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The Ability of Endophytic Bacteria in Phosphate Solubilization and IAA Production on Chili Plant Growth

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Abstract: Biofertilizer is considered an alternative to chemical fertilizers for enhancing plant productivity while minimizing ecological damage. Endophytic bacteria have the ability to solubilize phosphate and produce IAA (indole acetic acid). The aim of this research is to assess the phosphate solubilization and IAA production abilities of bacterial isolates applied to chili plants. Seven bacterial isolates collection from the Microbiology Laboratory, Universitas Sumatera Utara, were tested. In quantitative tests conducted in vitro, isolates MB10 and MB11 demonstrated the highest phosphate solubilization capabilities, yielding solubilization indices of 16.2 mm and 9.5 mm, respectively. Additionally, the ability of bacterial isolates to produce IAA in vitro was evaluated using calorimetry, revealing that isolates MB8 and MB2 produced the highest concentrations of IAA, measuring 58.58 ppm and 54.64 ppm, respectively. Furthermore, in vivo application involved the inoculation of isolates MB10 and MB11 on chili plants for 30 days. Seed treatment with suspensions of isolates MB10 and MB11 resulted in decreased plant performance compared to the control. These findings indicate that some endophytic bacteria originating from the mangrove root Avicennia marina have potential for producing relatively high levels of secondary metabolites. However, direct application did not significantly enhance plant growth performance.

Keywords: Biofertilizer; Chili; IAA producing bacteria; Phosphate solubilizing bacteria

Introduction

Phosphorus is an essential macronutrient that aids in the growth and development of plants. Phosphate assists in plant metabolism, including cell division, the growth and development of new tissues, energy formation, biosynthesis, and photosynthesis (Elhaissoufi et al., 2022; Rawat et al., 2021). However, the availability of phosphorus (P) sometimes becomes limited in the soil because most soils have sufficiently high levels of P, while some soils have low or even unavailable amounts (Husen et al., 2022).

The use of phosphate-solubilizing microbes such as phosphate-solubilizing bacteria (PSB) is employed to enhance phosphorus (P) requirements in plants (Husen et al., 2022). Many genera of bacteria are reported as phosphate solubilizers, including *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Azospirillum*, *Serratia*, *Flavobacterium*, *Pseudomonas*, *Microbacterium*, and *Rhizobium* (Kumar et al., 2020). Several studies report that inoculation of phosphate-solubilizing endophytic bacteria contributes to enhanced plant growth (Emami et al., 2019; Oteino et al., 2015).

The hormone IAA (indole-3-acetic acid) also plays a crucial role in the growth and development of plants. IAA is considered an active form of auxin, one of the major phytohormones, involved in root initiation, cell division, cell enlargement, extension, and differentiation. Bacteria also have effects on

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photosynthesis, regulate vegetative growth, initiate lateral and adventitious roots, enhance xylem function, pigmentation, and various metabolic processes. Numerous studies demonstrate the ability of endophytic bacteria to produce IAA and subsequently influence plant growth enhancement (Kumar et al., 2020). Additionally, in research by Mu'minah et al. (2015), it was reported that several bacteria producing exopolysaccharides had the capability to produce IAA, for example, exopolysaccharide-producing bacteria like *Pseudomonas aeruginosa, Erwinia, Ralstonia*, and *Azotobacter vinelandii.*

Various potentials of phosphate-solubilizing bacteria and IAA-producing bacteria were investigated to assess their effectiveness as PGPR (Plant Growth Promoting Bacteria). One of the applications could be conducted with chili plants. Chili (*Capsicum annum*) is a horticultural plant widely utilized in society. Chilies are cultivated in agriculture sectors or domestically by farmers and communities. Thus, the growth and development of chili agriculture, which is widely utilized, are greatly needed. Safe natural biofertilizers need to be applied for plant growth.

Previous studies have reported that several microbes were capable of producing compounds that could solubilize phosphate and produce IAA, thereby enhancing plant growth and serving as plant nutrients. Bacteria could serve as biofertilizers, offering a safe alternative to various chemical fertilizers to boost plant productivity while minimizing ecological damage. The aim of this study was to test bacteria for their phosphate-solubilizing abilities and their capacity to produce IAA, followed by application to chili plants.

