Research Article

Isolation and Functional Characterization of Indigenous Phosphate-Solubilizing Microbes from Shallot Rhizosphere in Andisol of Karo, Indonesia

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Abstract | Phosphorus (P) availability for plants is low in Andisol due to its high retention phosphate capacity. It can be increased by utilizing phosphate-solubilizing microbes (PSMs) that live in the rhizosphere of plants, including shallots. This study aims to qualitatively assess P solubilization by PSMs from shallot rhizospheres cultivated in a highland Andisol. The research was conducted from May to December 2023 in Karo Regency, North Sumatra, Indonesia. Isolates were obtained via serial dilution and cultivation on Pikovskaya agar, then observed for their ability to form phosphate-solubilization zones (halo zones) as an indicator of phosphate solubilization activity. The results showed that based on the results of the isolation of phosphate-solubilizing microbes in Andisol soil in Karo, Indonesia, 8 isolates (6 bacteria and 2 fungi) were obtained that were able to dissolve phosphate based on a qualitative P dissolution test. The results of the identification of the eight types of isolates from each sample location were *B. thuringiensis* (T1 1), *B. cereus* (T2 1), *B. substilis* (BJ1 1), *B. cereus* (BJ1 2), *B. gladioli* (BJ2 2), *B. cereus* (SE), *A. pseudodeflectus* (BJ2 1) and *A. niger* (BJ2 3). The eight isolates can produce organic acids, phosphatase enzymes, and phytohormones of different types and amounts. The hemolysis results obtained that B. substilis (BJ1 1) were pathogenic to living things. Each bacteria could not be consorted with fungi based on the inhibition test. Further research should be focused on seven types of phosphate-solubilizing microbes, namely *B. thuringiensis* (T1 1), *B. cereus* (T2 1), *B. substilis* (BJ1 1), *B. gladioli* (BJ2 2), *B. cereus* (SE), *A. pseudodeflectus* (BJ2 1) and *A. niger* (BJ2 3), which should be tested on plants in the field to determine their potential in P solubilization and increasing yield.

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Introduction

Low phosphorus availability is a common problem in tropical soils, especially those found in Andisol. With 127 active volcanoes, Indonesia is home to a sizable portion of Andisol, or volcanic ash soil (Saing *et al*. 2020). About 5.395 million hectares, or 2.9% of the total land area, are covered by this type of soil (Sukarman and Dariah, 2014) and used for agriculture (Anda *et al*., 2021). However, plants access to phosphorus is greatly diminished by Andisol's high phosphorus retention capacity, which is mostly caused by reactive clay minerals like allophane (Marpaung *et al*., 2021). Only around 0.1% of the total phosphorus can be absorbed by plants since allophane can retain up to 97.8% of it (Zhu *et al*., 2018). Large amounts of soluble phosphorus fertilizers must be applied since the limited solubility of phosphorus in these soils frequently inhibits plant growth.

Additionally, phosphorus (P) is essential for controlling physiological reactions and improving resistance to abiotic stressors (Hawkesford *et al*., 2023); (Lambers 2022). P is essential for photosynthesis and the growth of roots and is the second primary nutrient after nitrogen (N) (Billah *et al*., 2019). A lack of phosphorus in the soil has a negative impact on root formation, vegetative growth, and fruit production, which eventually lowers agricultural yields (Jezek *et al*., 2023); (Lopez *et al*., 2023); (Abobatta and Abd Alla, 2023). The majority of phosphorus in soil is insoluble, thus even if it is abundant, its availability is poor.

A viable substitute for increasing the amount of phosphorus in soil is the application of phosphate-solubilizing microorganisms (PSMs) (Tian *et al*., 2021). Because they mineralize organic phosphorus, solubilize inorganic phosphorus minerals, and store significant amounts of phosphorus in their biomass, PSMs, which comprise a diverse group of soil microflora, play a critical role in the soil phosphorus cycle (Gross *et al*., 2020); (Liang *et al*., 2020). The processes of phosphate solubilization include the release of organic acids, phosphatase enzymes, lowering the pH of the soil, and boosting chelation activity, which creates additional phosphorus adsorption sites. According to Billah *et al*. (2019). these microorganisms convert insoluble phosphorus into forms that plants can readily absorb, such as orthophosphates (PO_4^{3-}, HPO_4^{2-}, dan $H_2PO_4^{-}$).

The rhizosphere, the soil area close to plant roots, is home to the majority of phosphate-solubilizing microorganisms and provides a space for root exudation (Hassan *et al*., 2020). Bacterial species like *Rhizobium*, *Arthrobacter*, *Burkholderia*, and *Rahnella aquatilis* HX2 ((Liu *et al*., 2019); (Zhang *et al*., 2019)), *Pseudomonas*, *Enterobacter*, *Bacillus* (Biswas *et al*., 2018), *Rhizobium*, *Arthrobacter*, and *Burkholderia*, as well as fungal species like *Penicillium brevicompactum* and *Aspergillus niger* (Perea Rojas *et al*., 2019), are effective phosphate-solubilizing microorganisms. Sembiring and Sabrina (2022) research identified the bacteria *Burkholderia cepacia*, *Bacillus subtilis*, *Burkholderia cenocepacia*, *Burkholderia seminalis* which are able to dissolve phosphate from Andisol soil affected by the eruption of Mount Sinabung. An environmentally responsible and sustainable method of treating phosphorus deficit in agricultural soils is to use phosphate-solubilizing microorganisms (Kalayu, 2019).

