Acta Solum 3, Number 3 (July 2025), 144–150. DOI: 10.20527/actasolum.v3i3.3419
http://jtam.ulm.ac.id/index.php/actasolum e-ISSN: 2987-5145

Exploration of Bacteria in Red Chili Plant Soil with Potential as *Plant Growth Promoting Rhizobacteria (PGPR)*

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Article Information

Received August 1st 2025
Accepted September 25th 2025
Published September 30th 2025
Online September 30th 2025

Keywords:

Nitrogen; *Phosphate; Plant growth-gromoting rhizobacteria (PGPR); Rhizobacteria*

Abstract

Soil fertility depends on the availability of sufficient nutrients for plant absorption. Chemical fertilizers can be used to provide sufficient N and P, but this affects soil health. The presence of rhizobacteria act as biofertilizers by increasing nutrients and altering soil formation, especially in the phosphate and nitrogen cycles. Phosphate-solubilizing and nitrogen-fixing bacteria play a role in increasing soil fertility and improving unstable soil properties. This study aims to identify rhizobacteria that have the potential as PGPR to increase N and P levels in the soil. Soil samples were collected randomly at a depth of 10 cm around chili plant roots. Bacteria were isolated using serial dilution and cultured on *Pikovskaya medium*. A total of 27 isolates produced clear zones with the highest solubility index and were inoculated on *Nitrogen-Free Bromthymol Blue Agar (NFB)* to evaluate N fixation through a color change from green to blue, as well as hypersensitivity tests on tobacco plants. Isolates showing the highest P solubility, highest N fixation ability, and no necrosis in tobacco were inoculated into sterile soil to analyze compound changes. Soil N content was measured using the Kjeldahl method, while phosphate levels were analyzed using the Bray I and HCl methods. The results of the study showed that the addition of KE2.15 isolate to the soil after 4 weeks of application increased the total organic N and available P content, making it a potential biofertilizer.

1. Introduction

Red chili peppers *(Capsicum annum* L.*)* are a type of horticultural crop used as a food seasoning that contains nutrients such as vitamins and minerals (Amalia and Ziaulhaq, 2022). The increasing demand for red chili peppers in the market has resulted in a decrease in the supply of red chili peppers, leading to an increase in local cultivation and farming. However, the decline in red chili pepper production is influenced by several factors, one of which is soil fertility. Fertile soil is soil that is able to provide sufficient nutrients that are readily available for absorption by plants (Wei *et al*., 2024). Essential nutrients required by plants in large quantities are nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur (Sirappa et al*.,* 2019).

Red chili plants grow well in a pH range of 5.5-6.8, with an optimum pH of 6.0-6.5. Chili plants in acidic soil conditions can experience aluminum (Al), iron (Fe), and manganese (Mn) toxicity. These elements can also bind with phosphate, limiting the availability of phosphate in the soil and adversely affecting agricultural yields (Adnan et al*.,* 2025). Meanwhile, alkaline soils have relatively low levels of nitrogen (N), iron (Fe), manganese (Mg), copper (Cu), and zinc (Zn) (Daniel *et al*., 2022).

Nitrogen (N) and Phosphate (P) are important macronutrients in supporting the growth and development of red chili plants. Nitrogen functions as a component of protoplasm, chlorophyll molecules, nucleic acids, and amino acids, which are components of proteins (Prakoso et al*.,* 2022), Phosphorus has important functions in plants, namely during respiration, photosynthesis, stimulating root development, and germination (Khastini et al*.,* 2024). The availability of phosphate and nitrogen in the soil can be achieved by applying inorganic fertilizers, but this has an impact on the soil environment and is relatively expensive (Zhang et al*.,* 2022; Wu and Ge, 2019). Therefore,

various efforts have been made to reduce the use of inorganic fertilizers, one of which is the application of biofertilizers. Several soil microbes are known to have the potential to be used as biofertilizers.

