight of plants and roots, and root length were observed after 7 days (Yesuf et al. 2021).

Biochemical characters

The mechanism of antagonistic microbes as biological control agents and plant growth promoters was analyzed for their ability to produce hydrolysis enzymes (protease, cellulase, lipase) and the ability to dissolve phosphate, according to Al-Talebi et al. (2022). In addition, lipase enzyme and HCN assay were also estimated according to method of Kandasamy et al. (2023) and Sehrawat et al. (2022), while detection of siderophore production with Chroma Azurol Sulfonate (CAS) agar media according to Murakami et al. (2021).

Data analysis

Data were subjected to the descriptive analysis and ANOVA. Data on root length, seedling height, root fresh weight, and seedling fresh weight were analyzed with the F test. Any significant difference from the results was analyzed with the Duncan Multiple Range Test (DMRT) at a 5% error rate.

RESULTS AND DISCUSSION

The exploration, isolation, and characterization of endophytic antagonistic bacteria of lowland creeping-sensitive plant yielded 15 isolates of fluorescent *Pseudomonas*. These isolates included 4 isolates (PE12-PE15) from the North Purwokerto District of Banyumas, 8 isolates (PE21-PE28) from Kembaran Banyumas, and 3 isolates (PE 34, PE36, PE 37) from Nusawungu District of Cilacap (Table 1). The colonies of fluorescent *Pseudomonas* on King's B media had a round shape, flat edges, and a greenish-yellow color. On Kings B media with 3% KOH and ultraviolet light, the Gram test result showed that all the bacteria were gram-negative, rod-shaped, produce fluorescence, and had no spores. All bacterial isolates also produce oxidase and catalase enzymes (Table 1 and Figure 1). A similar findings was observed by Labhasetwar et al. (2019) and Khan et al. (2021) they reported that *P. fluorescens* exhibits round colonies, flat edges, fluidity, and the secretion of greenish-yellow pigments on King's B media. Individually, bacteria were rod-shaped with a size of 0.5-1.0 µm in diameter -1.5-4.0 µm in length. *P. fluorescens* was gram-negative that can form catalase enzymes and positive oxidase, essential components for aerobic growth. The hypersensitivity test results showed that all the bacterial isolates were incapable of causing necrotic symptoms on tobacco leaves (Figure 1.F). This indicates that the bacteria isolated from the endophytes of creeping-sensitive plant roots were not plant pathogens.

The in vitro test results (Table 2) showed that almost all isolates of fluorescent *Pseudomonas* were able to inhibit the growth of test pathogenic fungi. The fluorescent *Pseudomonas* isolate that had the highest percentage of inhibition in preventing the growth of several pathogenic fungi was PE13. Isolate PE37 failed to inhibit the fungus *Colletotrichum* sp. while three isolates (PE 21, PE25, and PE37) could not inhibit the growth of *Xanthomonas* sp. and *R. solanacearum*. The presence of inhibition zone around the colony of antagonistic bacteria or the inability of fungal mycelium to grow near the bacteria colony indicated the inhibited growth of test pathogen (Figure 2).

These results indicate that fluorescent *Pseudomonas* can produce bioactive compounds that inhibit fungal growth through antibiosis, competition, or lysis mechanisms. According to Maurya et al. (2024), bacteria can produce secondary metabolites which inhibit the growth or damage pathogens. Phenazine, 2,4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin, cyclic lipopeptides, and volatile organic compounds, including hydrogen cyanide, are some compounds that inhibit the growth of bacteria. *P. fluorescens* can produce various types of antibiotics, including phenazine-1-carboxylic acid, pyocyanin, pyrrolnitrin, pyoluteorin, 2,4-diacetylphloroglucinol (Phl). Phl is a phenol metabolite that has antibacterial and antifungal properties (Stepanov et al. 2022; Johnson et al. 2023).