This study seeks to identify and describe putative phosphate-solubilizing microorganisms in the rhizosphere of shallots cultivated in Andisol soil from Karo regency, Indonesia. By separating and screening microbial strains, evaluating their capacity to solubilize phosphate, and examining their morphological, physiological, and biochemical traits.

Materials and Methods

Sampling location determination for andisol soil in karo, indonesia

The research was conducted in May until December 2023 in Karo Regency, North Sumatra, Indonesia. To establish exact coordinates, the sampling locations for Andisol soil in Karo regency, Indonesia, were identified using the soil/geological maps (1:50.000). The color of soil using android-based Munsell soil color chart (Priandana *et al*., 2014). Samples of soil were taken from the shallot rhizosphere that were two months old. Five locations, each encompassing the rhizosphere of five plants, were used to collect composite soil samples at depths ranging from 0 to 15 cm. Coordinates the each samples from Karo Regency were taken from two points in Dolat Rayat (T) with coordinates 3°12'6,921"N 98°32'23,774"E (T1) and 3°11'41,562"N 98°32'33,379"E (T2); two points in Barusjahe (BJ) with coordinates 3°10'59"N 98°32'57"E (BJ1) and 3°10'59"N 98°32'54"E (BJ2); and one point in Simpang Empat with coordinates 3°9'46,659"N 98°29'7,608"E (SE).

Phosphate-solubilizing microorganisms potential from andisol soil in the karo regency of north sumatra

Isolation of phosphate-solubilizing microorganisms from andisol soil

The Microbiology Laboratory of Universitas Sumatera

Utara (USU) processed Andisol soil samples from ten pre-selected locations. In a 250 mL Erlenmeyer flask, 10 g of dirt was weighed for each sample, and 90 mL of 0.85% saline solution was added. At room temperature, the mixture was shaken for half an hour at 120 rpm. To guarantee the isolation of phosphate-solubilizing fungus, serial dilutions (10^{-1} to 10^{-7}) were made, and suspensions from three dilutions were utilized. The spread plate method was used to isolate microorganisms on solid Pikovskaya's media (Pikovskaya, 1948). For two to three days, plates were incubated at 28°C. Clear zones (halozones) encircling colonies were indicative of bacterial and fungal phosphate solubilization. Purified halozone-containing colonies were then incubated for three more days to verify their phosphate-solubilizing capabilities. On Pikovskaya's medium, purified isolates were obtained following multiple streakings.

Qualitative assessment of phosphate-solubilizing activity

For qualitative phosphate-solubilization experiments, bacterial cultures cultured for 24 hours on nutritional broth (NB) and fungal cultures on potato dextrose agar (PDA) were employed. Pikovskaya's medium was inoculated with 5 μL and let to sit at room temperature for 1–7 days. Measurements were made of the clear zones surrounding the colonies on Pikovskaya's medium, which show that $Ca2(PO_4)_2$ has been phosphate solubilized. The following formula was used to determine the solubilization index (SI) (Paul and Sinha, 2017).

$$SI = \frac{\text{Holozone Diameter (mm)}}{\text{Colony Diameter (mm)}}$$

Molecular identification of phosphate-solubilizing microorganisms

The BRIN lab, the isolates with the highest phosphate solubilization activity underwent molecular identification. A genomic DNA Wizard kit (Promega) was used to extract DNA. For bacterial isolates, PCR amplification focused on the 16S rRNA gene; for fungal isolates, it targeted the ITS4 and ITS5 sections. Cultures of bacteria and fungi cultivated in liquid Luria Bertani broth were used to extract DNA. Agarose gel electrophoresis (0.8% gel) was used to analyze the PCR products, and nucleotide sequences were then ascertained by sequencing. The RDP-II database's CLASSIFIER program was used to accomplish taxonomic classification with 95% confidence (Cole *et al*., 2014).

Characterization of potential phosphate-solubilizing microorganisms

Macroscopic and microscopic characterization

Nutrient agar (NA) was streaked with pure bacterial cultures, and they were cultured for three days. Along with cell shape and the results of Gram staining, colony morphology including size, shape, color, and edge was studied. To observe colony morphology, including hyphal development features, colony color, and colony shape, fungal cultures were cultivated on PDA for three days. The hyphal traits, branching patterns, and conidia features were examined under a microscope through microscopic observations.

Biochemical characterization

Organic Acid Production Test: High-Performance Liquid Chromatography (HPLC) was used to quantify organic acids. Chromatograms were examined using retention durations and peak regions after samples were injected.

Phosphatase Enzyme Activity Test: Using p-nitrophenyl phosphate as a substrate, phosphatase activity was measured. At 400 nm, the resultant p-nitrophenol was measured using spectrophotometry (Margesin, 1996).

