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Research Article

The Effectiveness of Phosphate Solvent Rhizosphere Bacteria in Stimulating the Growth of Rice Plants

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ABSTRACT
Phosphate is a macronutrient that is needed in plant growth. Rhizosphere bacteria are very helpful in the availability of phosphate in the soil. Phosphate solvent microbes including soil microorganisms that can release P bonds can also dissolve phosphate so that it is available for plant growth and development. This study aims to obtain phosphate solvent rhizosphere bacteria that have the potential to spur plant growth. Bacteria were isolated through dilution. Bacterial isolation results obtained 40 bacterial isolates that can grow on nutrient agar medium. From the morphological characterization, eighteen isolates were obtained with different morphological characteristics. Rhizosphere bacteria isolates can dissolve phosphate in pikovskayas media, produce clear zones around bacterial colonies, and have high concentrations of phosphate dissolution. The results of the phosphate dissolution test were selected by five bacterial isolates tested on rice seeds, namely AHR11MS, AHR15MS, AHR2LS, AHR3LS, AHR4KS, and AHR7KS. AHR15MS isolates had a significant effect on root length of 14.44 cm and a plant dry weight of 3.01 grams compared to controls with a root length of 8.75 cm and a dry weight of 1.03 cm. In conclusion, AHR15MS isolates have the potential to stimulate rice plant growth.

Keywords: phosphate, rhizosphere, bacteria

INTRODUCTION
Development of rice productivity as a major food crop needs to be given great attention to meet the food needs of communities such as soil fertility, the availability of essential nutrients such as phosphate, the presence of microbes that play a role in the process of dissolving phosphate in the soil. Meeting the nutritional needs of plants can be done with a biological approach that is utilizing rhizosphere microbes that can dissolve phosphate and secrete plant growth hormones, which can minimize the use of inorganic fertilizers and improve environmental quality [1], [2]. Rhizosphere microbes live and are associated with plant roots and provide benefits for plant growth known as Plant growth-promoting rhizobacteria (PGPR) [3], [4], [5]. PGPR can trigger plant growth by producing phytohormones, providing phosphate, and fixing nitrogen [6], [7] so that it can function as induction of plant resistance to pathogens [8], [9], [10].
Phosphate solvent microbes, including soil microorganisms, can release P bonds that can also dissolve phosphate so that it is available for plant growth and development. The application of phosphate solubilizing microbes significantly increased the P uptake, the number of leaves, and dry weight of corn plants [11], [12], . Inoculation of phosphate solvent bacteria can increase plant growth and reduce the use of synthetic fertilizers in lowland rice plants, [13]. This study aims to obtain phosphate solvent rhizosphere bacterial isolates that can stimulate the growth of rice plants.

MATERIALS AND METHODS
Isolation and morphological characterization of rhizosphere bacteria
Isolation of rhizosphere bacteria comes from the soil around the roots of healthy rice plants, carried out by serial dilution methods. A total of

one gram of soil is homogeneous with 10 ml of sterile water and put into five test tubes in series. Every 1 ml of dilution 10^{-3} and 10^{-4} was put into a petri dish containing the nutrient agar (NA) medium aseptically and incubated for 48 hours at 28^0 C.

Morphological characterization of rhizosphere bacterial isolates on NA media was observed based on the method: color (white, cream and yellow), size (pinpoint, small, moderate and large), form (circular, irregular, filamentous and rhizoid), margin (entire, undulate, serrate, curled and lobate), elevation of the colony (raised, convex, flat and umbonate) [14].

The ability of rhizosphere bacterial isolates to dissolve phosphate ability of rhizosphere bacterial isolates to dissolve phosphate in agar media

The ability of bacterial isolates to dissolve phosphate using pikovskayas agar with the addition of tri- calcium phosphate (TCP). The isolates tested were qualitatively evaluated based on the formation of clear zones around bacterial colonies [15]. Observations of the dissolution index [3] and dissolution efficiency (EP) [16] are as followed.

$$\text{Dissolution Index} = \frac{\text{clear zone diameter} - \text{colony diameter}}{\text{colony diameter}} \quad (1)$$

$$\text{Dissolving Efficiency} = \frac{\text{clear zone diameter}}{\text{colony diameter}} \times 100\% \quad (2)$$

Determination of phosphate solubilizing concentration of rhizosphere bacterial isolates

Determination of P dissolved in the media ie bacterial isolates were inoculated in 30 ml of liquid pikovskayas media, in a shaker and incubated for seven days at a speed of 120 rpm. The pH was measured at the end of the incubation period. The cultures were centrifuged at 10,000 rpm for 15 minutes [17].

The ability of rhizosphere bacterial isolates spurred the growth of rice seeds

Rhizosphere bacterial isolates that can produce phosphate are inoculated in 33 varieties of inpari rice seeds by soaking the seeds. The study was arranged in a Completely Randomized Design (CRD), repeated three times.

