BILINGUAL PUBLISHING GROUP

Research in Ecology

https://journals.bilpubgroup.com/index.php/re

ARTICLE

Isolation and Identification of the Plant Growth-Promoting Bacterium *Pseudomonas fluorescens* by 16S rRNA Sequence Analysis Its Efficacy as a Bioinoculator

Naseer Jawad Kadhim * **, *Jawad Abdul Kadhim Kamal***

College of Agriculture, University of Al-Qadisiyah, Al Diwaniyah 58001, Iraq

ABSTRACT

The isolation of bacteria from the rhizosphere soil of different plants and locations in Diwaniyah Governorate and their diagnosis by two methods. Isolation and routine molecular diagnosis revealed ten bacterial isolates with the attributes of *P. fluorescens* out of fifteen local isolates that are represented by the following codes and sequences (P.f9, P.f8, P.f6, P.f5, P.f4, P.f2, P.f1, P.f14, P.f13, P.f11). Results also confirmed the diagnosis of bacterial isolates by biochemical and molecular tests using a specialized primer to amplify the bp698 region of the 16S ribosomal RNA gene, approved by Macrogen/Korea. The test efficiency in dissolving solid phosphate by *P. fluorescens* bacteria showed that the most effective is the (P.f1) isolate, giving the highest score effectiveness in mineral phosphate dissolution by the diameter of the clear zone around the colony, which was effective in phosphate dissolution up to 6.95 mm. The efficiency of the Nitrogen Fixation Test showed that the isolate (P.f5) scored the highest nitrogen-fixing efficiency amount with a value of 6.81 mg L^{-1}. The quantitative amount of the hormone for each of Auxins, Cytokinins, and Gibberellins was assayed; the results with isolate (P.f1) for IAA (Auxins) gave a concentration up to 28.6 µg ml^{-1}, which was the most, while the production of GA3 by isolate (P.f1) gave the maximum value of 36.7 µg ml^{-1}, and for synthesis of the hormone of Cytokinins represented by isolate (P.f2), the highest value in the production of Cytokinins hormone was recorded at 26.3 µg ml^{-1}.

Keywords: *Pseudomonas fluorescens*; PCR; Phosphate Dissolution; Nitrogen Fixation; Hormones

*CORRESPONDING AUTHOR:

Naseer Jawad Kadhim, College of Agriculture, University of Al-Qadisiyah, Al Diwaniyah 58001, Iraq; Email: naseer.jawad@qu.edu.iq

ARTICLE INFO

Received: 28 March 2025 | Revised: 22 April 2025 | Accepted: 23 April 2025 | Published Online: 22 May 2025
DOI: https://doi.org/10.30564/re.v7i2.9267

CITATION

Kadhim, N.J., Kamal, J.A.K., 2025. Isolation and Identification of the Plant Growth-Promoting Bacterium *Pseudomonas fluorescens* by 16S rRNA Sequence Analysis Its Efficacy as a Bioinoculator. Research in Ecology. 7(2): 83–92. DOI: https://doi.org/10.30564/re.v7i2.9267

1. Introduction

Polymerase chain reaction (PCR) is used in the diagnosis of bacteria. PCR technology is a qualitative multiplication of a specific piece of DNA enzymatically millions of times outside the body of the living organism in the presence of primers in a short time. This multiplication process depends on the ability of the DNA polymerase enzyme (DNA-Polymerase) to build complementary sequences to the template DNA piece in the presence of the primer and phosphorylated nitrogenous bases (dNTPs) in the reaction mixture. This activates a chain reaction in which an exponential amplification of the DNA template occurs.

Molecular diagnosis is one of the sensitive and important indicators and means of confirming microscopic and biochemical diagnosis of bacteria. It is one of the highly efficient methods of classification in the assessment of the evolutionary origin of bacterial species [1, 2]. Therefore, it has become very important to find a fast and accurate way to diagnose bacteria in general, including *P. fluorescens*, which is one of the most important microorganisms that has received special attention among the types of microorganisms as the largest and most powerful groups of plant growth-promoting bacteria (PGPR) found in the areas surrounding the root rhizosphere [3]. It is important in increasing the amount of dissolved phosphate, as many types of bacteria found in the soil play an effective role in dissolving phosphate minerals.