IAA and Gibberellin Test: PLC was used to measure the amounts of gibberellin and indole-3-acetic acid (IAA) (Jimtha *et al*., 2014). Using a reverse-phase column with methanol as the mobile phase, extracts were made, filtered, and subjected to HPLC analysis.

Hemolysis Test: The hemolysis test is performed using blood agar. A clear zone formed around the colony on the medium indicates that the microbe is pathogenic (Zimbro *et al*., 2009).

Inhibition test

The dual plate method was used to assess interactions between fungi and bacteria that solubilize phosphate. In the middle of a Petri dish filled with a 1:1 combination of PDA and NA medium, a fungal mycelium plug (6 mm in diameter) was positioned (Goswami and Deka, 2020). After streaking bacteria on both sides of the plate, the plates were left to incubate for seven to fourteen days at room temperature. The ability of fungi and bacteria to coexist and form a single growth pattern was used to

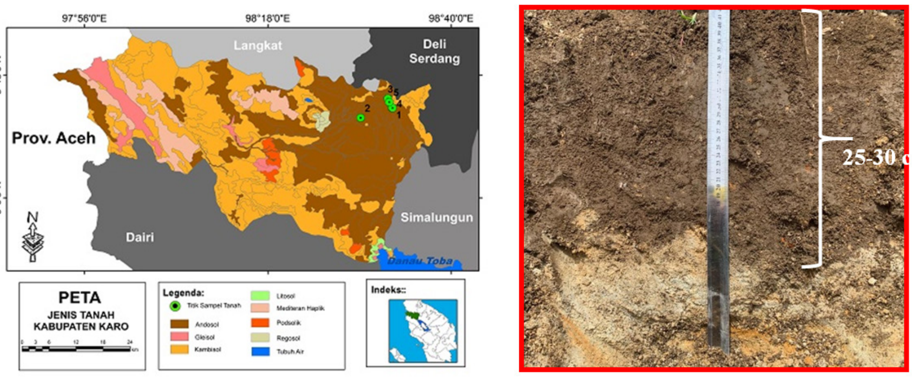

Figure 1: *Geology map (1:50.000) and andisol soil solum in karo regency*

Table 1: *Chemical content of soil from shallot rhizosphere at 3 sample locations*

Locations	Soil pH	Organic carbon (%)	Phosphate total (mg 100 g^{-1})	Available phosphate (ppm)
Dolat Rayat, Karo	6.14	4.79	51.88	3.28
Barusjahe, Karo	6.56	5.75	37.98	3.79
Simpang Empat, Karo	5.45	3.72	44.60	4.71

determine the good interaction activity.

Results and Discussion

Determination of sampling locations for acidic andisol soil in the karo regency of north sumatra using soil type maps and taxonomy

Soil samples were collected as composite samples from Andisol soils based on geological maps (Figure 1) and characterized by a black soil solum depth of 25–30 cm. Based on the Munsell Soil Chart, the soil colors from Dolat Rayat were identified as 10YR 3/2 (dark grey), 2.5Y 4/4 (bright brown) for Barusjahe, and 2.5Y 3/6 (bright brown) for Simpang Empat. These findings align with the range of Andisol colors in Indonesia, which vary from black (10YR 2/1) to dark reddish-brown (10YR 3/4) (Sukarman and Dariah, 2015).

Soil samples' chemical analysis showed that the amount of phosphorus (P) varied depending on the location (Table 1). At the sample location in Dolat Rayat, it was found that the chemical properties of the soil were very low in available P content (3.28 ppm), high total P content (51.88 mg 100g^{-1}), high organic carbon content (4.79%), and slightly acidic pH (6.14). In Barusjahe, very low available P content was also obtained (3.79 ppm), moderate total P content (37.98 mg 100g^{-1}), very high organic carbon (5.75%), and acidic pH (6.56). In Simpang empat, the soil contained very low available P (6.36 ppm), high total P content (44.60 mg 100g^{-1}), high organic carbon content (3.72%), and slightly acidic pH (5.45).

Isolation and identification of potential phosphate-solubilizing microorganisms in andisol soil from the highlands of north sumatra

Isolation of phosphate-solubilizing microorganisms from andisol soil

Microbial colonies were measured for every sample location following four days of incubation (Table 2). The largest bacterial population was found in Barusjahe 1 (8.10 × 10^6 CFU/g soil), whereas the highest fungal population was found in Dolat Rayat 1 (3.2 × 10^7 CFU/g soil). Eight isolates; two fungi and six bacteria were taken from Karo Regency's Andisol soils.