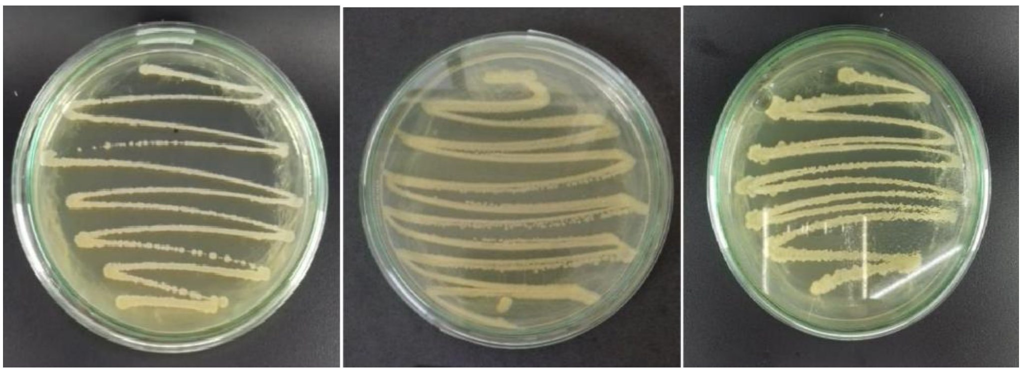
Rhizosphere bacterial isolates were grown on the NA mediums for 48 hours. The bacterial colony was dissolved with 10 ml of distilled water and put into a 50 mL erlenmeyers with a concentration of 108 cfu mL^{-1}. Rice seeds were soaked for 12 hours. Seeds were then planted in a sterile filter media in a petri dish and stored at 28^0 C. Observations include the root length, shoot length, and plant dry weight on the 14th day after planting.

RESULTS AND DISCUSSION

Isolation and morphological characterization of rhizosphere bacterial isolates

The results from the phosphate solvent bacteria isolations found 40 isolates that can grow well on NA media. NA media is a source of nutrients that can support the growth of rhizosphere bacteria because they contain carbohydrates, nitrogen, proteins, and vitamins needed by microorganisms to grow and develop [18], [19].

A total of 18 bacterial isolates that showed different morphological characters showed the color, shape, edge, and elevation characteristics of each isolate different from each other (Figure 1). The isolated colony is dominated by circular (white), white, beige, and yellow, flat, and clear edges (Table 1). Although the shape of bacterial cells varies, they generally have a stem (bacillary), round (coccus), and spiral [20], [21].



A B C

Fig.1: Results of isolation of rhizosphere bacteria in rice plants. A. AHR5MS, B. AHR2LS, C.

AHR3LS

Table 1. Morphological characterization of rhizosphere bacteria in rice plants.

Isolates	Size	Shape	Edge	Elevation	Color
AHR1MS	Large	Irregular	Lobate	Raised	Cream
AHR7MS	Large	Circular	Entire	Convex	Cream
AHR10MS	Small	Irregular	Lobate	Raised	White
AHR11MS	Moderate	Irregular	Lobate	Raised	White
AHR15MS	Moderate	Irregular	Entire	Convex	White
AHR1LS	Small	Circular	Entire	Convex	White
AHR2LS	Pinpoint	Circular	Lobate	Flat	White
AHR3LS	Pinpoint	Circular	Entire	Convex	White
AHR6LS	Small	Circular	Entire	Flat	White
AHR8LS	Small	Circular	Lobate	Flat	Krem
AHR9LS	Small	Irregular	Entire	Flat	White
AHR3GS	Moderate	Circular	Entire	Convex	Yellow
AHR4GS	Pinpoint	Circular	Entire	Convex	Cream
AHR3KS	Moderate	Circular	Entire	Flat	Cream
AHR4KS	Moderate	Circular	Entire	Flat	Yellow
AHR7KS	Moderate	Circular	Entire	Flat	Yellow
AHR8KS	Moderate	Circular	Entire	Flat	Cream
AHR9KS	Moderate	Circular	Entire	Flat	Yellow

The capability of rhizosphere bacterial isolates to dissolve phosphate

The capability of rhizosphere bacterial isolates to dissolve phosphate in agar media

The ability of rhizosphere microbes to dissolve phosphate is characterized by the formation of clear zones around bacterial colonies that have different clear zone diameters and colony diameters (Figure 2). Observations showed that all rhizosphere bacterial isolates could dissolve phosphate with a phosphate dissolving index that varied between 0.45 to 2.26 (Table 2). The presence of the enzyme phosphatase produced causes the rhizosphere bacteria to have the ability to dissolve inorganic phosphates such as tricalcium phosphate found in the media, thus forming clear zones around bacterial colonies [22], [23], [24].