The role of a number of soil microorganisms in converting insoluble phosphate compounds into forms ready for the plant has become known. In this field, *P. fluorescens*, belonging to the genus Pseudomonas, is one of the microorganisms that analyze phosphate from its insoluble compounds in the soil to more ready forms. This is done through the biological production of organic acids or mineralization of organic phosphorus by phosphatase [4]. Vinod Babu et al. [5] also found that *Pseudomonas* spp. bacteria contain ammonia in the liquid culture medium compared to the control treatment, which is evidence that these bacteria fix atmospheric nitrogen. Many studies have also indicated the importance of these bacteria as a bioinoculation that increases nitrogen and phosphorus fixation in the soil and improves soil properties [6]. Nitrogen enters into the composition of many organic acids important in biological processes and also enters into the composition of nucleic acids DNA and RNA and works to stimulate the production of auxins, cytokinins, gibberellins, amino acids, proteins and chlorophyll synthesis. It has also been proven that some species of *Pseudomonas spp*. bacteria fix nitrogen in liquid cultures and produce hormones [7]. This study aimed to isolate and identify Pseudomonas fluorescens from rhizosphere soils in different areas of Diwaniyah Governorate using traditional (morphological, biochemical) and molecular (PCR of 16S rRNA gene) methods. The functional abilities of the isolates were evaluated, including phosphate solubilization, nitrogen fixation, and hormone (IAA, cytokinins, GA_3) production. Ten isolates were confirmed as P. fluorescens. Among them, P.f1 showed the highest phosphate solubilization and hormone (IAA, GA_3) production, while P.f5 had the highest nitrogen fixation, and P.f2 excelled in cytokinin production

2. Materials and Methods

Fifteen soil samples were collected from the rhizosphere of plants in different areas of Diwaniyah Governorate for the isolation and identification of *P. fluorescens*. The plants were extracted with their roots and associated soil, by making a circle around the plant which increases in its diameter to fit the root of the plant, then removed plants were shifted by hand until the soil adhered completely to the roots, and then the samples were transferred to polyethylene plastic bags. Several biological replicates were taken for each sample, and all those details were documented. The rhizosphere soil samples of different plants and locations were collected and processed for bacterial isolation using the decimal dilutions and plate counting technique. A loopful of each dilution was spread on the Pseudomonas agar medium, and the plates were incubated at 28 °C for 36 hours **Table 1**.

The distinct fluorescent dye-producing colonies were picked and streaked on King's B selective medium to obtain pure colonies for confirmatory diagnostic tests for *P. fluorescens*. Thereafter, the bacterial isolates were subjected to microscopic, cultural, and biochemical tests according to the methods of Palleroni [8], by studying their morphological features as per the manual of Atlas, Parks and Brown [9], and by studying their cultural characters, in view of confirming the identification of the bacterial isolates related to the species of *P. fluorescens* **Table 2**.

The genomic DNA samples of bacterial isolates (10 out of 15) were subjected to the Polymerase Chain Reaction

Table 1. Biological materials & chemicals.

No.	Biological Materials & Chemicals	Company/Country
1	FavorPrep Total DNA Mini Kit	FAVORGEN/Korea
2	Master Mix or GoTaq® Green Master Mix	Promega/USA
3	TAE buffer 10 X	Carl Roth/Germany
4	Red safe	Mebep Bio Science/China
5	6X Loading dye	Intron/Korea
6	Agarose	Carl Roth/Germany
7	Ladder 100	Transgen/China
8	Primer	Macrogen/Korea

Table 2. The sequence of primer used in this study.

Primer	Sequence	Primer Sequence	Size of Product (bp)
P. fluorescens;	F	TCAACCTGGGAACTGCATCC	698
16S ribosomal RNA gene	R	CAGACTGCGATCCGGACTAC	Primer design

technique based on the extraction of genomic DNA utilizing a Genomic DNA extraction kit for Gram-negative bacteria from the (FAVORGEN/Korea) company and the primer was prepared following the manufacturer's instructions **Tables 3** and **4**.

Table 3. Reaction components of PCR.

Component	25µL (Final Volume)
Component	25 µL (Final volume)
Master mix	12.5 µl
Forward primer	10 picomols μl^{-1} (1 µl)
Reverse primer	10 picomols μl^{-1} (1 µl)
DNA	1.5 µl

The isolates were tested for their ability to dissolve phosphates by inoculating them into Pikovskaya medium and incubating the plates at 30 ± 10 °C for 5 to 7 days. Bacterial colonies that dissolve phosphates can be recognized by the formation of a clear transparent halo around the colonies (**Figure 1**), which means insoluble phosphates are being dissolved. An equation was applied to express phosphate-dissolving ability in bacteria[10].

$$\text{Dissolution coefficient (IS)} = h'' + Hd/C \quad (1)$$

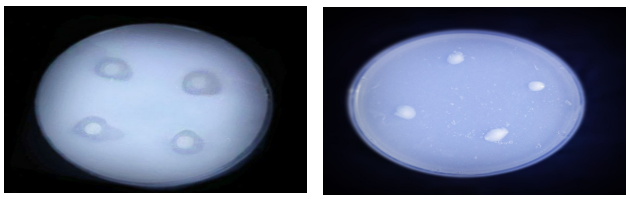

Figure 1. Formation of a clear, transparent halo around colonies on Pikovskaya agar medium.