Method

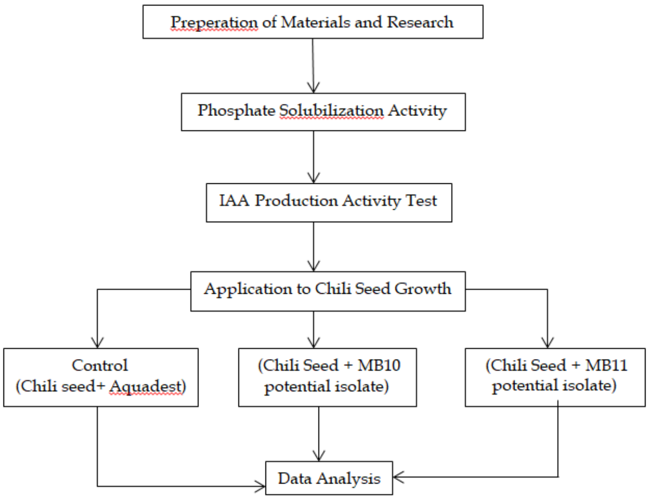

Figure 1. Research work scheme

This study used experimental method. The aim of this research is to assess the phosphate solubilization and IAA production abilities of bacterial isolates applied to chili plants.

Materials and Research Tools

The materials used in this study were bacterial isolates from the Microbiology Laboratory collection at Universitas Sumatera Utara: 7 endophytic bacterial isolates capable of phosphate solubilization from the roots of mangrove plants Avicennia marina (MB1, MB2, MB7, MB8, MB10, MB11, and MB12). The media included Nutrient Agar (NA), distilled water (Aquadest), Pikovskaya Agar, pure IAA (indole-3-acetic acid), Luria Bertani (LB) media, tryptophan, Salkowski reagent, and seeds of red chili peppers (Capsicum annum). The tools used in this research included knives, calipers, vortex mixer, shaker, microscope, centrifuge, and spectrophotometer.

Phosphate Solubilization Activity Test

The ability of endophytic bacteria to solubilize phosphate was tested using the spot inoculation method. Bacterial isolates were inoculated onto solid Pikovskaya media by dotting and then incubated for 7 days. The growth of phosphate-solubilizing bacterial colonies was indicated by the formation of clear zones around the colonies (Asril et al., 2020). The clear zones formed around the colonies were measured, and the solubilization index was determined using the following equation:

$$\text{IPF} = \left(\frac{\text{DZB} - \text{DK}}{\text{DK}}\right) \tag{1}$$

Description:
IPF : Phosphate Solubilization Index
DZB : Diameter of Clear Zone
DK : Diameter of Colony

IAA Production Activity Test

The ability of bacteria to produce IAA hormone was tested using calorimetry. A volume of 1-2 mL of endophytic bacterial culture was taken at standard Mac Farland and added to 10 mL of sterile NB media containing 0.1% L-tryptophan. It was then shaken for 24 hours at 120 rpm. A 10 mL aliquot of the inoculum was taken and centrifuged at 5500 rpm for 10 minutes. 1 mL of the supernatant was transferred to a reaction tube and mixed with 4 mL of Salkowski reagent in a 1:4 ratio (supernatant: salkowsky). This mixture was incubated for 20 minutes. The concentration of IAA was measured using a spectrophotometer at a wavelength of 535 nm and calculated using a standard IAA curve (Sharma et al., 2015).

The standard curve of IAA was prepared by dissolving 0.001 g of pure IAA in 10 ml of distilled water and transferring it into separate tubes with concentrations of 0, 5, 10, 15, 20, and 25 ppm. Then, 4 ml of solution from each concentration was mixed with 1 ml of Salkowski reagent, homogenized, and the absorbance was measured using a spectrophotometer at a wavelength of λ=530 nm (Ardiana et al., 2022; Herlina et al., 2015).