It was observed that the pathogenic mycelia of the test fungus were degraded in the inhibition zone area (Figure 2). Hydrolysis enzymes produced by *Pseudomonas* influenced the destruction or lysis of mycelia. Riseh et al. (2024) stated that some antagonistic bacteria produce hydrolysis enzymes. These enzymes changed hyperparasitic activity which included the growth of biological control agents on the target organism, as well as the entanglement and destruction of the target cell wall. Some microbes, including *Pseudomonas*, produce many enzymes that affect pathogen wall breakdown (Bhunia and Meshram 2022).

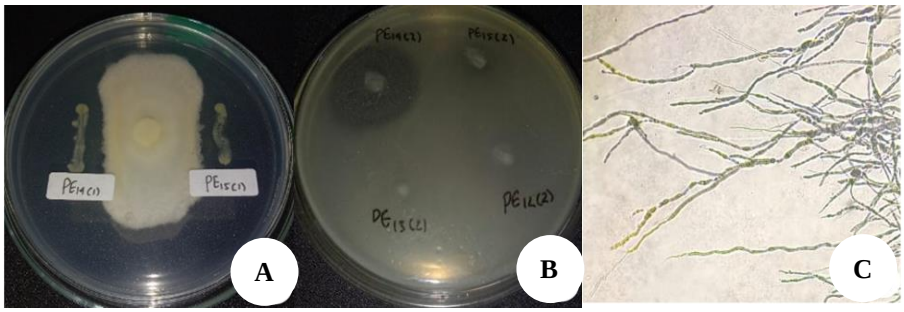

Figure 2. Pathogen growth inhibition. A. Fungal pathogen inhibition; B. Bacterial pathogen inhibition; C. Pathogen mycelia damage

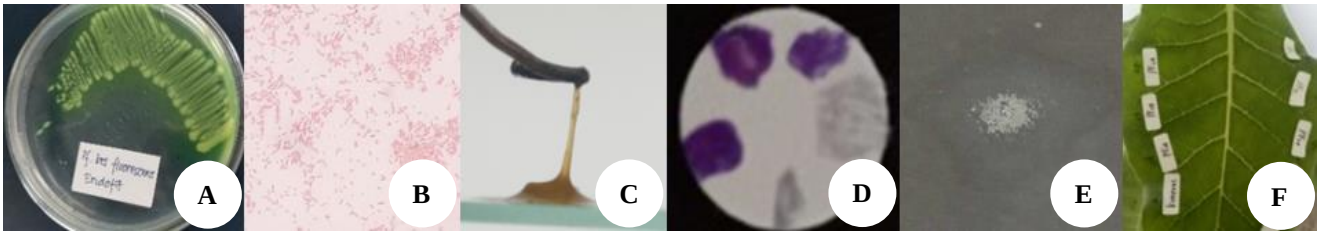

Figure 1. Morphological characters, biochemical test, and hypersensitive test of fluorescent *Pseudomonas*. A. Bacterial colonies; B. Rod shape bacterial cells; C. Gram-negative test; D. Positive catalase test results; E. Positive oxidase test results; F. Hypersensitivity test results

Table 1. Morphological, and hypersensitivity test of fluorescent *Pseudomonas*

Isolates	Size (mm)	Shape	Elevation	Margin	Pigmentation/ color	Fluor-escent	Gram test	Oxidase test	Catalase test	Hyper-sensitivity test
PE12	Small (2-3)	Circular	Flat	Entire	Yellow	+	-	+	+	-
PE13	Pinpoint (<1)	Circular	Flat	Entire	Yellow	+	-	+	+	-
PE14	Small (2-3)	Circular	Flat	Entire	Greenish yellow	+	-	+	+	-
PE15	Small (2-3)	Circular	Flat	Entire	Greenish yellow	+	-	+	+	-
PE21	Small (2-3)	Circular	Flat	Entire	Greenish white	+	-	+	+	-
PE22	Small (2-3)	Circular	Flat	Entire	Yellow	+	-	+	+	-
PE23	Small (2-3)	Circular	Flat	Entire	Yellowish white	+	-	+	+	-
PE24	Medium (4-5)	Circular	Flat	Entire	Yellow	+	-	+	+	-
PE25	Small (2-3)	Circular	Flat	Entire	Green	+	-	+	+	-
PE26	Small (2-3)	Circular	Flat	Entire	Yellow	+	-	+	+	-
PE27	Pinpoint (<1)	Circular	Flat	Entire	Yellow	+	-	+	+	-
PE28	Small (2-3)	Circular	Flat	Entire	Yellow	+	-	+	+	-
PE34	Pinpoint (<1)	Circular	Flat	Entire	Yellowish white	+	-	+	+	-
PE36	Small (2-3)	Circular	Flat	Entire	Yellowish white	+	-	+	+	-
PE37	Small (2-3)	Circular	Flat	Entire	Yellowish white	+	-	+	+	-