Efficiency in nitrogen fixation by isolates Liquid media for *P. fluorescens* bacteria were prepared using Liquid Nutrient Broth while ensuring that a control sample is maintained without inoculation. The 250 ml conical flasks were filled with 50 ml of liquid media after which 1% mannitol solution was added to each of them. Inoculation was carried out by adding 1 ml of liquid culture of the different isolates into the bottles, after which they were incubated in a shaking incubator for three weeks at a temperature of 28 ℃. The ammonia formed in the media was estimated by pipetting out 2 ml of the media and estimating using the Micro-Kildahl apparatus[11]. Extraction of hormones produced by *Pseudomonas fluorescens* bacteria was estimated by preparing the nutrient liquid medium (N.B) and then placing 100 ml of this medium in (250) ml bottles and adding 0.2% of tryptophan to each bottle and sterilizing it by autoclaving at a temperature of 121 ℃ and a pressure of 1.5 bar for 15 minutes. Then the bottles were inoculated after cooling with a swab of *P. fluorescens* bacteria isolates, and then the isolates were incubated in a shaking incubator for 24 hours at a temperature of 27 ± 2 ℃. After the incubation period 1.5 ml of the bacterial culture was placed in Eppendorf tubes and centrifuged for 7 minutes at 7000 rpm. 1 ml of the filtrate was transferred to test tubes and 2 ml of reagent A was added and the tubes were left for (20–25) minutes for the reaction and change to occur Chromatography, absorbance was measured by HPLC[4], and the concentration of auxins, gibberellins and cytokinins was calculated according to Nepali, Bhattarai and Shrestha[12]. Salkowski A reagent: It is prepared by mixing 2 ml of 0.5 M

Table 4. The optimum condition of detection Lin0454.

No.	Phase	Tm (°C)	Time	No. of Cycle
1	Initial Denaturation	95 °C	3 min	1 cycle
2	Denaturation-2	92 °C	45 s	
3	Annealing	66 °C	45 s	30 cycles
4	Extension-1	72 °C	45 s	
5	Extension-2	72 °C	7 min	1 cycle

$FeCl_3$ with 98 ml of 35% perchloric acid[13].

3. Results and Discussion

Isolation and Identification of *Pseudomonas fluorescens* Bacteria The results presented in **Table 5** concerning the fifteen local isolates of *Pseudomonas* spp. obtained from the soil of the rhizosphere of various plants and localities of Diwaniya Governorate, based on cultural, microscopic, and biochemical characteristics, reveal that all isolates were found to show growth on MacConkey Agar medium and fluoresced under ultraviolet rays for their ability to produce Pyoverdin. These isolates were able to grow in King's B medium, which is a selective medium for these bacteria, and gave positive results for the oxidase and catalase tests, gelatin enzyme production, urease enzyme, Simmons citrate test, and growth at a reaction temperature of pH = 7.5–6.5, and were negative for the Gram stain test. As for the hydrogen sulfide production test, the isolates showed that they varied in the production of hydrogen sulfide (H_2S) and the indole production test. While the results of examining the Gram-stained slides under the microscope showed that these cells have straight, single or double rod-shaped, and mobile shapes, the morphological examination also showed that they are small, smooth, convex, raised, circular colonies with some large colonies, in addition to the presence of a distinctive smell for some isolates.

Table 5. Bacterial isolates identified as *Pseudomonas fluorescens*.

Test	Type of Isolation									
	P.f2	P.f3	P.f4	P.f5	P.f6	P.f8	P.f9	P.f11	P.f13	P.f4
Gram stain	-	-	-	-	-	-	-	-	-	-
Motility test	+	+	+	+	+	+	+	+	+	+
Cell shape	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods
Voges-Proskauer	+	+	-	+	+	+	+	+	+	+
H_2S production	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	-	-	+	-	-	-	-	-	-	-
Nitrate reduction	-	-	+	-	-	-	-	-	-	-
Indole production	-	-	-	-	-	-	-	-	-	-
Catalase test	+	+	+	+	+	+	+	+	+	+
Oxidase test	+	+	+	+	+	+	+	+	+	+
Urease test	+	+	+	+	+	+	+	+	+	+
Methyl Red test	-	-	-	+	-	+	-	-	+	-
MacConkey agar	+	+	+	+	+	+	+	+	+	+
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+
Citrate Simmons	+	+	+	+	+	+	+	+	+	+
Growth in 4 °C	+	+	-	+	+	+	+	+	+	+
Growth in 42 °C	-	-	+	-	-	-	-	-	-	-
pH = 6.5	+	+	+	+	+	+	+	+	+	+
pH = 7.5	+	+	+	+	+	+	+	+	+	+