Phosphate solubilizing bacteria can grow on pikovskayas media and form clearer colored areas around bacterial colonies, indicating that bacterial isolates can form organic acids that can bind to Ca- ions to produce Ca3 (PO4) 2 compounds and free H2PO4 ions [25], [26], [22].

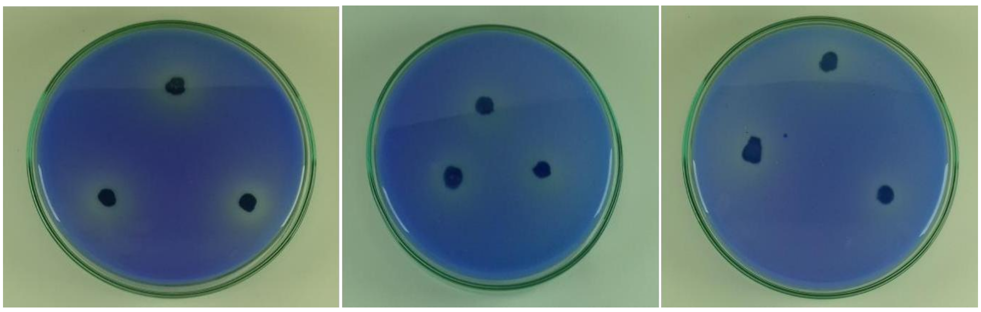


Fig.2: The capability of rhizosphere bacterial isolates to produce clear zones on pikovskayas

media

Table 2. Analysis of the capability of phosphate dissolution of rhizosphere bacteria on pikovskaya media.

Isolate Name		Phosphate solubility capability		
	Clear Zone Diameter (cm)	Colony Diameter (cm)	Phosphate Dissolution Efficiency (%)	Phosphate Dissolution Index
AHR1MS	1,30	0,70	185,71	0,86
AHR7MS	1,33	0,66	201,52	1,02
AHR10MS	1,30	0,70	185,71	0,86
AHR11MS	1,33	0,56	237,50	1,38
AHR15MS	1,26	0,46	273,91	1,74
AHR1LS	1,33	0,66	201,52	1,02
AHR2LS	1,50	0,46	326,09	2,26
AHR3LS	1,30	0,63	206,35	1,06
AHR6LS	1,20	0,63	190,48	0,90
AHR8LS	1,10	0,56	196,43	0,96
AHR9LS	1,50	0,76	197,37	0,97
AHR3GS	1,03	0,53	194,34	0,94
AHR4GS	1,33	0,56	237,50	1,38
AHR3KS	1,80	1,03	174,76	0,75
AHR4KS	1,66	0,90	184,44	0,84
AHR7KS	1,70	0,73	232,88	1,33
AHR8KS	1,30	0,70	185,71	0,86
AHR9KS	1,60	1,10	145,45	0,45

Determination of phosphate solubilizing concentration of rhizosphere bacterial isolates

The bacterial isolates' capability to dissolve phosphate is indicated by the level of phosphate contained in each isolate. The highest phosphate solubility was produced by AHR15MS isolate with 9,932 mg/l phosphate content and the lowest was in AHR9KS isolate with phosphate content 5,4414 mg/l (Figure 3). Each isolate can dissolve different phosphates suspected of the bacteria' capability to adapt and utilize nutritional sources in the growth medium [27], [28], [23] and differences in morphological, physiological and biochemical characteristics, and differences in the growth ability of each isolate [16].

In releasing organic compounds the capability of different microbes, and if the availability of phosphate is low, it can be fulfilled by the enzyme phosphatase produced by phosphate solvent microbes that function to release phosphate bound by organic compounds [29], [30], [31]. The amount of rhizosphere microbes in the soil is influenced by the activity of root metabolism in removing metabolites into the soil in the form of root exudates. Microbes in the soil will utilize root exudates for their development so that it affects the diversity of phosphate solvent microorganisms in the soil [32], [33]. Earlier research reported that the ability to dissolve phosphate by bacteria varies depending on the strain [34], [35], [36]. Rhizosphere microbes are indispensable by plants in providing phosphate nutrition [37], [38], [39].