Table 2: *Number of phosphate-solubilizing microbial populations in andisol soil in karo*

Soil Source	Bacteria (Cfu/g tanah)	Fungi (Cfu/g tanah)
Dolat Rayat 1	3.6 x 10^6 ± 0.02	3.2 x 10^7 ± 0.00
Dolat Rayat 2	4.3 x 10^6 ± 0.09	9.9 x 10^6 ± 1.05
Barusjahe 1	8.1 x 10^6 ± 0.26	2.2 x 10^7 ± 2.63
Barusjahe 2	5.7 x 10^6 ± 0.23	2.8 x 10^7 ± 0.79
Simpang Empat	6.8 x 10^6 ± 0.09	1.1 x 10^7 ± 0.07

Qualitative phosphate-solubilization test

The 8 isolates were subjected to qualitative phosphate-solubilization tests. All of isolates were found capable of forming clear zones around their colonies on Pikovskaya's medium (Bacteria: T11,

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T21, BJ11, BJ12, BJ22, SE; Fungi: BJ21, BJ23). In general, the bacteria solubilising index is hingher than fungi solubilising index. The solubilization index (SI) showed that bacterial isolate T21 exhibited the highest SI among all isolates during 1-4 days after inoculation. However, from 5-7 days, the solubilization index of bacteria isolat is variation. In fungi it is obtained that isolate BJ23 had the highest SI than BJ21 (Figure 2). In general, the SI produced by bacteria increased from 1 to 6 days and decreased on the 7th day, except for bacterial isolates T21, BJ12, and BJ22, which remained elevated. Meanwhile, the SI of fungi decreased starting at 4 days.

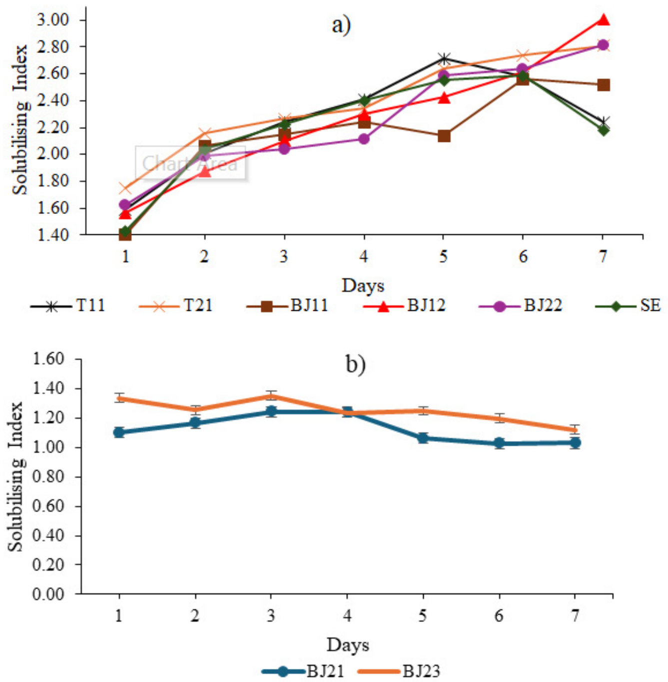


Figure 2: *P solubilization index of phosphate-solubilizing bacteria (a) and fungi (b) aged 1-7 days*

Table 3: *Identification of 8 isolates of phosphate-solubilizing microbes*

Soil Source	Isolate	Identification	Solubilization indeks 3 DAI
Dolat rayat, Karo	T11	*Bacillus thuringiensis*	2.25
	T21	*Bacillus cereus*	2.27
Barusjahe, karo	BJ11	Bacillus substilis	2.15
	BJ12	Bacillus cereus	2.10
	BJ22	Burkholderia gladioli	2.04
	BJ21	Aspergillus pseudodeflectus	1.24
	BJ23	Aspergillus niger	1.35
Simpang empat, Karo	SE	*Bacillus cereus*	2.22

DAI: Days after inoculation

Identification of phosphate-solubilizing microorganisms

Molecular identification of eight phosphate solubilizing isolates showed that *Bacillus thuringiensis* and *Bacillus cereus* were found in Dolat Rayat; *Bacillus substilis*, *Bacillus cereus*, *Burkholderia gladioli*, *Aspergillus niger*, and *Aspergillus pseudodeflectus* were found in Barusjahe; and *Bacillus cereus* was found in Simpang empat (Table 3; Figure 3 and Figure 4). In general, bacterial isolates produce a higher solubility index than fungi. These demonstrate the isolates varying capacities for solubilization, as seen by their capacity to produce clear zones on Pikovskaya's medium.