Based on what was shown by studies that dealt with *Pseudomonas* bacteria, these characteristics are consistent with the microscopic and morphological characteristics of *Pseudomonas* spp. bacteria. Based on the results of the cul-

tural and microscopic examinations and biochemical tests shown in the table, it was shown that the ten isolates that took the following symbols and sequences (Ps1, Ps2, Ps4, Ps5, Ps6, Ps8, Ps9, Ps11, Ps13, Ps14) carry the characteristics of *Pseudomonas fluorescens* bacteria. The diagnostic results of *Pseudomonas fluorescens* bacteria were characterized by their production the fluorescent (yellowish green) pigments that were distributed in the medium when grown on King's B medium and under UV radiation, indicating their ability to produce Pyoverdin pigment, **Figure 2**. They could also grow at 4 °C but not at 42 °C. The isolates showed negative test results for Voges-Proskauer, starch hydrolysis, methyl red, and nitrate reduction. Similar results were reported by Kamil, ALjasani and ALShammari[13] and Al-Sajad and Alsalim[14].

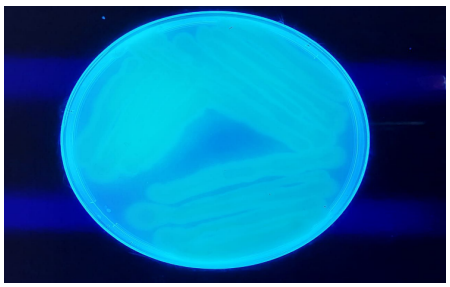

Figure 2. *Pseudomonas fluorescens* bacteria under UV light growing on King's medium.

The following isolates (Ps12, Ps10, Ps3) showed that they belong to the *Pseudomonas aeruginosa* species and their ability to grow in King's A medium and produce an orange phenazine pigment. They were also able to grow at a temperature of 4 °C. They were unable to grow at 42 °C and were positive for the starch hydrolysis test, which is consistent with what Polse, Khalid and Mero[15] found. The diagnostic results for the isolates Ps7 and Ps15 showed that they belong to the species Burkholderia cepacia and did not show any fluorescence characteristics, were positive for the hydrogen sulfide production test (H_2S) as well as for the indole test and starch hydrolysis. To confirm the diagnosis of the studied isolates, the Vitek 2 compact system was used to confirm the diagnosis of the species belonging to the genus *Pseudomonas* spp., as shown by the diagnostic forms in **Figure 2**.

3.1. Molecular Diagnosis of *Pseudomonas fluorescens* Isolates

Molecular diagnosis is one of the most sensitive and important indicators and means that confirm the microscopic and biochemical diagnosis of bacteria. It is one of the very efficient methods of classification with respect to determining the evolutionary origin of bacterial species because of its efficiency in molecular diagnosis and sensitivity to detect bacterial species with specialized primers, which has became a benchmark for bacterial classification[16]. After selection and microscopic, cultural, and biochemical diagnosis of ten isolates of *Pseudomonas fluorescens* from fifteen bacterial isolates, the following codes and sequences were obtained (Ps1, Ps2, Ps4, Ps5, Ps6, Ps8, Ps9, Ps11, Ps13, Ps14) to be molecularly tested by using polymerase chain reaction (PCR) technology to know the DNA sequence for these genes. The primers (16SPSEfluF, 16SPSEfluR) were applied to amplify the 16S rRNA gene. The analysis of the initial study of the extracted DNA of *Pseudomonas fluorescens* isolates showed DNA bands, as apparent in **Figure 3**. This proves the method of extraction for accuracy. After the bacterial strains produced clear bands of molecular weight at 698 bp for 16S rRNA, which is the size expected with this primer couple pair from the DNA of *Pseudomonas fluorescens* bacteria, with other complementary sequences on the template DNA chain while not from others. This was evident in the electrophoresis results of the agarose gel for amplification products that verified all these isolates to be bacteria of the *Pseudomonas fluorescens* type. This finding was affirmed by what was obtained[17, 18].

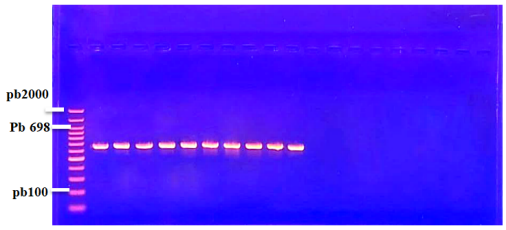

Figure 3. Products of DNA extracted from Pseudomonas fluorescens and phased on 1.5% agarose gel.