Plant growth-promoting rhizobacteria (PGPR) are a group of soil bacteria around plant roots or rhizosphere that can act as biological fertilizers by increasing nutrients for plants (Hasan et al., 2024). Microbes are involved in various processes that influence soil formation, particularly in the phosphorus and nitrogen cycle (Timofeeva et al., 2023). Phosphate-solubilizing bacteria act as biological agents in dissolving phosphate minerals because they are capable of secreting phosphatase enzymes that play a role in the hydrolysis of organic P into inorganic P. Phosphate-solubilizing bacteria also produce organic acids, thereby lowering the pH (Tarigan et al., 2023). Nitrogen-fixing bacteria are mostly symbiotic with legumes, but there are also non-symbiotic bacteria (Patra and Mandal, 2023). Nitrogen-fixing bacteria produce the enzyme nitrogenase, enabling them to naturally convert atmospheric nitrogen (N_2) into harmless soluble forms (mainly NH_4^+ and NH_3) that plant cells use to synthesize various biomolecules (Shah and Wu, 2019; Greed, 2023).

Phosphate-solubilizing rhizobacteria and nitrogen-fixing rhizobacteria can improve soil formulation by increasing total P and total N in the soil (Saida et al., 2024). The application of PGPR can increase plant growth and red chili yield in terms of plant height, total number of branches, number of fruits, and fruit weight (Ichwan et al., 2021). Crop quality also improves with the application of PGPR. Management of nutrient availability is essential to avoid adverse effects on soil fertility and crop production (Xing et al., 2025). Therefore, it is important to note that rhizobacteria have the potential to act as PGPR, dissolving phosphate and fixing nitrogen, thereby increasing the availability of phosphate and nitrogen in the soil.

2. Research Method

2.1. Research Location

The material used was soil samples from red chili *(Capsicum annum* L.*)* plantations in Cirebon, taken using a composite method from five points. The points were selected randomly based on land elevation, with soil samples taken at a depth of approximately 10 cm from the soil surface and approximately 5 cm from the plants. Testing and analysis of the samples were carried out at the Laboratory of the Department of Microbiology and the Laboratory of the Department of Soil Science and Land Resources, IPB University.

2.2. Research Implementation

Isolation and purification of phosphate-solubilizing bacteria using *Pikovskaya Agar* medium by forming clear zones (Tarigan et al., 2023). Isolates of phosphate-solubilizing bacteria on *Pikovskaya Agar* medium were measured for clear zones after 2 days of incubation at room temperature. The solubilizing index (SI) was calculated based on the diameter of the clear zone of the bacterial colony according to the equation.

$$\mathrm{SI} = \frac{\text{Total clear zone diameter} - \text{Colony diameter}}{\text{Colony diameter}}$$

Colony identification of isolates was observed based on shape, edges, elevation, and color, while morphological identification of phosphate-solubilizing bacteria was performed using Gram staining. The ability of nitrogen-fixing bacteria was inoculated on *Nitrogen Free Bromthymol Blue Agar (NfB)* medium and then incubated at room temperature (30°C) for 7 days. A positive reaction was indicated by a color change from greenish-yellow to blue on the NfB Agar medium (Widya et al., 2024). Test the hypersensitivity of bacterial isolates with a density of $\pm 10^8$ cells/mL in liquid culture infiltrated on the underside of tobacco leaves using a 1 mL syringe (without a needle). Pseudomonas syringae, a plant pathogen, was used as a positive control, while distilled water and media were used as negative controls (Tarigan et al., 2023).

2.3. Data Analysis

Soil analysis was conducted to determine changes in available P and total N content in the soil before and after the addition of selected isolates. The addition of isolates to the soil was carried out using a 100 mL syringe (without a needle) with a density of ±108 cells/mL. The isolates were administered once a week for 4 weeks. Total N content in soil samples was analyzed using the Kjeldahl method, while available P content was analyzed using the Bray I method (Sukamto and Rahmat, 2023).