Application to Chili Seed Growth

A planting medium consisting of a mixture of sterilized soil and compost (ratio 3:1) weighing 2 kg was prepared and placed into polybags. Subsequently, bacterial suspensions of potential phosphate-solubilizing and IAA-producing bacteria were prepared using 10 mL of culture at standard Mac Farland. Chili seeds were soaked in each suspension of potential endophytic bacteria (OD600=0.5) for 30 minutes. As a negative control, chili seeds were soaked in sterile distilled water (Aquadest) without bacterial suspension for 30 minutes. Three treatments were conducted: untreated/control, with addition of phosphate-solubilizing bacteria (MB10), and with addition of phosphate-solubilizing bacteria (MB11). Plant growth was observed over 4 weeks using a Completely Randomized Design (RAL) with 5 replicates selected from 10 planted chili seeds. Growth parameters measured included: number of germinated seeds, plant height (cm), number of leaves, fresh weight of plants (g), and dry weight of plants (g) at the end of the observation period.

The calculation of the number of seedlings that experienced growth was conducted by sowing 10 chili seedlings for each treatment, and counting the successful growth rate after 30 days. Plant height was measured using the RAL (Completely Randomized Design) method with 5 replications selected from the germinated results. Measurements started from the lowest stem precisely at the soil surface to the uppermost stem tip, using a meter following the stem curves. The total number of leaves was measured by counting the leaves on the growing medium. Wet and dry weights of the plants were measured at the end of the observation period through plant weighing. Wet weight was calculated by weighing all parts of the plant. Dry weight of the plants involved drying them in an oven at 60-70°C for 1-3 days until a constant dry weight was obtained.

Result and Discussion

Endophytic Bacteria Activity in Phosphate Solubilization

The ability to solubilize phosphate by 7 phosphate-solubilizing endophytic isolates, capable of solubilizing $Ca3PO4$, was tested, indicated by the formation of clear zones around bacterial colonies. After incubation for 7 days, varying sizes of clear zones were observed. Based on the area of the clear zones formed, two bacterial isolates were identified as the most potent, with the largest clear zones: isolate MB10 (16.2 mm) and MB11 (9.5 mm) (Table 1).

Table 1. Phosphate Solubilization Activity by Endophytic Bacteria

Isolate Code	Colony Diameter (mm)	Clear Zone Diameter (mm)	Phosphate Solubilization Index (mm)
MB1	1.6	1.9	0.2
MB2	3.6	3.6	0.1
MB7	5.5	8.2	2.8
MB8	5.0	6.4	1.4
MB10	5.5	21.7	16.2
MB11	4.4	14.0	9.5
MB12	3.8	6.0	2.1

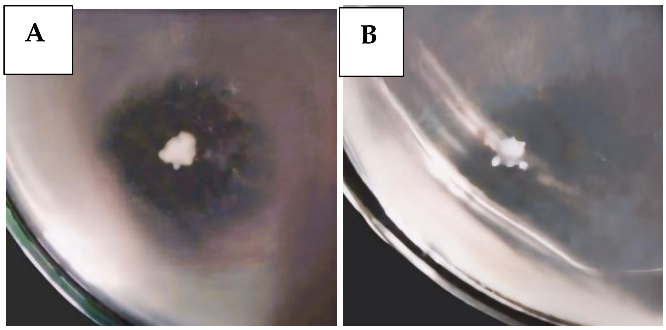

Figure 2. Phosphate solubilization activity of endophytic bacterial isolates on day 7 in Pikovskaya media incubated at room temperature; (A) Colony of MB10 (B) Colony of MB11 capable of phosphate solubilization, indicated by clear zones around the colonies

The clear zone was formed because the endophytic bacterial isolate had the ability to produce an enzyme called phosphatase (Figure 1). Phosphate-solubilizing microorganisms have various mechanisms that aid in the solubilization of bound or unavailable phosphorus. Various mechanisms of inorganic phosphate solubilization include acidification due to the production of organic acids, inorganic acids, and $H+$ excretion, production of exopolysaccharides, and siderophore production. Enzymes such as phosphatase, phytase, and C-P lyase assist in the solubilization of organic phosphates (Prabhu et al., 2019).