The results of **Table 6** reveal the identification of the isolates by the polymerase chain reaction technique after DNA extraction of the bacterial isolates and sending them to Microgen company in South Korea for performing Sconcesink analysis. The results were showed the matching rates between 87–99% based on the 16S rRNA genetic sequence. *Pseudomonas fluorescens* isolates showed, in addition to their ability to increase phosphorus availability, a tendency to fix nitrogen and produce hormones. Accordingly, the isolate Ps1 was chosen as superior to the rest of the isolates

based on the set of tests. Its DNA was extracted and the genetic sequence was obtained for the purpose of classifying it and indicating its groups in the genetic kinship tree, which was registered by us under the name *Pseudomonas fluorescens* Iraq according to the data of the Gene Bank (NCBI) No. PQ393131.

Table 6. Results of isolate diagnosis using the 16S rRNA gene polymerase chain reaction technique.

No.	Sequence ID with Compare	Source	Identities
Ps1	ID: MG819229.1	>*Pseudomonas fluorescens* strain AL120 16S rRNA gene,	99%
Ps2	ID: MG819229.1	>*Pseudomonas fluorescens* strain AL120 16S rRNA gene,	99%
Ps4	ID: MG819229.1	>*Pseudomonas fluorescens* AL120 16S rRNA gene,	88%
Ps5	ID: MG819229.1	>*Pseudomonas fluorescens* AL120 16S rRNA gene,	88%
Ps6	ID: MG819229.1	>*Pseudomonas fluorescens* AL120 16S rRNA gene,	97%
Ps8	ID: MG819229.1	>*Pseudomonas fluorescens* AL120 16S rRNA gene,	95%
Ps9	ID: MG819229.1	>*Pseudomonas fluorescens* strain AL120 16S rRNA gene,	95%
Ps11	ID: MG819229.1	>*Pseudomonas fluorescens* strain AL120 16S rRNA gene,	88%
Ps13	ID: MG819229.1	>*Pseudomonas fluorescens* strain AL120 16S rRNA gene,	97%
Ps14	ID: MG819229.1	>*Pseudomonas fluorescens* strain AL120 16S rRNA gene,	95%

The evolutionary history was inferred using the maximum likelihood method and the model [19], and the *Pseudomonas fluorescens* isolates represent the tree with the highest log likelihood (314.69–) in which the percentage of clusters (groups) in which related taxa are grouped together next to the branches is displayed. The initial tree for the experimental search was obtained by applying the neighbor-joining method to the genetic dimension matrix between pairs estimated according to the Jukes-Cantor method. **Figure 4** shows that the isolate showed a high similarity rate of up to 100%. The evolutionary analyses were conducted in MEGA11 [20].

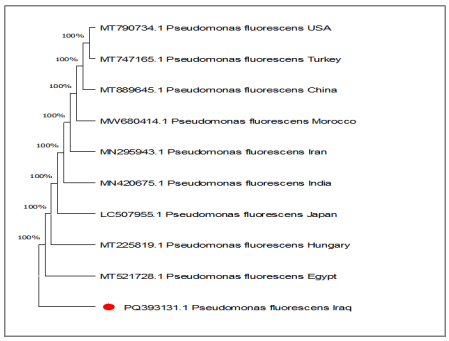

Figure 4. Phylogenetic tree from MEGA11 analysis of *Pseudomonas fluorescens* isolates [21].

3.2. Results of Testing the Efficiency of *Pseudomonas fluorescens* Isolates in Dissolving Phosphate and Fixing Nitrogen

The results in **Table 7** indicated that all bacterial isolates identified as *Pseudomonas fluorescens* showed varying efficiency in dissolving solid phosphate, which was indicated by the formation of a clear, transparent halo around the colonies. The dissolution area around the colonies on solid Pikovskaya agar medium after three days of incubation at 28 °C ranged between 2–11 mm, while the size of the bacterial colonies varied and was within the range of 2–4 mm. Among all the isolates, some isolates were found to be highly effective in dissolving phosphate. The efficiency of the isolate Ps1 in dissolving phosphate minerals through the diameter of the clear area around the colony was effective in dissolving phosphate, reaching 6.95 mm. Good followed it, in this regard, with Ps11 isolates where the dissolution factor reached 6.50 mm while the least effectiveness in dissolving phosphate was for the isolate Ps4 of the same bacterial type with a value of 2.18 mm. This test confirms the role of *Pseudomonas fluorescens* bacteria in solid phosphate dissolution, whereas the size variation of the dissolved zone might give an indication of their ability to produce organic acids. These organic acids reduce the pH around the bacterial colonies, which means that the phosphatase enzyme responsible for phosphate dissolution as well as the type and nature of the other organic acids are led by lactic acid mainly, which is formed in a great concentration and proved very effective in dissolving insoluble compounds of phosphorus. Although it is qualitative and not quantitative, this test confirms that these organisms have efficiency in dissolving mineral phosphorus in tricalcium phosphate medium which in part explains their efficiency to increase the availability of phosphorus in the inoculated soil.