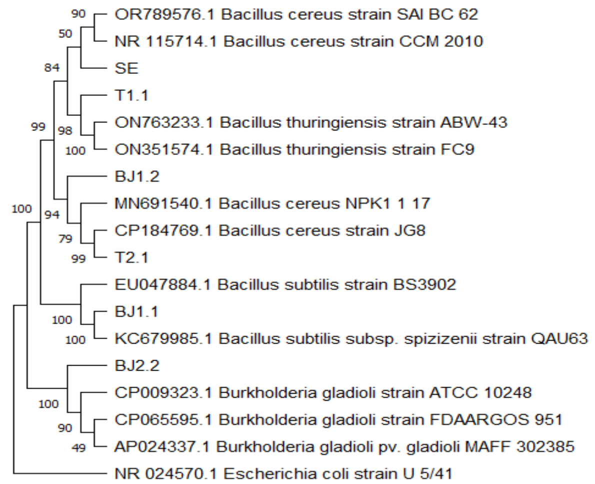


Figure 3: *Phylogenetic tree for bacteria*

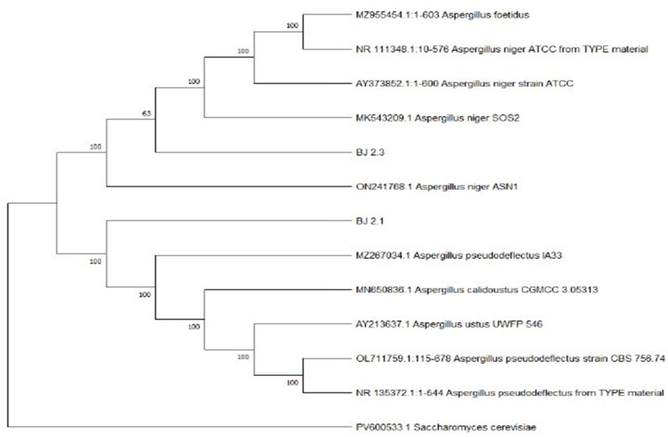


Figure 4: *Phylogenetic tree for fungi*

Characterization of Potential Phosphate-Solubilizing Microorganisms from Andisol Soil in the Karo Regency, North Sumatra

Macroscopic and microscopic characterization of phosphate-solubilizing microorganisms

The macroscopic characterization of phosphate-solubilizing *Bacillus* isolates revealed circular colonies with white to milky-white coloration, large size, and

Table 4: *Macroscopic and microscopic characteristics of 6 selected bacterial isolates*

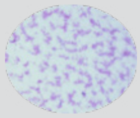
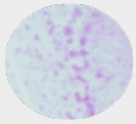
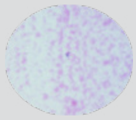
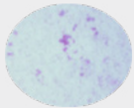
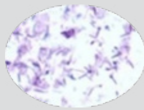
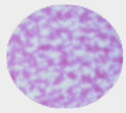
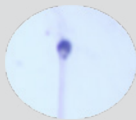
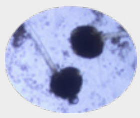
Isolate code	Identification	Macroscopis				Isolate	Microscopic		
		Colony shape	Colony color	Colony edge	Colony size		Cell Shape	Gram staining	Microcopis
T1 1	*B. thuringiensis*	Circular	White	Entire	Big		Bacill	Positive	
T2 1	*B. cereus*	Circular	Milky white	Undulate	Big		Bacill	Positive	
BJ1 1	B. substilis	Circular	Milky white	Undulate	Big		Bacill	Positive	
BJ1 2	B. cereus	Circular	White	Undulate	Big		Bacill	Positive	
BJ2 2	B. gladioli	Circular	White	Undulate	Big		Bacill	Positive	
SE	B. cereus	Circular	White	Undulate	Big		Bacill	Positive	

Table 5: *Macroscopic and microscopic characteristics of 2 fungi isolates*

Iso-late	Identifi-catio	Macroscopis				Microscopis			
		Colony Shape	Upper col-ony color	Basic col-ony color		Hyphae char-acteristics	Hyphae branching	Conidia char-acteristics	
BJ2 1	A. pseudo-deflectus	Round	White	White		-	Un-branched	-	
BJ2 3	A. niger	Round, coarsely fibrous	Brownish black	White Yellowish		Septa	Un-branched	Round, forms vesicles	

undulate colony margins. Different strains exhibited varying macroscopic characteristics. Microscopically, all six bacterial isolates had rod-shaped cells. Gram staining revealed that *Bacillus* strains and *Burkholderia* were gram-positive (Table 4).

The macroscopic characterization of fungi isolates showed distinct features. *A. niger* BJ23 had circular colonies with fine filamentous structures, small spores, dark brown-black coloration on the upper colony, and yellowish-white coloration on the lower colony surface. Hyphae were septate. *A. pseudodeflectus* BJ21 formed circular colonies, no spore, whit coloration on the upper colony, and the lower colony surface. Microscopically, *A. niger* BJ23 fungal isolates exhibited septate hyphae with unbranched structures and conidia forming round vesicles (Table 5).

Biochemical characterization of phosphate-solubilizing microorganisms

Organic acid production test

All eight phosphate-solubilizing isolates could produce organic acids (Table 6), including citric, oxalic, malic, lactic, and acetic acids. The quality and quantity of organic acids produced varied among the isolates. The highest production of citric acids was observed