Notes: +: Positive reaction; -: Negative reaction

Table 2. Growth inhibition of plant pathogenic microbes by endophytic fluorescent *Pseudomonas*

Isolates	*Pythium* sp.[a]	*Fusarium* sp.[a]	*Colletotrichum* sp.[a]	*Xanthomonas*[b]	*Ralstonia solanacearum*[b]
PE12	45.08 ± 10.33	44.34 ± 0.83	40.15 ± 0.21	0.00 ± 0.00	0.29 ± 0.06
PE13	54.13 ± 11.45	50.27 ± 4.80	48.12 ± 5.83	0.00 ± 0.00	0.20 ± 0.05
PE14	45.55 ± 0.78	50.00 ± 0.00	33.08 ± 2.90	0.67 ± 0.08	0.45 ± 0.14
PE15	40.81 ± 5.93	44.14 ± 5.85	39.24 ± 1.85	0.05 ± 0.07	0.43 ± 0.18
PE21	38.86 ± 11.43	28.44 ± 0.19	25.4 ± 2.64	0.00 ± 0.00	0.00 ± 0.00
PE22	36.82 ± 8.55	7.12 ± 2.06	32.89 ± 4.91	0.00 ± 0.00	0.41± 0.13
PE23	44.42 ± 6.78	38.10 ± 6.74	30.50 ± 6.77	0.15 ± 0.07	0.00 ± 0.00
PE24	42.89 ± 7.62	45.08 ± 10.32	39.08 ± 5.35	0.05 ± 0.07	0.00 ± 0.00
PE25	47.49 ± 2.27	14.45 ± 3.80	41.37 ± 16.91	0.00 ± 0.00	0.00 ± 0.00
PE26	41.94 ± 10.12	11.51 ± 7.96	48.92 ± 10.96	0.15 ± 0.07	0.00 ± 0.00
PE27	25.90 ± 1.94	60.36 ± 0.50	44.25 ± 15.44	0.00 ± 0.00	0.08 ± 0.12
PE28	31.18 ± 11.94	26.75 ± 2.47	38.58 ± 13.70	0.00 ± 0.00	0.63 ± 0.05
PE34	56.79 ± 4.55	52.16 ± 8.95	37.57 ± 3.44	0.00 ± 0.00	0.13 ± 0.05
PE36	41.67 ± 3.92	26.13 ± 8.67	36.71 ± 1.41	0.00 ± 0.00	0.53 ± 0.04
PE37	30.51 ± 11.53	19.35 ± 0.00	0.00 ± 00.00	0.00 ± 0.00	0.00± 0.00

Notes: a: Percentage inhibition; b: Antibiosis Index

Fluorescent *Pseudomonas* exhibited a diverse range of inhibition levels. On average, fluorescent *Pseudomonas* PE13 was the best isolate to inhibit the growth of pathogenic fungi with an average inhibition of 50.83%, followed by PE43, PE27, PE12, PE14, PE 24, PE15 with an average inhibition above 40%. Fluorescent *Pseudomonas* PE14 demonstrated the best inhibition against both pathogenic bacteria, with an antibiosis index of 0.67 and 0.45 (Table 2). The varying ability of fluorescent *Pseudomonas* to produce fungal inhibitory compounds, either antibiotics, enzymes, or other toxic compounds, likely explains the differences in their abilities. According to Hossain et al. (2024), the ability of a biological agent to inhibit other microorganisms depends on the concentration of the material and the type of antimicrobial produced. The higher concentration of antimicrobial material, the greater its ability to inhibit pathogens, and the more expansive the clear zone or zone of inhibition.