3. Results and Discussion

3.1. Testing the Ability of Phosphate-Solubilizing Bacteria (PSB)

The results of phosphate-solubilizing bacteria isolation in soil samples around red chili plantations yielded 27 phosphate-solubilizing isolates marked by clear zones around the colonies (Figure 1). Phosphate-solubilizing bacteria solubilize bound P due to the dissolution of $Ca_3(PO_4)_2$ in *Pikovskaya Agar* medium (Tarigan et al., 2023).

Based on the observation results of 27 bacterial isolates, different elevation and colony color characteristics were observed, while Gram staining results generally showed that they belonged to the gram-negative bacteria group, characterized by red-colored colonies in the form of bacilli and coccus (Figure 1. Table 1).

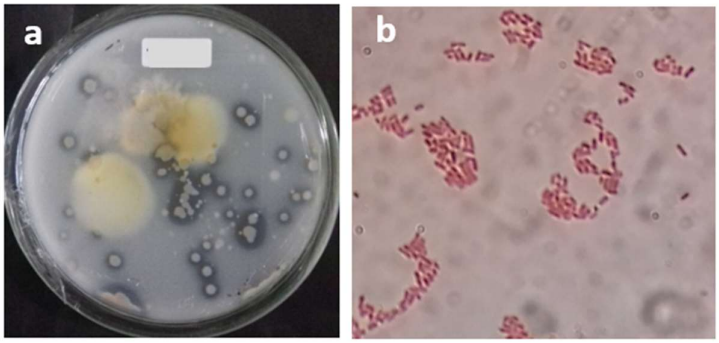

Figure 1. (a) Bacterial isolates on pikovskaya medium and (b) gram staining

Qualitative testing of the isolates' ability to dissolve phosphate was indicated by the presence of a clear zone around the colony by calculating the Dissolution Index (DI). Based on the dissolution index values of the isolates capable of dissolving the highest P (Figure 2, Table 1), there were differences in the dissolution index values of each isolate. The differences in the dissolution index of colonies growing on *Pikovskaya Agar* in terms of the speed of clear zone formation and the area of the clear zone suggest that there are differences in the quantity and quality of organic acids excreted by each bacterial species (Saida et al*.,* 2024).

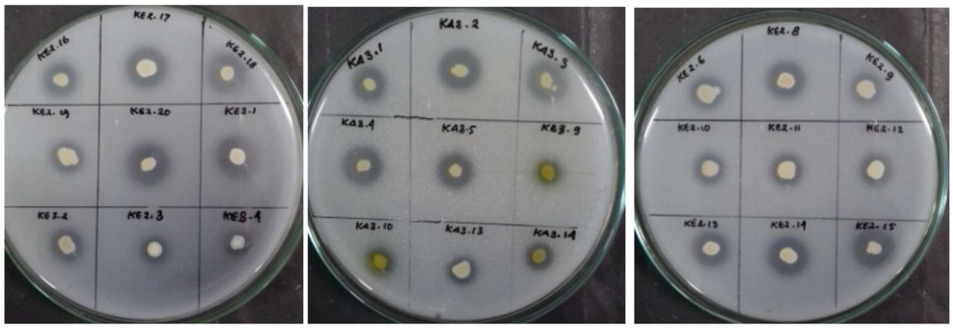

Figure 2. Testing the ability of bacterial isolates to dissolve P in pikovskaya medium

The variation in solubility index (SI) values observed among isolates can be explained by differences in phosphate solubilization mechanisms. The phosphate solubilization mechanism is determined by the ability of microbes to produce organic acids and protons, through the binding of hydroxyl groups with phosphate cations, thereby converting them into soluble forms (Pan and Cai, 2023). According to Mohamed et al*.,* 2018) states that the dissolution process of P occurs due to the release of protons such as H+, which react with tricalcium phosphate $Ca_3(PO_4)_2$ present in the Pikovskaya medium, described as follows: $Ca_3(PO_4)_2 + 2H^+ \rightarrow 2CaHPO_4 + Ca_2^+$. In addition, there are variations in phosphate solubility test values due to differences in the ability of bacteria to produce organic acids, including citric, malic, oxalic, and acetic acids, which function as catalysts, chelating agents, and complexing agents for phosphate absorption. (Timofeeva et al*.,* 2023).

