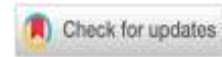




## Isolation and characterization of phosphate-solubilizing bacteria in land managed with the dusung system



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### ABSTRACT

Soil microorganisms play a crucial role in nutrient cycling and soil fertility, particularly through their ability to solubilize insoluble phosphate compounds. This study aims to isolate and characterize phosphate-solubilizing bacteria (PSB) on land managed under the dusung agroforestry system. Soil samples were collected from five types of land use: forests, mixed orchards, clove hamlets, nutmeg orchards, and shrubs. PSB isolation was performed using Pikovskaya medium with the spread plate method, followed by characterization of colony morphology (shape, edge, color, elevation) and Gram staining. The results showed that the highest PSB population occurred in forest land ( $8.3 \times 10^4$  CFU/ml), followed by mixed orchards ( $6.2\text{--}6.7 \times 10^4$  CFU/ml), clove orchards ( $5.3\text{--}5.4 \times 10^4$  CFU/ml), nutmeg orchards ( $3.2\text{--}5.6 \times 10^4$  CFU/ml), and the lowest in shrubs ( $7.1 \times 10^3$  CFU/ml). The characteristics of PSB varied, with isolates from forests and nutmeg orchards predominantly consisting of coccus-shaped Gram-positive bacteria, while those from mixed and clove orchards were dominated by bacillus-shaped Gram-positive bacteria. The population and diversity of PSBs were influenced by the availability of organic matter, soil pH, and land management practices. These findings highlight the ecological importance of the dusung system in sustaining soil fertility and promoting ecosystem resilience.

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## INTRODUCTION

In Maluku, the dusung system has long served as the backbone of community-based forest management, playing a vital role in maintaining ecosystem balance and soil fertility. However, in Seith Village, Leihitu District, Central Maluku Regency—one of the regions with the widest distribution of dusung on Ambon Island—the system is currently facing serious threats due to land conversion into settlements and open areas caused by illegal logging, leading to a decline in canopy cover over the past decades. This degradation has had a significant impact on soil health, including reduced microbial biomass, phosphatase enzyme activity, and diversity of phosphate-solubilizing bacteria (Putra et al., 2025; Raiesi & Beheshti, 2015). Such conditions reflect the real challenges of achieving sustainable land management, which has become a global focus to address soil fertility degradation and ecosystem damage caused by over-reliance on synthetic chemical fertilizers (Khan & Adnan, 2021; Şahin & Şahin, 2023).

Amid global efforts to restore environmental balance, local wisdom-based approaches such as the dusung system in Maluku have emerged as innovative solutions (Girsang et al., 2023). Dusung is a traditional agroforestry system that integrates forest management with agriculture, utilizing organic waste to improve soil quality and optimize the use of natural resources (Homeshwari-Devi et al., 2024; Njurumana et al., 2021). This system not only conserves biodiversity but also revitalizes sustainable agricultural practices that are in harmony with the ecosystem (Altieri, 2018).

The integration of the dusung system in land management provides holistic benefits that go far beyond the utilization of organic waste. Through natural fermentation processes, the system produces bioactive compounds such as organic acids that lower soil pH and enhance enzymatic activity, thereby creating a favorable environment for the growth of beneficial microorganisms, including phosphate-solubilizing bacteria (Tian et al., 2021). The resulting microclimatic conditions not only enrich soil organic matter but also facilitate nutrient mobilization—particularly phosphorus—which is vital for optimal plant growth (Silva et al., 2020). Phosphorus, as an essential nutrient, is often locked in insoluble mineral forms such as calcium phosphate, making it unavailable to plants (Lizcano-Toledo et al., 2021; Mendrofa & Lase, 2025). At this point, the crucial role of phosphate-solubilizing bacteria becomes apparent, as these microorganisms convert insoluble phosphate compounds into available forms through the production of organic acids and phosphatase enzymes (Chakraborty & Tribedi, 2019). This activity supports plant metabolic processes ranging from energy synthesis (ATP) to the formation of biomolecules such as DNA and cell membranes, which ultimately enhance agricultural productivity (Goswami et al., 2024).