The results are in corroboration with the findings of Fouzia et al.[22] and Nagpal[23] in their laboratory study of bacteria belonging to the genus *Pseudomonas* sp., including the species *Pseudomonas fluorescens*, which recorded the ability of this type of bacteria to dissolve phosphorus in a laboratory experiment. The effectiveness of dissolving phosphate is estimated by the ability of biochemical organisms to form organic and inorganic acids. This process can be considered one of the most important mechanisms agreed upon by some researchers, and inorganic phosphate compounds are dissolved through it, as most microorganisms in the soil carry out the dissolution process by producing inorganic acids (phosphoric, sulfuric and nitric) as well as organic acids (humic, fulvic, citric, oxalic, licinic acid)[24]. Regarding the nitrogen fixation efficiency of the Pseudomonas fluorescens isolates described in **Table 8** below, these results indicated that these isolates fix nitrogen at varying efficiencies after one week of incubation on liquid nutrient medium N.B at 30 °C. Isolate Ps1 was best at accumulating a large quantity of fixed nitrogen, as it managed to accumulate the highest quantity of biologically fixed nitrogen, which was as high as 6.81 mg L^{-1}. Isolate Ps14 recorded the least amount of fixed nitrogen, which was as low as 2.1 mg L^{-1}.The efficiency of nitrogen fixation by *Pseudomonas fluorescens* is due to the role of the biological secretions secreted by this type of bacteria, including enzymes, organic acids, inorganic acids, and plant hormones that work to increase the fixation of atmospheric nitrogen, and this was confirmed by Vinod Babu et al.[5] and Tian, Zhang and Ju[25]. The difference in the values of phosphate solubilization coefficient and fixed nitrogen between bacterial isolates of the species Pseudomonas fluorescens may be due to genetic differences between the isolates, which may affect the ability and efficiency of microorganisms to solubilize insoluble phosphate, or the reason may be due to the conditions of the growth environment or the difference in the dissolution mechanisms by organisms and the variation in their ability to produce organic acids and the nature and type of organic acid produced, which is what Saad, Ghanem and Hassan[26] claimed.

Table 7. Efficiency of *Pseudomonas fluorescens* bacterial isolates in dissolving phosphate and fixing nitrogen.

Isolation Symbol	Soluble Phosphate			Amount of N_2 Fixed mg L^{-1}
	Colony Diameter mm	Zone of Halo	SI	
Ps1	2.0	11.9	6.95	6.81
Ps2	2.2	8.21	4.73	5.53
Ps4	2.6	2.2	2.18	2.33
Ps5	2.2	7.77	4.55	6.62
Ps6	4.0	8.7	5.77	4.72
Ps8	3.7	8.54	5.66	4.25
Ps9	3.5	2.6	2.79	1.71
Ps11	4.11	10.2	6.50	3.54
Ps13	3.1	6.5	4.36	5.33
Ps14	3.2	2.7	2.69	2.1

(SI) = (colony diameter + zone of halo)/colony diameter

3.3. Testing the Efficiency of *Pseudomonas fluorescens* Isolates in Hormone Production

The results presented in **Table 8** reflect the potentials of *Pseudomonas fluorescens* isolates in hormone production determined by HPLC method as in the chromatogram depicted in **Figure 5** for the isolate Ps1. The results indicate that Pseudomonas fluorescens isolates varied in their concentrations of respective hormone productions i.e., indole-3-acetic acid, gibberellins and cytokinins. The highest concentration of the indole-3-acetic acid hormone was recorded by the isolate Ps1 at 28.6 mg L^{-1}, while the lowest concentration of this hormone was also produced by the isolate Ps6 at a value of 18.5 mg L^{-1}. In terms of the concentration of the hormone, isolate Ps1 had the highest concentration of gibberellins at 36.7 mg L^{-1} and the lowest concentration of the hormone was in isolate Ps9 at 22.3 mg L^{-1} while the production of cytokinins hormone, isolate Ps2 had the highest concentration of the hormone at 22.9 mg L^{-1}, and the lowest concentration of the hormone in the isolate Ps14 was 13.9 mg L^{-1}. The

results show that the isolates belonging to the same type of bacteria differed in their production level of each of indole-3-acetic acid, gibberellins and cytokinins, as some isolates have a high production amount of gibberellins and low indole-3-acetic Acid and vice versa for cytokinins, as well as there is a difference in the concentration of hormones among the ten isolates. The difference between the isolates may be due to the genetic variation of these isolates among themselves and the environment from which they were isolated and the extent of its reflection on the efficiency of bacteria, and this difference is often reflected in their biological properties, including their secretions in the growth medium. These results are consistent with Ng et al. [27], Basmal and Kusumawati [28], and Kejela [29].

