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Isolation and characterization of phosphate solubilizing bacteria from corn rhizosphere

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Abstract. The aim of this study was to isolate and characterize the Phosphate solubilizing bacteria from the rhizosphere of *Zea mays* L., Jeneponto Regency. This research was conducted in several stages; i.e, sampling, medium preparation, sample dilution, isolation, characterization in the form of gram staining, biochemical tests, and quantitative tests of phosphate solubility. Soil samples were diluted in 0.9% NaCl and soil containing microbes was isolated on the Picovskaya medium. Three isolates were obtained which could dissolve phosphate, namely J2KN1, J3KR2, and J3TG3 isolates. The isolates were generally round in shape with raised elevations, white, slimy, smooth, shiny surface, milky white, shape like coccus and bacillus, and gram-negative. Some of the isolates had positive motility, indole, voges, methyl red, glucose, and sucrose fermentation in the biochemical test. The quantitative tests of the ability to dissolve phosphate showed that J2KN1 isolate had the highest concentration of 51.1 μM , and the J3KR1 and J3TG3 isolates had a concentration of 45.2 μM and 37.6 μM , respectively.

1. Introduction

Corn is a horticultural commodity that has a high demand, particularly in Indonesia. The steady increase in demand makes farmers produce more corn crops every season. However, this is not directly proportional to the growth and productivity of corn in Indonesia. The low growth and productivity of corn can be caused by several factors, namely the application of unsuitable plant cultivation technology, climatic condition, and low soil fertility. One of the efforts to increase productivity is using superior varieties and optimal fertilization of inorganic fertilizers, which is the fastest and easiest way to deal with plant nutritional needs. It is easily biodegradable and can be directly absorbed by plants. Therefore, farmers rely on inorganic fertilizers. However, inorganic fertilizers have several drawbacks, such as they are expensive and cause environmental pollution if given inappropriately and excessively. Organic fertilizers are an alternative to overcome problems caused by excessive use of inorganic fertilizers [13].

The increase in organic acid levels also causes a decrease in the pH of the rhizosphere that P ions can dissolve and be available to plants. Apart from phosphatase and phytase, phosphate solubilizing bacteria also produce other enzymes capable of producing free P, such as pyrophosphatase and metaphosphatase [8]. Phosphatase solubilizing bacteria are nonpathogenic soil bacteria, and they include in the category of plant growth-promoting bacteria. These bacteria produce vitamins and phytohormones to improve plant root growth and increase nutrient uptake [6].



Phosphate solubilizing bacteria are soil microorganisms that can release P bonds and play a role in dissolving P, which is not available to become available. Plants can use it to grow and develop the availability of P elements, especially in acid soils. In addition, these P-solvent microorganisms will produce organic acids capable of chelating Al, Fe, Ca, and Mg to form a stable organometal complex. Phosphate becomes available for plants and solvent for organic phosphate and mostly in root areas [4]. Based on the description above, a study entitled isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of corn in the area of marginal land in Jeneponto Regency, South Sulawesi were carried out.

2. Materials and Methods

The soil sample was taken from corn plantations located in 3 points, namely Bangkala District, Tamalatea District, and North Bangkala District in Jeneponto Regency, South Sulawesi. The Pycovskaya medium has a composition of glucose 10 g, yeast extract 0.5 g, $(\text{NH}_4)_2\text{SO}_4$ 0.5 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1 g, 5 g Ca_3PO_4 , 0.2 g KCl, 0.02 g $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$, 0.02 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 20 g agar, and a pH of 6.8 were dissolved in 1000 ml distilled water [20]. Then heated to boiling and sterilized using an autoclave. Soil samples were weighed as much as 5 grams and then carried out stratified dilutions. In the 10^{-1} UC bottle, as much as 45 ml of physiological 0.9% NaCl solution. They are then transferred to UC bottles 10^{-2} - 10^{-3} containing 25 ml Physiological NaCl solution of 0.9%. Isolates were grown on Pycovskaya medium and form a clear zone are then subjected to a purification or purification stage. Isolates were incubated at 300°C for 72 hours [1].

Characterization of phosphate solubilizing bacteria using the gram stain method, biochemical tests, and quantitative analysis of phosphate solubilizing bacteria were carried out using the direct method using a Haemocytometer. Counting the number of dead cells is blue, while the living cells are opaque [12]. A quantitative test of the ability of bacteria to dissolve phosphate was carried out using a colorimetric method; two round-shaped needle of each selected isolate were inoculated into 40 mL of liquid Pikovskaya media, then incubated using a shaker at a speed of 120 rpm at room temperature for 72 hours. The analysis was carried out by reacting 1 mL of the supernatant to a color-forming reagent (2.5 mL of 2.5% sodium molybdate and 1 mL of 0.3% hydrazine sulfate). Phosphate concentration was measured using a spectrophotometer at a wavelength of 830 nm. The control used was liquid Pikovskaya media without the inoculants [11].