Given the critical role of phosphate-solubilizing bacteria in maintaining nutrient availability and soil fertility, this study aims to examine the influence of the dusung system on the population and activity of these bacteria. By exploring the interaction mechanisms between dusung practices and soil microbial ecosystems, this research seeks to formulate an environmentally friendly and sustainable land management strategy (Bhattacharyya & Furtak, 2022; Dewi & Trimulyono, 2024). This approach not only incorporates modern scientific principles but also revitalizes traditional ecological knowledge that has proven effective in supporting land productivity (Kurnia et al., 2022).

## RESEARCH METHODS

### Research Design

This research includes two activities, namely field observation and soil sampling on several types of land use in Seith Village, Leihitu District, Central Maluku Regency, and analysis of soil biological properties in the laboratory at the Laboratory of the Biology Study Program, Faculty of

Teacher Training and Education, Pattimura University. The medium used for the isolation of Phosphate Solubilizing Bacteria is pikovskaya using the spread plate method.

### Population and Samples

Soil samples were collected from Seith Village, Leihitu District, Central Maluku Regency, representing five land-use types: forest soils, clove hamlets, nutmeg hamlets, mixed orchards, and shrub areas. For each land-use type, soil was taken from four points surrounding the base of representative trees, then composited to obtain a homogeneous sample. The composite samples were placed in polyethylene plastic bags, labeled, and stored in a cool box to preserve their physical and biological properties before further analysis.

### Instruments

This study uses a set of tools and materials to support the process of isolation and characterization of phosphate-soluble bacteria (PSB). The tools used include: a soil hoe or drill for soil sampling, sterile polyethylene plastic as a sample storage container, and a coolbox to maintain the temperature of the sample during transportation to the laboratory. In the laboratory, the process of isolation and enumeration of bacteria involves tools such as Erlenmeyer's flasks, test tubes, micropipettes, shakers for homogenizing samples, sterile petridishes, and incubators for growing bacterial colonies. Morphological characterization and staining of Grams were carried out with the help of light microscopes, bacterial ose for inoculation, glass objects, and preparation clamps. The main ingredients used consist of Pikovskaya agar media as a selective medium for PSB growth, physiological solution (NaCl 0.85%) for the sample dilution series, as well as Gram staining reagents such as violet crystals, iodine, 96% alcohol, and saffron.

### Procedures

#### Soil Sampling

Soil samples were collected at a depth of 0–25 cm, placed in polyethylene bags, and stored in a cool box to preserve their physical and biological integrity.

#### Isolation and Enumeration of Bacteria

A total of 10 grams of soil samples were put into 90 ml of physiological solution, then cubed using a shaker for 3 hours at room temperature with an agitation speed of 120 rpm. A total of 1 ml of the extract is put into a test tube containing 9 ml of sterile physiological solution, then beaten until homogeneous, and 1 ml transferred to the next tube, and so on until the  $10^{-1}$  –  $10^{-7}$  dilution series. A total of 0.1 ml of extracts from the dilution series was put into sterile petridishes that had been poured into their respective selective media and incubated at a temperature of 25–30°C for 3–7 days. The colonies that are formed are then observed and counted. The growing bacterial population was calculated using the TPC (total plate count) method.

#### Characteristics of Bacteria

Macroscopic Characteristics refers to Bergey's Manual. Single colonies that grow in the Pikovskaya medium were identified macroscopically by observing the morphology of PSB, namely color, colony shape, colony surface, colony edge, and colony elevation.

The PSB and BPN Gram tests were carried out using the coloring technique. The selected PSB isolate is taken using an ose needle aseptically and then scraped on the object smoothly and not too thick so that it is easy to read when observed using a microscope, then fixed with heat. The preparation is dripped with violet crystals for 1 minute and then rinsed using aquades gently. After that, the preparation is dripped in iodine grams and left for 1 minute, and rinsed with aquades. The

alcohol is dripped on the preparation little by little and rinsed, then given safranin dye and left to sit for 1 to 2 minutes, and then rinsed. The final stage of the preparation is dried and observed using a microscope. The bacteria identified as gram-positive are purple, and the gram-negative are red. Observation of the shape of the cell is also carried out to find out whether the bacteria are round (coccus), rod (bacilli), or wavy (spiral).