3.2. Testing the Ability of Nitrogen-Fixing Bacteria

Testing of 27 isolates for nitrogen fixation using *Nitrogen Free Bromthymol Blue (NFB) Agar* medium yielded 19 isolates capable of nitrogen fixation, indicated by a color change in the media from green to blue around the colonies (Figure 3). This color change was due to the activity of the nitrogenase enzyme in bacteria that were fixing nitrogen, causing an increase in pH, with bromothymol blue acting as an indicator of higher pH in the medium.

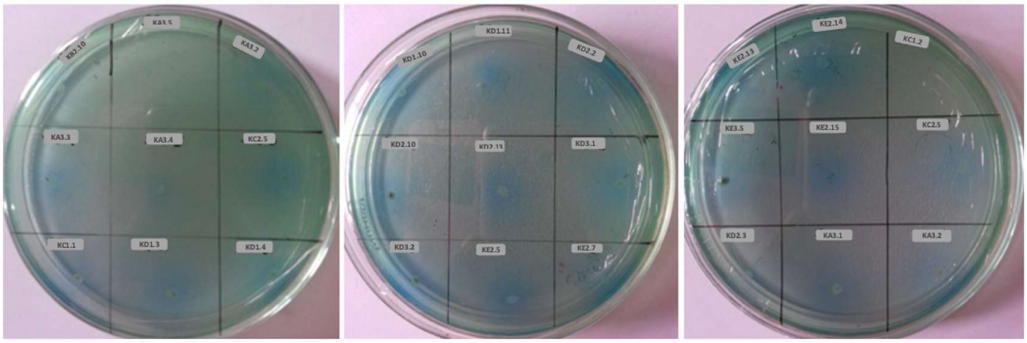

Figure 3. Testing the ability of N-fixing isolates on *Nitrogen Free Bromthymol Blue (NfB) Agar* medium

Nitrogen is one of the most important elements in plant growth and productivity. Naturally, plants are unable to directly absorb free nitrogen (N_2) from the air. Therefore, the availability of nitrogen-fixing bacteria capable of producing the nitrogenase enzyme can convert N_2 into ammonia (NH_3), which can be absorbed by plants (Greed, 2023). Enzim nitrogenase terdiri dalam beberapa bentuk molibdenum (Mo) nitrogenase, vanadium (V) nitrogenase, dan nitrogenase khusus besi (Fe). Gen nitrogenase meliputi Nif, seperti nifRLA, nifHDK, nifENB, nifJ, nifUSVM, dan nifWF, berperan penting dalam fiksasi nitrogen, sintesis komponen, dan regulasi enzim (Usman and Wali, 2024).

3.3. Hypersensitivity Test on Tobacco Plants

The hypersensitivity test results of 27 isolates on tobacco plants showed that 9 isolates were pathogenic to plants, marked by necrosis or discoloration to yellow or brown in the injected leaf area for 2x24 hours, similar to the positive control reaction using *Pseudomonas syringae* bacterial isolates, which showed necrosis on the injected leaf surface. This bacterium is capable of inducing a hypersensitivity reaction in tobacco leaves, with yellow spots or necrosis symptoms appearing in the injected areas. Meanwhile, the negative control using medium and distilled water showed negative results, with no necrosis or discoloration occurring on the injected leaf surface (Figure 4).