3. Result and Discussion

3.1. Isolation and purification of phosphate solubilizing bacteria

The results showed that 3 types of isolates were found, i.e. J2KN1, J3KR2 and J3TG3 which were characterized by the formation of a clear zone around the bacterial colony in the Pycovskaya medium (Figure 1).

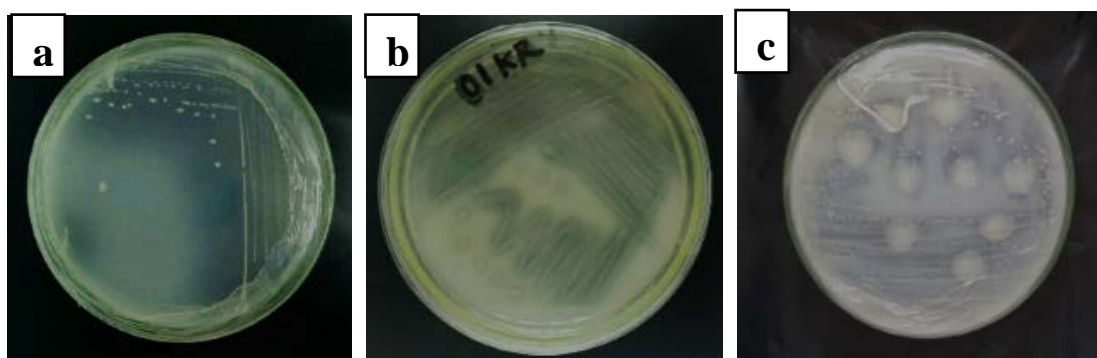


Figure 1. Isolate J2KN1 which forms a clear zone on the medium (a); Isolate J3KR2 which forms a clear zone on the medium (b); Isolate J3TG3 which forms a clear zone in the Pycovskaya medium (c);

Based on this research, it was found that 3 sample isolates were included in the phosphate solubilizing bacteria, which was marked by the formation of a clear zone around the bacterial colony in the Picovskaya medium. The formation of a clear zone is caused by the activity of phosphate solubilizing bacteria in dissolving bound phosphate. The research showed that phosphate solubilizing bacteria significantly affected the increase in available phosphate in the soil; besides producing organic acids, phosphate solubilizing bacteria also produce phosphatase enzymes that can dissolve phosphate that phosphate becomes available to plants [16]. Widiawati and Suliasih reported [18] that phosphate solubilizing bacteria has the excellent ability as a biofertilizer by dissolving phosphates that are still entangled in the soil, such as Fe's elements, Mg Ca, so that these elements can be dissolved by bacteria which become available to plants. Some soil bacteria that live around the rhizosphere can excrete organic acids such as formic acid, propionic acid, lactic acid, and fumaric acid and bind to Fe^{2+} and Al^{3+} ions; they can free bound phosphate ions to become phosphate which plants can absorb.

3.2. Morphological characteristics of phosphate solubilizing bacteria

All isolates had a round shape (Table 1), Some isolates have wavy edges, except for J3KR2 isolates. The color of the isolates varied; white and milky white; some isolates were not slimy, except for 32TG3 isolates. The nature of the colony is round. Gram staining showed that J2KN1 was coccus, and J3KR4 and J3TG3 isolates had the form of bacilli form. J2KN1 and J3TG3 isolates was gram-negative bacteria, and J3KR2 isolates were gram-positive bacteria.

Table 1. Colony Characteristics of Phosphate solubilizing bacteria isolates from the rhizosphere of maize (*Zea mays* L.) in Jeneponto district.

isolates	Characteristics of the bacterial colony							
	Elevation	surface	edge	color	mucus	Colony nature	shape	Gram
J2KN1	arise	smooth	flat	Milk white	Not slimy	round	coccus	negative
J3KR2	arise	Shiny smooth	choppy	white	Not slimy	round	Basil	positive
J3TG3	arise	Shiny smooth	flat	Milk white	slimy	round	Basil	negative