### Data Analysis

Data processing was performed through systematic tabulation of observational and quantitative results. Quantitative descriptive analysis was carried out by determining the total population of phosphate-solubilizing bacteria (PSB) grown on Pikovskaya media using the colony-forming unit (CFU/ml) method. The number of colonies was counted after incubation, and the mean value from two replicates was calculated to ensure accuracy and reproducibility.

## RESULTS

### Total Population of Azotobacter and Phosphate-Solubilizing Bacteria

Based on the results of the study, it is known that the highest total population is in the type of forest land use with a total population of phosphate-solubilizing bacteria  $8.3 \times 10^4$  CFU/ml, in mixed plantation land use (MGI, MG2) the total population of phosphate-solubilizing bacteria ranges from  $6.2 \times 10^4$  CFU/ml –  $6.7 \times 10^4$  CFU/ml, land use of clove hamlets (CG1, CG2)  $5.3 \times 10^4$  CFU/ml –  $5.4 \times 10^4$  CFU/ml, land use of nutmeg orchards (NG1, NG2) total population of phosphate-solubilizing bacteria  $3.2 \times 10^4$  CFU/ml –  $5.6 \times 10^4$  CFU/ml, and for bush land the total population of phosphate-solubilizing bacteria  $7.1 \times 10^3$  CFU/ml.

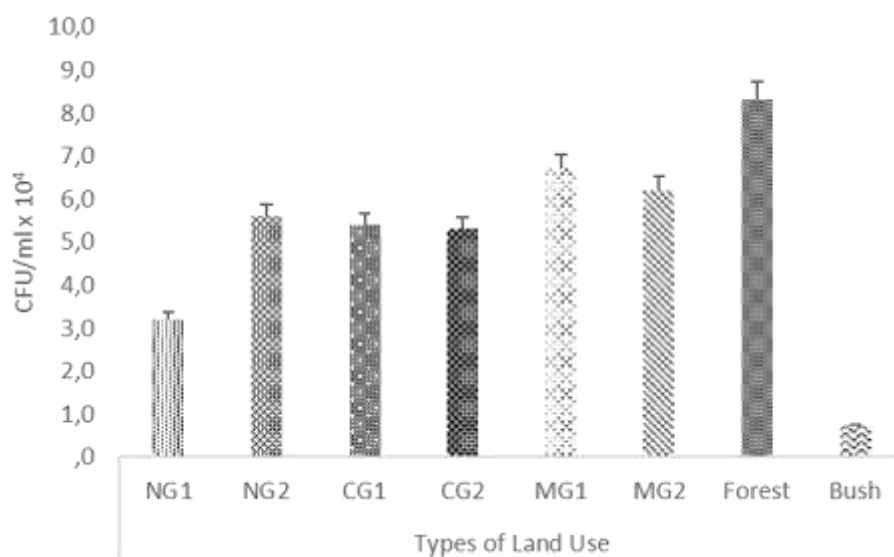


Figure 1. Total population of phosphate-solubilizing bacteria on different land-use types

### Characteristics of Phosphate Solubilizing Bacteria

In each soil sample taken, 1 isolate was selected to represent the type of land use to carry out the characteristics of phospho-solubilizing bacteria. Observation of the morphology of bacterial colonies was carried out by observation of colonies in the medium. The bacteria are first inoculated on the medium, where the phosphate-solubilizing bacteria are inoculated on the pikovskaya medium. Observations of bacterial morphology include colony shape, colony margin, colony color, elevation, gram, and cell shape. Of the eight isolates of phosphate-soluble bacteria observed, it is known that the colony form consists of filamentous, entire, and lobate. For the edges of the colony

consisting of filamentous, undulate, and entire, the color of the phosphate-soluble bacterial colony consists of beige, yellow, and white. The elevation form of bacterial colonies consists of flat and convection.