Different results were observed in the production of hydrolytic enzymes like protease, lipase, chitinase, and cellulase by *Pseudomonas* bacteria (Table 3). All isolates of fluorescent *Pseudomonas* were able to produce lipase, with an index of 1.08-3.04. Fluorescent *Pseudomonas* isolates PE12, PE13, PE14, PE15, PE21, and PE22 produced protease, cellulase, and chitinase enzymes (Table 3 and Figure 3).

Clear zones around bacterial colonies grown on SMA media indicate the ability to produce protease enzymes. The presence of lipase enzymes was represented with a milky white color around the bacterial colonies grown on media containing 1% Tween 80. The ability of antagonistic bacteria to act as biological control agents is associated with protease and lipase enzymes (Gow et al. 2017). According

to Olanrewaju et al. (2017), protease enzymes can degrade fungal cell wall proteins, and lipase enzymes can degrade some lipids associated with the cell wall. The combination of two enzymes can help lyse the fungal cells. Lipids on the plasma membrane are important regulators of fungal pathogenicity. Several fungal species have been shown to confer virulence and to various glycolipids (Rizzo et al. 2021). Protease extracellular enzymes also play a role in inhibiting various pathogenic bacterial and fungal communities. Protease enzymes are effective for direct and indirect biocontrol of pathogenic fungi (Asad 2022; Riseh et al. 2024). Extracellular protease enzymes can inactivate antibiotic compounds produced by pathogens (Aqel et al. 2023).

Isolate PE14 demonstrated the ability of fluorescent *Pseudomonas* to produce chitinase enzyme, with a chitinolytic index of 4.0 (Table 3). The clear zone around the bacterial colony, visible on 4th day after inoculation (Figure 3), indicates the presence of chitinase enzyme. Chitinase is an enzyme that can degrade chitin, which, according to Veliz et al. (2017), is an important component of insect and fungal cell walls, nematode eggs, and some protists. The presence of chitinase enzymes weaken and degrade the cell walls of many pests and pathogens, thus exhibiting antibacterial, antifungal, insecticidal, or nematicidal activities.

Fluorescent *Pseudomonas* isolates PE12, PE13, PE14, PE15, PE21, and PE22 demonstrated the ability to produce cellulase enzyme. The clear zone around bacterial colonies grown on CMC-containing media revealed the presence of cellulase enzyme from the sixth day after inoculation (Figure 3). The hydrolytic enzyme cellulase degrades cellulose which is a constituent of the cell wall of plants. This helps endophytic microbes to enter and penetrate plant tissues. Generally, endophytic bacteria come from epiphytic rhizosphere and phylloplane bacterial communities, as well as from seeds or planting materials, which then live as endophytes. Endophytic bacteria can get into plants through natural holes or wounds, but they can also penetrate plant tissues with the hydrolytic enzyme cellulase (Rana et al. 2020; Dogan and Taskin 2021). There are some Oomycota fungi that make cellulose a part of the plant cell walls (Fawke et al. 2015), so cellulase enzymes inhibit the growth of some pathogenic fungi.

All fluorescent *Pseudomonas* isolates had the ability to produce siderophores (Table 3) with an ability index of 0.4-3.1. The media around the bacterial colonies change from blue to orange or yellowish, indicating the presence of siderophores (Figure 3.E). According to Himpsl and Mobley (2019), CAS agar media uses Chrome Azurol S (CAS) and Hexadecyl Trimethyl Ammonium Bromide (HDTMA) as indicators. The CAS/HDTMA complex with iron ions produces a blue color. When there is a strong iron-chelating compound such as siderophore, it removes iron from the dye complex, so the color changes from blue to orange.

Siderophores and their derivatives have wide applications in agriculture to improve soil fertility and biological control of pathogenic fungi (Xie et al. 2024). Iron ions are virulence components of microbes that infect plants (Pandey 2023). Secreted siderophores are perceived to play a role in the solubilization of this iron in soil because they have a strong affinity with the substrate and the ability to sequester it (Berenguer et al. 2019). The complex between iron and bacterial siderophores facilitates the availability of iron. This can be utilized for plant growth, and conversely, these molecules limit iron acquisition by phytopathogens, thus preventing their proliferation and virulence (Deb and Tatung 2024). Siderophores are also reduce the level of metal pollution in the environment, especially from soil and water (Roskova et al. 2022; Gomes et al. 2024).