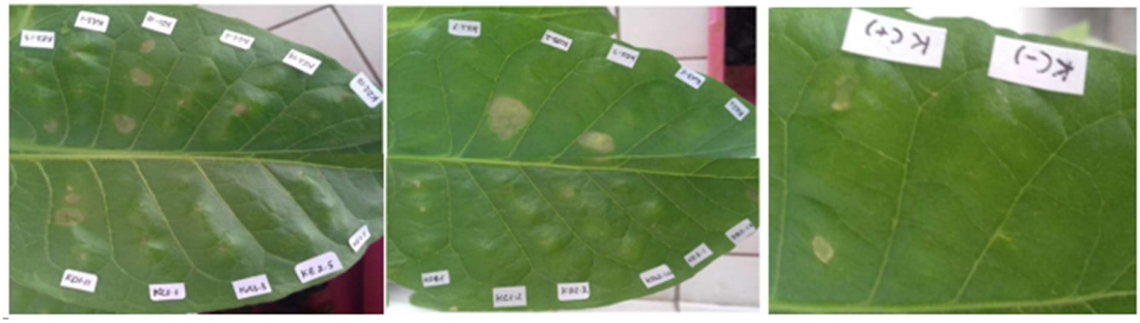

Figure 4. Results of hypersensitivity testing of selected bacteria against tobacco plants during 2x24 hours of incubation. Control (-) distilled water and Control (+) *Pseudomonas syringae*

The hypersensitivity test results showed that 18 isolates exhibited a negative reaction to tobacco plants after 2x24 hours. A negative hypersensitivity reaction is defined as no necrosis or discoloration to yellow or brown at the injection site (Figure 4, Table 2). The negative control using sterile distilled water and *Pikovskaya Broth* medium produced a negative reaction, while the positive control using *Pseudomonas syringae* bacterial isolates showed a positive reaction with yellow spots or necrosis at the injection site. A hypersensitive reaction is a rapid and localized process of cell death. This reaction occurs in infected plants upon pathogen recognition as an attempt to inhibit pathogen growth. The induction of hypersensitivity and pathogenicity is influenced by the hrp gene, which is commonly found in Gram-negative plant pathogenic bacteria, including the *Pseudomonas syringae* group (Tarigan et al., 2023).

Soil analysis was performed using one isolate, namely KE2.15, which had the highest phosphate solubilization index on *Pikovskaya Agar* medium and was capable of fixing nitrogen, as indicated by a color change from green to blue on *Nitrogen Free Bromthymol Blue Agar (NfB)* medium. Furthermore, it did not cause necrosis in tobacco plants.

Table 1. Characteristics of isolates based on morphological observations and tests of P solubilization, N fixation, and hypersensitivity

No	Code Isolate	DI Zone PSB	Nitrogen-Fixing Bacteria	Characteristics of the colony				Colony Form	Gram Bacteria	Hyper-sensitivity 2x 24 Hours
				Bentuk	Elevasi	Margin	Warna			
1	KA3.1	1.33	+	Circular	Raised	Entire	Cream	Coccus	Negative	-
2	KA3.2	1.77	+	Irregular	Raised	Undulate	Cream	Bacil	Negative	-
3	KA3.3	1.66	+	Spindle	Raised	Entire	Cream	Bacil	Positive	-
4	KA3.4	1,6	+	Circular	Raised	Entire	Cream	Coccus	Negative	-
5	KA3.5	1.75	-	Circular	Convex	Entire	Cream	Coccus	Negative	+
6	KB2.10	1.75	-	Circular	Convex	Undulate	Yellow	Bacil	Negative	+
7	KC1.1	1.88	-	Circular	Convex	Undulate	Yellow	Coccus	Negative	-
8	KC1.2	1.88	-	Circular	Convex	Undulate	Cream	Bacil	Negative	-
9	KC2.5	1	+	Circular	Umborate	Undulate	Cream	Bacil	Negative	-
10	KD1.3	1.5	+	Circular	Convex	Entire	White	Coccus	Negative	+
11	KD1.4	1.5	+	Circular	Convex	Entire	White	Coccus	Negative	+
12	KD1.10	1.6	+	Circular	Convex	Entire	White	Coccus	Negative	+
13	KD1.11	1.5	+	Circular	Convex	Entire	White	Coccus	Negative	+
14	KD2.2	1.33	+	Circular	Convex	Entire	White	Bacil	Negative	-
15	KD2.3	1.09	-	Circular	Convex	Undulate	White	Coccus	Negative	-
16	KD2.10	1.4	+	Irregular	Raised	Undulate	White	Coccus	Positive	-
17	KD2.13	1.44	+	Circular	Raised	Undulate	White	Bacil	Negative	-
18	KD3.1	1.22	+	Circular	Raised	Undulate	White	Bacil	Negative	-
19	KD3.2	1	+	Spindle	Raised	Entire	Cream	Bacil	Negative	+
20	KE2.5	1	+	Circular	Raised	Entire	Cream	Bacil	Negative	-
21	KE2.7	1	+	Circular	Raised	Entire	Cream	Coccus	Negative	-
22	KE2.13	1.09	+	Circular	Raised	Entire	White	Coccus	Negative	+
23	KE2.14	1.27	+	Circular	Undulate	Entire	White	Coccus	Negative	+
24	KE2.15	1.77	+	Irregular	Raised	Entire	White	Coccus	Negative	-
25	KE3.5	1	+	Irregular	Raised	Entire	Cream	Bacil	Negative	-
26	KA3.1	1	+	Irregular	Raised	Entire	Cream	Bacil	Negative	-
27	KA3.2	1.2	+	Irregular	Raised	Entire	Cream	Bacil	Negative	-