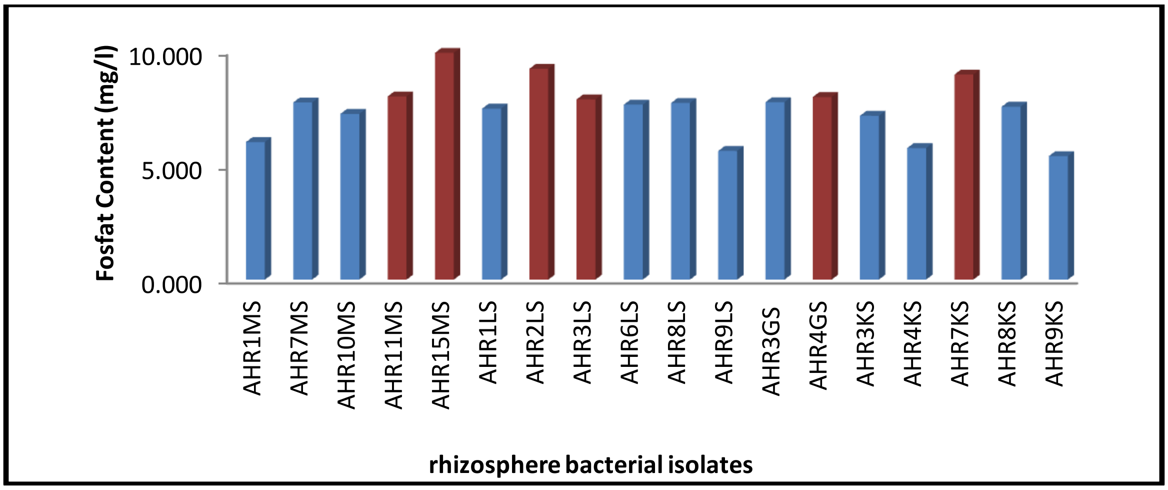


Fig.3: Concentration of phosphate dissolution of rhizosphere bacteria isolates

The capability of rhizosphere bacterial isolates spurred the growth of rice seeds

Bacterial isolates that are inoculated into rice seeds are isolates that have the highest phosphate dissolving ability. From 18 isolates selected, six isolates were inoculated into rice seeds, namely AHR11MS, AHR15MS, AHR2LS, AHR3LS, AHR4GS, and AHR7KS bacterial isolates. Observation results showed that rhizosphere bacterial isolates were able to spur the growth of leaf length, root length, and dry weight of rice seeds. Based on the ANOVA analysis, bacterial isolates had no significant effect compared to controls on leaf length but had a significant effect on root length and dry weight of rice plants (Table 3). Root extension will cause a short distance between the source of P with the root so that it can help increase P absorption.

Based on observations, the leaf length with the longest increase was found in AHR4GS isolates with a length of 11.70 cm but did not have a different effect with other bacterial isolates and controls. In the Duncan test at 0.05%, the AHR15MS bacterial isolate had a significant effect on the root length of 14.44 cm and a dry weight of 3.01 grams compared with controls with a root length of 8.75 cm and a dry weight of 1.03 cm. So AHR15MS isolates are isolates have a significant influence in spurring rice plant growth.

Increases in leaf number, plant height, leaf area, leaf area index, assimilation rate, and plant dry weight are caused by the availability and uptake of P elements derived from phosphate solvent microbes [40], [41]. Phosphate solvent microbes, in addition to producing metabolites, needed by plants such as siderophores, phytohormones and antibiotic compounds [42], [43] can also increase soil C-organic, enzyme activity, P absorption, phosphate solvent microbial populations to stimulate plant growth and increase yields [44], [45], [43], [11].

Table 3. Growth of rice seeds inoculated with phosphate solvent bacterial isolates.

Treatment	rice plant growth parameters		
	Leaf length (cm)	Root length (cm)	Dry weight (gram)
AHR11MS	10,80^{a}	10,30^{a}	1,74^{b}
AHR15MS	10,87^{a}	14,44^{c}	3,01^{bc}
AHR2LS	11,03^{a}	12,59^{b}	1,59^{b}
AHR3LS	10,77^{a}	11,83^{b}	1,03^{b}
AHR4GS	11,70^{a}	12,08^{b}	1,36^{b}
AHR7KS	11,43^{a}	12,13^{b}	1,87^{b}
Kontrol	9,37^{a}	8,75^{a}	1,03^{a}

The numbers followed by the same letters in the same column are not significantly different based on the 5% DMRT test.

CONCLUSION

Rhizosphere bacterial isolation obtained 40 bacterial isolates that can grow on NA media. A total of 18 bacterial isolates have different morphological characteristics and are dominated by circular (white), white, beige, and yellow, flat, and clear edges. Rhizosphere bacteria isolates can dissolve phosphate in pikovskayas media, produce clear zones around bacterial colonies, and have high concentrations of phosphate dissolution. The five highest isolates of rhizosphere bacteria in phosphate dissolution were AHR11MS, AHR15MS, AHR2LS, AHR3LS, AHR4KS, and AHR7KS isolates tested to rice seeds. AHR15MS isolate had a significant effect on root length of 14.44 cm and a dry weight of 3.01 grams compared with controls with a root length of 8.75 cm and a dry weight of 1.03 cm. Leading to that AHR15MS isolates have the potential to stimulate rice plant growth.

Competing Interests

The authors declare no competing interests.

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