Of the 15, 11 (73%) isolates of the antagonistic bacteria were able to produce HCN, with weak to strong categories (Table 3 and Figure 3). According to Gupta and Sinha (2020) and Sehrawat et al. (2022), HCN is a toxic and volatile secondary metabolite produced by many microorganisms, can act as an antimicrobial, insecticide, nematicide and herbicide, and has repellent activity. HCN synthesized by biocontrol agents works synergistically with other biocontrol methods, such as the presence of antibiotics or cell wall-degrading enzymes (Sehrawat et al. 2022).

Table 3. Biochemical tests of endophytic fluorescent *Pseudomonas*

Isolates	Biochemical traits						
	Lipase	Cellulase	Protease	Chitinase	HCN	Dissolve phosphate	Siderophores index
PE12	1.08	2.00	1.08	2.00	Slightly red	1.13	1.8
PE13	1.35	2.00	2.40	2.00	Red	1.18	2.1
PE14	1.39	2.20	1.67	4.00	Slightly red	1.13	2.1
PE15	1.33	2.00	2.33	2.40	Red	1.30	1.7
PE21	1.33	1.60	2.08	1.60	Red	1.14	1.9
PE22	1.65	1.60	1.87	0.00	Red	1.22	2.1
PE23	1.56	0.00	2.20	0.00	Slightly red	1.13	2.4
PE24	1.87	0.00	2.00	0.00	Red	1.27	2.1
PE25	1.11	0.00	1.50	0.00	Red	1.20	3.1
PE26	1.71	0.00	0.00	1.60	Yellow	1.00	1.3
PE27	1.30	0.00	2.20	0.00	Slightly red	1.27	0.4
PE28	1.80	0.00	2.22	0.00	Red	1.20	0.9
PE34	1.45	0.00	0.00	1.60	Yellow	1.00	0.6
PE36	3.40	0.00	0.00	2.00	Yellow	1.00	2.1
PE37	1.70	0.00	0.00	0.00	Yellow	1.25	0.4

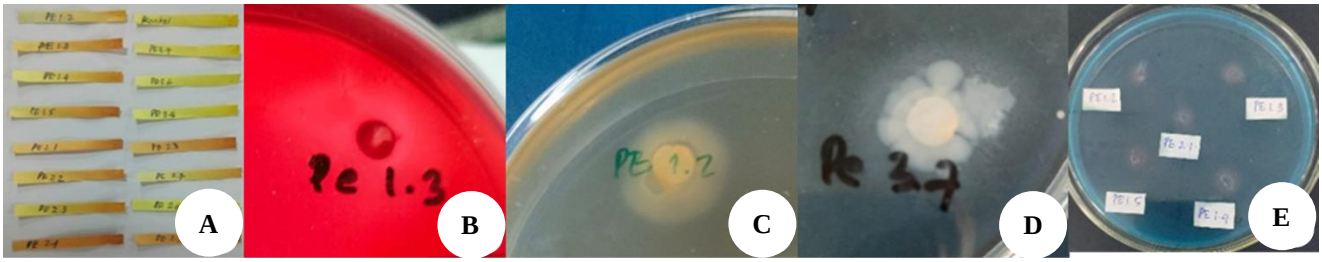

Figure 3. The results of metabolite compounds of fluorescent *Pseudomonas*. A. HCN; B. Cellulase test; C. Protease test; D, Lipase test; E. Siderophore test