Table 2. Soil analysis before and after inoculation of KE2.15 isolate in soil incubated for 4 weeks

Chemical Properties	Initial Soil	Final Soil
N total	0.21 %	2.83 %
Bray I P	8.93 ppm	10.33 ppm
HCl 25% P	194.71 ppm	217.63 ppm

Notes: Initial soil = Before the addition of rhizosphere isolates; Final soil = After the addition of rhizosphere isolates

Soil analysis was conducted before and after the addition of the selected KE2.15 isolate. There was a change in the content of available P and total N in the soil, although it was still in the very low category (Table 2). Nitrogen content analysis of the samples was performed using the Kjeldahl method by converting nitrogen in the soil to ammonia, which was then measured by titration to determine the total nitrogen content in the soil samples (Sukamto and Rahmat, 2023). The total N content before the addition of the isolate was 0.21%, after the addition of the isolate it changed to 2.83%, which is in accordance with the research (Purwaningtyas and Nuraini, 2022) states that rhizobacteria have the ability to bind free nitrogen from the atmosphere (N_2) and convert it into nitrate, thereby increasing the amount of nitrogen available in the soil.

Phosphate content analysis using the Bray I method and 25% HCl extracted phosphate from soil using NH_4F and HCl acid solutions (Sukamto and Rahmat, 2023). The Bray 1 P method showed an initial available phosphorus content of 8.93 ppm, which increased to 10.33 ppm after the addition of the isolate. Similarly, the 25% HCl method showed an initial available phosphorus content of 194.71 ppm, which increased to 217.63 ppm after the addition of the isolate. Phosphate-solubilizing bacteria can largely mineralize or hydrolyze insoluble phosphate in the soil by secreting acids and enzymes and forming complexes with metal cations (Ca^{2+}, Fe^{2+}, Al^{3+}) in the soil to release

phosphate ions (Pan and Cai, 2023). The availability of phosphate in the soil indicates that P-solubilizing bacteria have not optimally secreted enzymes, resulting in a lack of organic acids that can increase P availability (Timofeeva et al., 2023).

4. Conclusion

The results of bacterial exploration in red chili plant soil showed 15 isolates with the potential to be *Plant Growth Promoting Rhizobacteria (PGPR)*, with the ability to dissolve phosphate, fix nitrogen, and not be pathogenic in hypersensitivity tests using tobacco plants. Isolate KE2.15 showed potential as a biofertilizer with phosphate solubilization and nitrogen fixation capabilities. Soil analysis before and after the addition of the isolate showed changes in total N and available P content after a 4-week incubation period

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