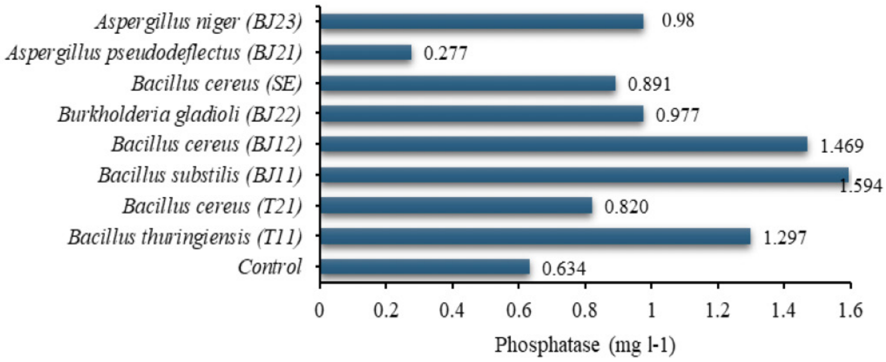


Figure 5: *Phosphatase activity of isolating from shallot rhizosfer*

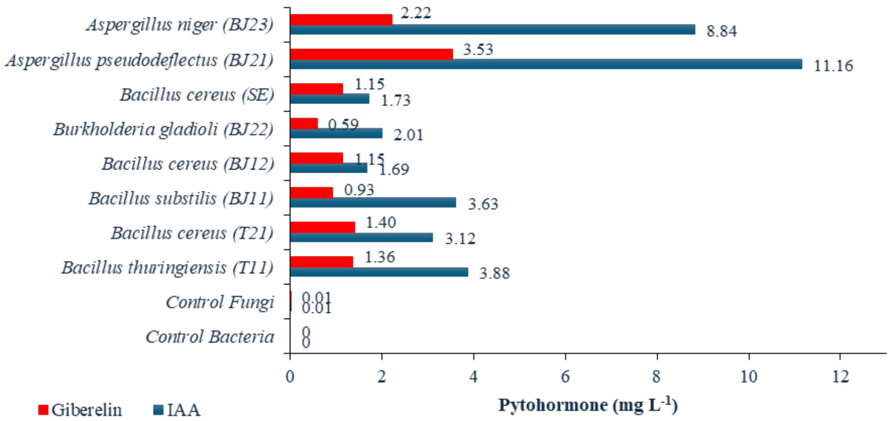


Figure 6: *Phytohormone content of isolation from the shallot rhizosphere*

in *B. cereus* (T21) (0.453 mg L^{-1}), oxalic acids in *B. substilis* (BJ11) and *B. cereus* (SE) (1.393 and 1.301 mg L^{-1} respectively), malic acid in *B. substilis* (BJ11) (9.94 mg L^{-1}), lactic acid in *A. niger* (BJ23) (12.076 mg L^{-1}), and acetic acid in *B. cereus* (T21) (6.768 mg L^{-1}). Notably, *B. gladioli* (BJ22) did not produce citric, oxalic and malic acid.

Table 6: *Organic acid tests on several phosphate-solubilizing microbial isolates*

Isolate Code	Consentration (mg L^{-1})				
	Citric	Oxalic	Malic	Lactic	Acetic
Control bacteria	Nd	Nd	Nd	Nd	Nd
Control fungi	Nd	Nd	Nd	Nd	Nd
Bacteria					
B. thuringiensis (T11)	0.199	0.611	7.829	Nd	Nd
B. cereus (T2 1)	0.453	0.565	9.672	11.031	6.768
B. substilis (BJ1 1)	0.386	1.393	9.940	11.613	5.502
B. cereus (BJ1 2)	0.399	0.858	9.622	7.701	2.147
B. gladioli (BJ2 2)	Nd	Nd	Nd	2.770	2.080
B. cereus (SE)	0.109	1.301	9.852	4.990	2.215
Fungi					
A. pseudodeflectus (BJ21)	0.248	0.973	5.971	6.063	5.608
A. niger (BJ2 3)	0.274	0.847	8.129	12.076	6.062

Nd = not detected

Phosphatase enzyme activity test

All types of bacteria and fungi can produce phosphatase enzymes. The phosphatase content of each microbe varies based on its ability to produce the enzyme. The three highest-ranking isolates producing phosphatase enzymes are *Bacillus substilis* (BJ11) with the highest (1.594 mg L^{-1}), followed by *Bacillus cereus* (BJ12) (1.469 mg L^{-1}) dan *Bacillus thuringiensis* (T11) (1.297 mg L^{-1}). The lowest phosphatase activity was found in *Aspergillus pseudodeflectus (BJ21)* (0.277 mg L^{-1}) (Figure 5).

Phytohormone (IAA and Gibberellin) test

All eight isolates produced higher concentrations of IAA compared to gibberellin. Fungal isolates, *Aspergillus pseudodeflectus* (BJ21) and *Aspergillus niger* (BJ23), produced higher IAA (11.16 mg L^{-1} and 8.84 mg L^{-1}) and gibberellin (3.53 mg L^{-1} and 2.22 mg L^{-1}) than bacterial isolates (Figure 6).

Hemolysis test

The results of the hemolysis test on eight isolates revealed that one isolate underwent lysis in *Bacillus substilis* (BJ11) blood agar (Table 7 and Figure 7). This indicates that both isolates are pathogenic to humans and animals because they can lyse red blood cells in the medium, as indicated by the formation of a clear zone in the growth medium.