Table 4. Cucumber seed growth in the treatment of fluorescent *Pseudomonas*

Isolates	Root length (cm)	Seedling height (cm)	Root fresh weight (mg)	Seedling fresh weight (mg)
Control	11.88 bc	17.00 abcd	20.75 g	119.88 g
PE12	9.94 bcde	15.00 bcdef	51.00 c	210.38 b
PE13	8.63 cde	13.31 cdef	50.63 c	190.38 c
PE14	12.13 bc	18.00 abc	51.00 c	170.38 e
PE15	11.50 bcd	17.56 abc	60.25 b	190.00 c
PE21	8.94 cde	14.75 bcdef	41.38 e	180.50 d
PE22	6.75 e	10.19 f	31.13 f	140.13 f
PE23	10.69 bcd	16.44 abcd	32.38 f	190.25 c
PE24	11.25 bcd	17.25 abcd	60.25 b	220.25 a
PE25	12.63 ab	19.00 ab	51.00 c	219.88 a
PE26	10.06 bcde	14.94 bcdef	41.63 e	190.38 c
PE27	8.06 de	11.38 ef	30.75 f	140.88 f
PE28	15.38 a	20.13 a	70.75 a	190.38 c
PE34	10.08 bcde	15.31 bcde	40.50 e	219.88 a
PE36	9.88 bcde	14.81 bcdef	40.13 e	190.38 c
PE37	7.98 de	12.75 def	40.25 e	180.13 d

Note: Numbers followed by the same letter in the same column indicate not significantly different according to DMRT at a 5% error rate

It was found that all isolates of fluorescent *Pseudomonas* were able to produce phosphate-solubilizing compounds with a medium solubility index (2-3). The clear zone around bacterial colonies grown on Pikovkaya media (Figure 3) demonstrates the ability to dissolve phosphate. It is perceived that the bacteria's phosphate solubilizing enzymes are correlated with their ability to dissolve phosphate. These enzymes convert insoluble organic and inorganic phosphate into a form that plants can easily use. According to Olanrewaju et al. (2017) and Elhaissoufi et al. (2022), the main phosphate solubilization mechanisms by plant growth-promoting microbes, include: (i) release of mineral solubilizing compounds such as organic acid anions, hydroxyl ions, protons, CO_2; (ii) release of extracellular enzymes for biochemical phosphate mineralization; and (iii) the release of phosphate during substrate degradation (biological phosphate mineralization). The genera *Bacillus, Rhizobium, Pseudomonas, Azotobacter, Arthrobacter, Serratia, Beijerinckia, Burkholderia, Enterobacter,* and *Azospirillum* are the most potent bacterial genera in aiding phosphate solubilization (Olanrewaju et al. 2017; Ushamalini et al. 2022).

The growth results revealed that isolate PE14, PE25, and PE28 had the ability to increase root length, plant height, root weight, and plant fresh weight, as compared to the control (Table 4). Fluorescent *Pseudomonas* isolates PE12, PE13, PE21, PE22, PE23, PE26, PE27, PE34, PE36, PE37 were not able to increase root length and seedling height, and all isolates of the *Pseudomonas fluorescens* group increased the fresh weight of roots and plants. These results indicate that all fluorescent *Pseudomonas* isolates tested have the potential to increase plant growth. These results are also supported by the ability of these bacteria to produce secondary metabolite compounds that can spur plant growth either directly or indirectly, such as their ability to produce phosphate-solubilizing compounds, compete with siderophores, HCN. According to Das et al. (2022) and Ali et al. (2024), the ability of microbes to increase plant growth is related to their ability to play a role in nutrient cycling, phytohormone synthesis, regulation of osmotic balance, stomatal regulation, modification of root morphology, increased mineral uptake and other changes in plant metabolism.

In conlusion, the exploration, isolation, and characterization of endophytic antagonistic bacteria of lowland creeping-sensitive plants obtained 15 isolates of endophytic fluorescent *Pseudomonas*. Exactly 80% of fluorescent *Pseudomonas* were able to inhibit the growth of several plant pathogens. Fluorescent *Pseudomonas* isolates demonstrate significant variability in their ability to inhibit pathogenic fungi and bacteria, with PE13 and PE14 showing the most effective inhibition and enzyme production capabilities. This ability was related to producing several hydrolysis enzymes (lipase, cellulase, protease, and chitinase), HCN, siderophores, and phosphate solubilizing compounds. Additionally, certain isolates positively influence plant growth parameters, highlighting their potential as beneficial agents in agricultural applications.

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