The formation of clear zones indicates that the isolates were capable of producing extracellular organic acids that could bind with $Ca2+$ ions bound in the form of $Ca3(PO4)2$ in Pikovskaya media, releasing $H2PO4-$ ions and creating clearer areas compared to areas with bound phosphorus (Bérdy, 2005). In the process of phosphate solubilization, phosphatase enzymes play a crucial role in catalyzing enzymatic hydrolytic mineralization reactions. Hydrolytic mineralization reactions involve the release of insoluble phosphate (Sridevi et al., 2013), resulting in the formation of clear zones. These clear zones also indicate phosphate solubilization activity through organic acid formation. Organic acids capable of binding with Ca2+ ions form compounds like Ca3(PO4)2 in Pikovskaya media, releasing H2PO4− ions to create clearer areas (George et al., 2002).

The phosphate solubilization index indicates the varying potential of bacteria in phosphate solubilization, as shown in Table 1 with diverse results. This is likely due to the origin of the endophytic bacterial isolates obtained from the roots of mangrove plants *Avicennia marina.* The source of bacterial isolates influences their abilities; mangrove plants are known to thrive in muddy, sandy, and even high-salinity environments, which are suspected to affect the phosphate solubilization capabilities of endophytic bacteria. The ability of phosphate-solubilizing bacteria can vary due to several soil factors such as soil nutrients, pH, moisture content, organic matter, and enzyme activities (Keneni et al., 2010).

Research by Suryanti et al. (2023) reported that bacteria originating from pineapple roots living in nutrient-poor lands like peatlands possess phosphate-solubilizing bacteria. According to Bashan et al. (2013), almost all types of bacteria can solubilize phosphate in the form of $Ca_3(PO4)_2$ by producing organic acids. The nutrient-poor conditions in peatlands serve as a selection mechanism; bacteria species with high phosphate solubilization capabilities thrive better in peatland environments compared to those without such abilities.

The Activity of Endophytic Bacteria in Producing IAA

The ability of endophytic bacteria to produce IAA was tested, with all isolates capable of producing IAA, with the highest concentrations found in isolates MB8 (58.58 ppm) and MB2 (54.64 ppm) (Figure 2). Endophytic bacteria's ability to produce IAA was confirmed using Salkowski reagent, which turns pink or red, indicating the high levels of IAA produced. Bacteria capable of producing IAA turn red when exposed to Salkowski reagent due to the interaction between IAA and iron, forming a complex compound $[Fe_2(OH)_2(IA)_4]$. The intensity of the pink color indicates the concentration of IAA produced by the bacteria (Kovavcs, 2009). The samples showed a positive reaction, indicating qualitative IAA production by observing the color change to pinkish red. The reaction of iron from the Salkowski reagent with IAA forms a complex compound. The interaction of these two compounds will appear pinkish red.

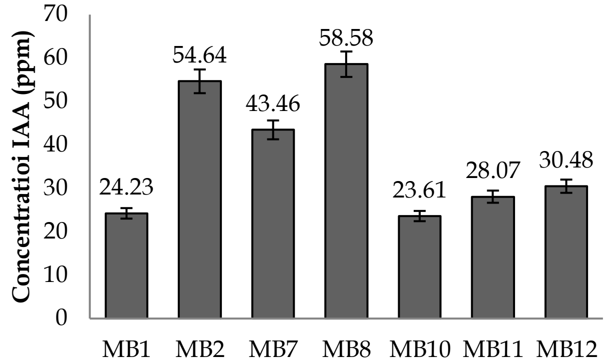

Figure 3. Concentration of IAA produced by endophytic bacterial isolates

The research findings suggest that endophytic bacteria may exhibit higher IAA production, likely due to the characteristics of the bacterial isolates used in this study, which were derived from the roots of mangrove plants, specifically *Avicennia marina*, demonstrating high IAA production. Mangrove plants adapt by forming specialized roots and thrive in diverse environments. PGPR (Plant Growth Promoting Rhizobacteria) are known as potential IAA producers due to their interaction with plant roots. Plant roots release exudates such as L-tryptophan to bacteria, enhancing root growth by incorporating exogenously synthesized IAA produced by rhizosphere bacteria into the plants (Silitonga et al., 2013).