Table 8. Results of the efficiency of *Pseudomonas fluorescens* isolates in hormone production.

Isolated Symbol	IAA mg L^{-1}	GA3 mg L^{-1}	CK mg L^{-1}
Ps1	28.6	36.7	24.9
Ps2	24.424	36.2	26.3
Ps4	24.6	32.5	15.3
Ps5	24.927	34.2	22.6
Ps6	8.51	26.7	22.1
Ps8	24.923	26.6	18.7
Ps9	24.725	22.3	18.8
Ps11	24.0	29.5	22.5
Ps13	20.2	28.0	20.7
Ps14	22.2	24.2	13.9

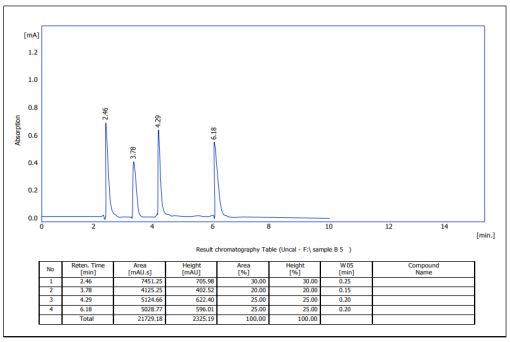

Figure 5. Chromatogram for the determination of hormones in the isolate Ps1 using HPLC technique.

4. Conclusions

Bacterial isolates belonging to the *P. fluorescens* type varied in their efficiency in dissolving phosphate, fixing nitrogen, and producing hormones, with a clear superiority for some of these isolates. The isolates P.f1 and P.f2 were the best among the local isolates isolated from the rhizosphere of the plant (alfalfa, barley).

Molecular diagnosis can be relied upon using polymerase chain reaction (PCR) technology to diagnose plant growth-stimulating bacteria spread in the rhizosphere of different plants as an alternative to conducting a series of different biochemical tests.

Author Contributions

Conceptualization, N.J.K. and J.A.K.K.; methodology, N.J.K. and J.A.K.K.; validation, N.J.K. and J.A.K.K.; formal analysis, N.J.K.; investigation, N.J.K. and J.A.K.K.; resources, N.J.K.; data curation, N.J.K. and J.A.K.K.; writing—original draft preparation, N.J.K. and J.A.K.K.; writing—review and editing, N.J.K. and J.A.K.K.; visualization, N.J.K. and J.A.K.K.; supervision, N.J.K. and J.A.K.K.; project administration, N.J.K. and J.A.K.K.; funding acquisition, N.J.K. and J.A.K.K. All authors have read and agreed to the published version of the manuscript.

Funding

This work received no external funding.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

Not applicable.

Conflicts of Interest

The author declares no conflict of interest.

References

[1] Xu, Y., Chen, W., You, C., et al., 2017. Development of a multiplex PCR assay for detection of Pseudomonas fluorescens with biofilm formation ability. Journal of Food Science. 82(10), 2337–2342.

[2] Drancourt, M., Bollet, C., Carlioz, A., et al., 2000. 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. Journal of clinical microbiology. 38(10), 3623–3630.

[3] Rojas-Sánchez, B., Castelán-Sánchez, H., Garfias-Zamora, E.Y., et al., 2024. Diversity of the maize root endosphere and rhizosphere microbiomes modulated by the inoculation with pseudomonas fluorescens UM270 in a milpa system. Plants. 13(7), 954.

[4] Yadav, A., Yadav, K., Vashistha, A., 2016. Phosphate solubilizing activity of Pseudomonas fluorescens PSM1 isolated from wheat rhizosphere. Journal of Applied and Natural Science. 8(1), 93.

[5] Vinod Babu, S., Gopal, A.V., Trimurtulu, N., et al., 2020. Isolation, screening and characterization of silica solubilizing bacteria from direct sown paddy (*Oryza sativa* L.) growing areas of Andhra Pradesh. International Journal of Current Microbiology and Applied Sciences. 9(10), 4054–4066.

[6] Hamid, M.Q., 2025. Response of physical properties of sandy soil treated with different levels of natural soil conditioners zeolite and perlite. Sarhad Journal of Agriculture, 41(2), 591–599.

[7] Shahzad, R., Khan, A.L., Bilal, S., et al., 2017. Plant growth-promoting endophytic bacteria versus pathogenic infections: An example of *Bacillus amyloliquefaciens* RWL-1 and *Fusarium oxysporum* f. sp. lycopersici in tomato. PeerJ. 5, e3107.

[8] Palleroni, N.J., 1984. Family I. Pseudomonadaceae Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith, 1917, 555. In: Krieg, N.R., Holt, J.G. (eds.). Bergey's Manual of Systematic Bacteriology. Williams & Wilkins: Baltimore, MD, USA. pp. 141–213.