Table 7: *Hemolysis test on several phosphate-solubilizing microbial isolates*

Isolates	Hemolysis
Bacteria	
B. thuringiensis (T1 1)	-
B. cereus (T2 1)	-
B. substilis (BJ1 1)	+
B. cereus (BJ1 2)	-
B. gladioli (BJ2 2)	-
B. cereus (SE)	-
Fungi	
A. pseudodeflectus (BJ2 1)	-
A. niger (BJ2 3)	-

Note: + hemolysis, - No hemolysis

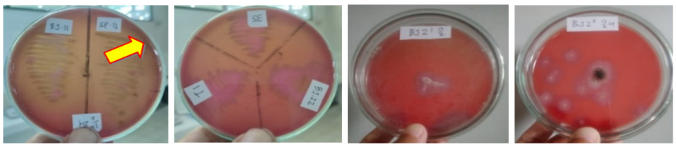


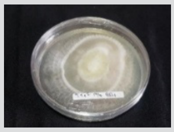
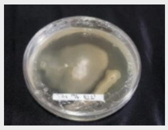
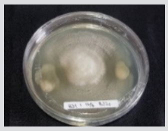
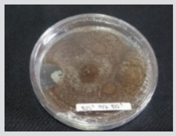
Figure 7: *Hemolysis test of phosphate-solubilizing microbial*

Inhibition test between phosphate-solubilizing bacteria and fungi

Inhibition test between bacterial and fungal isolates revealed that none of the bacterial isolates exhibited compatibility with either of the fungal isolates (Table 8). *Bacillus thuringiensis* (T11) and *Bacillus substilis* (BJ11) were tested with *Aspergillus pseudodeflectus*, fungal growth was dominant, suppressing bacterial growth. *Aspergillus niger* was test with *Bacillus thuringiensis* (T11), *Bacillus cereus* (BJ12) and *Burkholderia gladioli* (BJ2 2), produce the dominant growth of fungi than bacteria.

The research results showed that the chemical examination of soil samples from each location revealed that levels of organic carbon were high, total phosphate was high, and there were very low amounts of available P. This trait is consistent with Andisol soils' characteristics, which include little accessible phosphorus because of significant allophane phosphorus fixation (Zhu *et al*., 2018). According to (Nanzyo *et al*., 1993), Andisol soils are also known for having a high organic carbon content, which is in line with the high to extremely high quantities of organic carbon found in the soil samples. Andisol is appropriate for agricultural usage because of these qualities (Anindita *et al*., 2023). The pH values of the soil samples, which ranged from 5.45 to 6.56, were classified as somewhat acidic. Most Andisol soils in Indonesia are acidic (pH 4.5 to 5.5), with a typical pH range of 3.4 to 6.7 (Sukarman and Dariah, 2015).

Table 8: *Inhibition test of bacteria with fungi*

Isolates	Fungi	
Bacteria	*pseudodeflectus (*BJ2 1)	*A. niger* (BJ2 3)
B. thuringiensis (T1 1)		
B. cereus (T2 1)		
B. substilis (BJ1 1)		
B. cereus (BJ1 2)		
B. gladioli (BJ2 2)		
B. cereus (SE)		

Among the eight phosphate-solubilizing isolates tested, all were capable of solubilizing phosphate on Pikovskaya's medium, which was indicated by the formation of a clear zone around the isolate. These findings confirm the ability of phosphate-solubilizing bacteria to effectively solubilize phosphate in qualitative tests (Ulfiyati and Zulaika, 2015). One species of phosphate-solubilizing bacteria is *Bacillus* sp. (Kumar and Rai, 2020). The genus *Bacillus* is known for its phosphate-solubilizing capabilities (Prakash and Arora, 2019). Previous studies have also identified *Bacillus* and *Burkholderia* as effective phosphate solubilizers ((Biswas *et al*., 2018); (Liu *et al*., 2019)). Similarly, *Aspergillus niger* has demonstrated significant phosphate solubilization potential (Perea Rojas *et al*., 2019) on tricalcium phosphate media

(Bakri, 2019). According to Sembiring and Sabrina (2022) research, the *Burkholderia cepacia* resulted in an increase in the available P of the soil of 83.37%, and *Bacillus subtilis* by 25.28% than the control.

Organic acid release is the main mechanism by which microorganisms solubilize phosphate. Microorganisms can access labile inorganic phosphate (Pi) fixed in iron (Fe) or aluminium (Al) complexes by secreting organic acids, which chelate Fe and Al ions and release phosphorus (Baumann *et al*., 2018). In addition, organic acid anions produced by phosphate-solubilizing microorganisms can increase soil phosphorus availability and reduce phosphate fixation by blocking or competing for soil adsorption sites on iron and aluminum oxides (Alori *et al*., 2017). Organic acids like citric, acetic, propionic, and lactic acids are produced by *Bacillus* species (do Carmo *et al*., 2019). *A. niger* also secretes organic acids, such as formic and oxalic acids (Li *et al*., 2016); (Tian *et al*., 2019), which reduce the medium's pH and make it easier for phosphates to dissolve.

Enzymatic activities, including the formation of phosphatase and phytase, are involved in the mineralization of phosphorus in soil. (Billah *et al*., 2019) Many organisms have the ability to solubilize and mineralize phosphate, such as *Bacillus*, C*yanobacteria*, and *Pseudomonas*. According to, (Neira-Vielma *et al*., 2018) *Aspergillus niger* is also known to create phytase, which facilitates the consumption of phosphorus from phytates. Paul and Sinha, 2017 state that the production of phosphatase enzymes by phosphate-solubilizing fungus or the secretion of polysaccharides are frequently cited as the causes of the halo zones that surround fungal colonies.