Application on Chili Seed Growth

The application of endophytic bacteria on chili seed growth, specifically MB10 and MB11, showed less effective results compared to the control (Table 2). Based on the observed parameters, the control without the application of potential endophytic bacteria yielded the best overall results. This suggests that the bacterial isolates used led to a decrease in plant performance. This is likely due to variations in the required levels of phosphate and IAA for each plant. Additionally, the application of single endophytic bacteria on plants is suspected to cause detrimental effects on the plants.

Table 2. Average Measurements of Potential Endophytic Bacteria Applied to Chili Seeds Over 30 Days

Treatment	Control	MB10	MB 11
Seedling growth	8	3	4
Plant height (cm)	16.86	5.7	7.64
Number of leaves	18	4.4	4.8
Fresh weight (g)	4.81	1.44	2.42
Dry weight (g)	3.80	0.75	1.42

The study by Rangkuti et al. (2014) indicated that the treatment with single bacteria caused a significant number of seeds not to germinate. The normal flora balance of the plants is disrupted when endophytic bacteria live alone in the plant, leading certain endophytic bacteria to transform into pathogens. Haggag (2010) mentioned that endophytic microbes can be symbiotic or can exhibit slight pathogenic characteristics.

Excessive phosphorus could bind with micro-nutrients such as iron (Fe), copper (Cu), and zinc (Zn), rendering them unavailable to plants. This disrupted the nutrient processes necessary for healthy plant growth. Research of Jayanto et al. (2018) indicated that phosphorus (P) concentrations influenced teak seedlings. Phosphorus deficiency manifested in symptoms such as dull greenish-purple coloration and stunted growth. Optimal phosphorus doses resulted in good height and diameter with vibrant green leaves. Conversely, phosphorus excess led to symptoms such as drying from leaf tips to bases, stunted growth, and leaves turning dull purple.

The response to the hormone IAA was influenced by the concentration of IAA applied; excessive concentrations could inhibit plant growth. However, IAA levels varied significantly depending on the sensitivity of the plant organs (Sari et al., 2021). Research from Ismawanti et al. (2020) indicated that the ratio and application of IAA concentrations affected the soybean seed germination process; insufficient IAA or damaged IAA synthesis prolonged soybean germination. In another study Yulia et al. (2020), it was found that there was no interaction between the application of BAP and IAA regarding orchid shoot height, likely due to the differing use of plant growth regulator concentrations that did not meet the plant's needs.

Synchronization and alignment of nutrient and growth regulator requirements in plants had to be carefully considered. Provision of inappropriate nutrients could lead to plant deficiencies or toxicity. Improper nutrient supply, such as deficiency or excess, risked causing nutrient loss or conversion into unavailable forms (Nuryani et al., 2019).

Conclusion

The seven collections of endophytic bacterial isolates collection from the Microbiology Laboratory of the University of North Sumatra were found to possess the ability to solubilize phosphate and produce IAA in their entirety. The two most potent bacterial isolates in phosphate solubilization were MB10 and MB11, with solubilization indices of 16.2 mm and 9.5 mm, respectively. The two most potent isolates in IAA production were MB8 and MB2, with IAA concentrations of 58.58 ppm and 54.64 ppm, respectively. Application to chili seed growth with MB10 and MB11 showed decreased plant performance compared to the control (-). This indicated that nutrient concentrations and growth regulators affect plants. Additionally, bacteria living in healthy plants do not always contribute to plant health if they do not coexist with other bacteria. Treatment combining several endophytic bacteria is necessary to understand their growth-promoting abilities in plants.

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Author Contributions

Z.A; contributed as researcher and article writer, E.M; contributed as a research idea and article writing supervisor, and I.N; contributed as a supervisor in processing research data. All authors have read and agreed to publish versions of the manuscript.

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Conflicts of Interest

In writing this article, we sincerely declare that there are no conflicts of interest that may affect the objectivity and integrity of the results.

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