[9] Atlas, R.M., Parks, L.C., Brown, A.E., 1995. Laboratory Manual of Experimental Microbiology. Mosby-Year Book, Inc.: Saint Louis, MO, USA.

[10] Glickmann, E., Dessaux, Y., 1995. A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Applied and Environmental Microbiology. 61(2), 793–796.

[11] Gordon, S.A., Weber, R.P., 1951. Colorimetric estimation of indoleacetic acid. Plant Physiology. 26(1), 192.

[12] Nepali, B., Bhattarai, S., Shrestha, J., 2018. Identification of Pseudomonas fluorescens using different biochemical tests. International Journal of Applied Biology. 2(2), 27–32.

[13] Kamil, S.H., ALjasani, R.F., ALShammari, H.I., 2023. Isolation, identefecation and effecincy of Pseudomonas fluorescens bacteria to termite microcerotermis diversus. Iraqi Journal of Agricultural Sciences. 54(6), 1583–1593.

[14] Al-Sajad, M.S., Alsalim, H.A.A., 2024. Detection of genes responsible for heavy metals resistance in locally isolated *Pseudomonas* spp. Iraqi Journal of Agricultural Sciences. 55(1), 361–370.

[15] Polse, R.F., Khalid, H.M., Mero, W.M.S., 2024. Molecular identification and detection of virulence genes among Pseudomonas aeruginosa isolated from burns infections. Journal of Contemporary Medical Sciences. 10(1), 7–12.

[16] Hamid, M.Q., 2025. Mycorrhiza and Trichoderma fungi role in improving soil physical properties planted with maize (*Zea mays* L.). SABRAO Journal of Breeding and Genetics. 57(1), 260–269.

[17] Al-Zayadi, A.M., Jarallah, R.S., 2025. Effect of bioinoculum and crop waste at the concentration of N, P, K and the sustainability of natural resources in soil planted with wheat. IOP Conference Series: Earth and Environmental Science. 1449(1), 012109.

[18] Al-Zayadi, A.M., Jarallah, R.S., 2023. Impact of fertilizer type and vegetative growth duration on the availability of copper in clay loam soil planted of maize (*Zea mays* L.) in Iraq. IOP Conference Series: Earth and Environmental Science. 1259(1), 012002.

[19] Jukes, T.H., Cantor, C.R., 1969. Evolution of protein molecules. Mammalian Protein Metabolism. 3(21), 132.

[20] Kumar, S., Sindhu, S.S., Kumar, R., 2022. Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. Current Research in Microbial Sciences. 3, 100094.

[21] Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution. 38(7), 3022–3027.

[22] Fouzia, A., Allaoua, S., Hafsa, C.S., Mostefa, G., 2015. Plant growth promoting and antagonistic traits of indigenous fluorescent Pseudomonas spp. isolated from wheat rhizosphere and A. halimus endosphere. European Scientific Journal. 11(24), 201–201.

[23] Nagpal, S., Sharma, P., Sirari, A., et al., 2021. Chickpea (*Cicer arietinum* L.) as model legume for decoding the co-existence of *Pseudomonas fluorescens* and *Mesorhizobium* sp. as bio-fertilizer under diverse agro-climatic zones. Microbiological Research. 247, 126720.

[24] Hundi, H.K., Hamid, M.Q., Noori, A.A.M., 2025. Role

of Ochrobactrum Bacteria and Organic Matter in Plant Growth and the Content of N, P, and K Under Soil Salinity Stress. Journal of Environmental & Earth Sciences. 7(5), 130–139.

[25] Tian, X., Zhang, C., Ju, X., 2023. Crop responses to application of optimum nitrogen fertilizers on soils of various fertilities formed from long-term fertilization regimes. European Journal of Agronomy. 148, 126857.

[26] Saad, T.M., Ghanem, B.N., Hassan, J., 2016. Study of the effect of addition of bacteriological vaccine using Rizophyosarum strains and the addition of Vicia faba. Al-Muthanna Journal of Agricultural Sciences. 4(1), 1–16.

[27] Ng, C.W.W., Yan, W.H., Tsim, K.W.K., et al., 2022. Effects of Bacillus subtilis and Pseudomonas fluorescens as the soil amendment. Heliyon, 8(11), eq11674.

[28] Basmal, J., Kusumawati, R., 2023. The role of microorganisms in solid biofertilizer production. IOP Conference Series: Earth and Environmental Science. 1137(1), 012037.

[29] Kejela, T., 2024. Phytohormone-producing rhizobacteria and their role in plant growth. In: Ali, B., Iqbal, J. (eds.). New Insights into Phytohormones. IntechOpen: London, UK.