Phosphate-solubilizing microorganisms are generally classified as plant growth-promoting microorganisms (PGPM) due to their phytostimulatory capacities;. (Hassan, 2017; Compant *et al*., 2019). Phosphate-solubilizing microbes produce growth-promoting hormones such as auxins, cytokinins, and gibberellins that promote cell division, cell differentiation, shoot growth, root development, flowering, germination, and xylem differentiation (Puri *et al*., 2020). Among PGPMs, those capable of solubilizing phosphate demonstrate substantial benefits for plant growth and yield (Tang *et al*., 2020) (Fahad *et al*., 2015). For instance, *Bacillus* sp. STJP (Prakash and Arora, 2019) and *Bacillus* sp. CP h60 (Ditta *et al*., 2018) have been reported to produce the phytohormone indole-3-acetic acid (IAA). Additionally, *Bacillus* sp. and *Pseudomonas* sp. produce gibberellins (Setiawati *et al*., 2015) which are also linked to their phosphate-solubilizing abilities. Phosphate-solubilizing microbes, in addition to increasing P availability in the soil, can also improve plant growth indicators. This is consistent with the Sembiring *et al*. (2016) research results of that found that the addition of phosphate-solubilizing bacteria significantly increased shoot dry weight (50.07-113.73%) and P uptake (34.47-163.28%) of potato plants on Andisols.

Phosphate-solubilizing microbial strains produce, in addition to organic acids and phosphatase enzymes, hemolysis. Research shows that *Bacillus subtilis* produces lysis on blood agar media, which can be harmful to living organisms. *Bacillus subtilis* is pathogenic, producing β-type hemolysis (Gai *et al*., 2023).

In order to assess the compatibility of bacterial and fungal isolates a critical step in the development of biofertilizers based on microbial consortiums, it is imperative to test for inhibition between them. The efficiency of advantageous additives can be increased by adding two or more compatible microbial strains from various species; their synergism makes it easier to obtain several desired benefits (Louca *et al*., 2018). In this study, the results of the inhibition tests indicated that none of the bacterial isolates exhibited compatibility with either fungal isolate. When co-cultured on a single petri dish, a clear inhibition zone was observed between the bacterial and fungal colonies, suggesting the formation of an antagonistic zone. This inhibition likely prevents the two types of microorganisms from coexisting harmoniously.

Conclusions and Recommendations

Based on the results of the isolation of phosphate-solubilizing microbes in Andisol soil in Karo, Indonesia, 8 isolates (6 bacteria and 2 fungi) were obtained that were able to dissolve phosphate based on a qualitative P dissolution test. The results of the identification of the eight types of isolates from each sample location were *B. thuringiensis* (T1 1), *B. cereus* (T2 1), *B. substilis* (BJ1 1), *B. cereus* (BJ1 2), *B. gladioli* (BJ2 2), *B. cereus* (SE), *A. pseudodeflectus* (BJ2 1) and *A. niger* (BJ2 3). The eight isolates can produce organic acids, phosphatase enzymes, and phytohormones of different types and amounts, that can dissolve inorganic phosphat that bound in Andisol minerals and plant growth stimulation. The hemolysis results obtained that B. substilis (BJ1 1) were pathogenic to living things. Each bacteria could not be consorted with fungi based on the inhibition test. Further research should be focused on seven types of phosphate-solubilizing microbes, namely *B. thuringiensis* (T1 1), *B. cereus* (T2 1), *B. substilis* (BJ1 1), *B. gladioli* (BJ2 2), *B. cereus* (SE), *A. pseudodeflectus* (BJ2 1) and *A. niger* (BJ2 3), which should be tested on plants in the field to determine their potential in P solubilization and increasing yield.

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Novelty Statement

This research has obtained seven types of phosphate-solubilizing microbes, *B. thuringiensis* (T1 1), *B. cereus* (T2 1), *B. substilis* (BJ1 1), *B. gladioli* (BJ2 2), *B. cereus* (SE), *A. pseudodeflectus* (BJ2 1) and *A. niger* (BJ2 3, which are able to dissolve phosphate qualitatively. These microbes are also able to produce organic acids, phosphatase enzymes, and phytohormones, which can dissolve inorganic phosphate bound to Andisols minerals and stimulate plant growth.

Author's Contribution

Agustina E. Marpaung: Conceptualization, supervision, methodology, software, validation, formal analysis, investigation, data curation, writing-original draft and funding acquisition.
T. Sabrina: Methodology, validation, formal analysis, writing-review & editing and Funding acquisition.
Abdul Rauf: Methodology, validation, formal analysis and funding acquisition.
Dwi N. Susilowati: Methodology, validation, formal analysis, data curation and writing-review. All co-authors examined the final draft and provided its approval for the manuscript prior to submission.

Generative AI or AI assisted technology statement
During the preparation of this work, the author used Artificial Intelligence (AI). After using this tool/service, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Conflict of interest
The authors indicate no conflict of interest in this work.

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