Gram-positive bacteria with peptidoglycan cell walls will react with crystal violet and iodine to form a complex that is strong and difficult to penetrate by safranin. Gram-negative bacteria have a thin cell wall so that the primary paint color will dissolve so that the cell wall can be colored by safranin. Mukamto et al. [9] reported that isolation and characterization of the phosphate solvent *Bacillus* sp from the rhizosphere of the leguminosae plant showed a type of isolate similar to the genus *Azotobacter*. This isolate was characterized a gram-negative, rod-shaped, non-motile bacteria with a slimy surface. Gram-negative bacteria have a dual membrane system, namely plasma membrane and a permeable membrane that envelops the plasma membrane. Isolation and characterization of the phosphate solubilizing *Bacillus* sp from the rhizosphere of Leguminosae plants showed that several isolates had flat, raised and convex elevations [9]. The clear zone around the colony shows that there is an activity of phosphate solubilizing bacteria in dissolving bound phosphate; this is due to the dissolution of Ca^3PO^4 in the Picovskaya medium [10]. This is in line with the research of George et al [5] which states that phosphate solubilizing bacteria will dissolve phosphate in the form of PO^4 using the enzyme phosphatase so that a clear zone is formed around the colony of phosphate solubilizing bacteria [5].

Phosphate solubilizing bacteria isolated from three types of rhizosphere soil of banana (*Musa paradisiaca* var. Nipah) in Singkawang City showed wavy and irregular edges. The color of the bacterial isolates consisted of white, brown, and milky white. Some colonies are slimy, the colony nature was

irregular and round [3]. The surface of the isolate is smooth, shiny, and rough, and the edges of the colony show choppy and flat [18].

3.3. Biochemical test

The biochemical test data was obtained, namely motile, where isolates will grow around the puncture line; conversely, non-motile isolates will not grow around the puncture line. The results of the indole test showed that several positive isolates, namely J2KN1, J2TG3, and J3TG5 with the formation of dark red rings on the surface of the medium. Based on the methyl red test results, the J2KN1 and J3TG3 isolates showed positive results due to the formation of red color in the medium. Voges Proskauer test of the isolates J2KN1, J3KR2, and J3TG3 showed positive results because of the red color in the medium. Positive carbohydrate fermentation test for J2KN isolates, while found positive results for sucrose fermentation were found in J2KN1, J3KR2, and J3TG3 isolates. This is in line with the research of Etha et al [3] which states Phosphate solubilizing bacteria isolated from three types of rhizosphere soil of banana (*Musa paradisiaca* var. Nipah) in Singkawang City showed some isolates positive carbohydrate fermentation.

Table 2. Characteristics of the phosphate solubilizing bacteria isolates that were subjected to biochemical testing.

No	Isolates	Motility	Methyl red	Biochemical test			
				Voges-Proskauer	Indole	Glucose fermentation	Sucrose fermentation
1	J2KN1	-	+	+	+	+	+
2	J3KR2	+	+	+	-	-	+
3	J3TG3	+	+	+	+	-	+
4	control	-	-	-	-	-	-

Note: (-) = negative biochemical test, (+) = positive biochemical test

3.4. Test the ability of bacteria to dissolve phosphate

The isolate that dissolved the highest phosphate was J2KN1, an average absorbance of 51.1 μM or 1.020 $\mu\text{g} / \text{ml}$, and the isolate that dissolved the lowest phosphate was J3TG3, as much as 37.6 μM (Table 3). The determination of phosphate solubilizing bacterial showed a growth phase with the average isolate showing an exponential phase on the third day (72 hours) of incubation before testing the ability of phosphate solubilizing bacteria to dissolve phosphate in liquid pikovskaya medium in the form of Ca^3PO^4 . The highest dissolved phosphate content in the GGO1 bacterial isolate was 0.473 $\mu\text{g} / \text{ml}$ [17].

Table 3 Phosphate concentrations for 3 isolates of phosphate solubilizing bacteria

No.	Isolates	Phosphate concentration (μM)
1.	J2KN1	51,1 μM
2.	J3KR2	45,2 μM
3.	J3TG3	37,6 μM

Phosphatase enzyme activity is also determined by the clear zone formed around the colony. The phosphate dissolution index's value indicated the isolates' ability to dissolve the phosphate in liquid Pikovskaya media. The phosphate concentration obtained shows the concentration of phosphate that each isolate can dissolve. Bacteria such as *Pseudomonas*, *Bacillus*, and *Rhizobium* can dissolve phosphate [15]. Organic acids dissolve insoluble phosphates for use, thereby increasing the potential availability of phosphate in plants. Microorganisms isolated from the soil rhizosphere are adapted to plants and provide better growth [14].

4. Conclusion

Three bacterial isolates, namely J2KN1, J3KR2, and J3TG3 dissolved phosphate by forming a halo zone (clear zone) around the bacterial colony. Several isolates were positive in the Motility test, Indol test, Proskauer Voges test, Methyl red test, and carbohydrate fermentation test. The quantitative test of the ability to dissolve phosphate showed that J2KN1 isolate had the highest concentration of 51.1 μM therefore.

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