**Table I.** Characteristics of Phosphate Solubilizing Bacteria

Soil Sample Isolate	Characteristics					
	Colony Shape	Colony Edge	Colony Color	Elevation	Gram	Cell Shape
NG1	Filamentous	Filamentous	Beige	Flat	+	Coccus
NG2	Filamentous	Filamentous	Beige	Flat	+	Coccus
CG1	Entire	Filamentous	Yellow	Convex	+	Bacill
CG2	Entire	Undulate	White	Flat	+	Bacill
MG1	Entire	Entire	White	Convex	-	Bacill
MG2	Lobate	Filamentous	Yellow	Flat	+	Bacill
Forest	Entire	Undulate	Beige	Flat	+	Bacill
Bush	Undulate	Lobate	Yellow	Flat	+	Bacill

## DISCUSSION

In general, the total population of phosphate-solubilizing bacteria (PSB) in soil ranges from  $10^4$  to  $10^6$  cells per gram of soil and is predominantly found in the rhizosphere region. Phosphate-solubilizing bacteria are a group of microorganisms capable of releasing phosphate bound to soil colloid surfaces, making it available for plant uptake. The total population of bacteria is presented in Figure 1.

The highest PSB population was found on forest land ( $8.3 \times 10^4$  CFU/ml). This is in line with research (Goswami et al., 2024), which states that forest ecosystems have a diversity of highland microbes due to the stable availability of organic matter from leaf litter and the lack of anthropogenic disturbances. Organic matter provides a substrate for microbes to produce organic acids and phosphate-solubilizing enzymes, such as phosphatase (Das Mohapatra et al., 2024; Sahu et al., 2023; Sahu, 2021). In addition, relatively neutral soil pH conditions in forests (generally 5.5–7.0) support PSB activity, in contrast to agricultural land, which often experiences pH changes due to fertilization (Akinsemolu et al., 2024).

In mixed farms, the PSB population ranged from  $6.2 \times 10^4$ – $6.7 \times 10^4$  CFU/ml. The lower population rate than in forests, but still relatively high, is likely due to the diversity of vegetation that provides diverse root exudates as a source of carbon for microbes. According to (Zhao & Riaz, 2024), polyculture systems support more complex microbial communities due to the symbiotic interaction between plants and microorganisms. However, disturbances such as pruning or partial fertilization may reduce the optimal conditions for PSB.

The PSB population in clove orchards ( $5.3 \times 10^4$ – $5.4 \times 10^4$  CFU/ml) was lower than in mixed orchards. This decline can be attributed to monoculture practices that reduce the diversity of root exudate and increase the accumulation of allelopathic compounds from clove plants, potentially inhibiting microbial activity (Cardoso-Silva et al., 2024; Pinto et al., 2021). In addition, the use of inorganic fertilizers in plantations may suppress the PSB population because plant dependence on chemical fertilizers reduces the need for natural symbiosis (Buta-Hubeny et al., 2022).

The PSB population in nutmeg hamlets varies quite widely ( $3.2 \times 10^4$ – $5.6 \times 10^4$  CFU/ml). This variation may be due to differences in management practices between sites, such as the intensity of use of organic fertilizers or amelioration materials. Research (Wang et al., 2021)

suggests that compost or manure applications can increase the PSB population by up to 40%, while excessive use of herbicides or synthetic pesticides has the potential to suppress it.

Shrubs recorded the lowest PSB population ( $7.1 \times 10^3$  CFU/ml). This condition can be explained by the low input of organic matter and the dominance of pioneer vegetation that does not support the microbial activity of phosphate solubilizing. Soils in shrub lands are often acidic (pH <5) due to mineral weathering and lack of decomposition of organic matter, which inhibits the growth of PSB (Zhang et al., 2023). In addition, the absence of a root system of cultivated plants reduces the stimulation of root exudate required for PSB activity (Kaur et al., 2024).

The high total population of phosphate-soluble bacteria in forest soils and mixed plantation land use is due to the availability of substrates and environmental conditions, including crown density, temperature, humidity, and litter (Kour et al., 2023), which is on forest land. In addition, plant roots also have an effect on the population of phosphate-soluble bacteria because plant roots contain exudate that can be used by PSB as an energy source (X. Zhang et al., 2021). The type of vegetation also determines the amount of soil organic matter content that will affect the bacterial population (Lei et al., 2025; Y. Zhang et al., 2021). Soils with low organic matter content contain only 3% of the total phosphate in organic form, while soils with high organic matter contain 50% or more of the total phosphorus content in organic form (Janati et al., 2023).

The morphological characteristics of phosphate-solubilizing bacteria (PSB) isolates are presented in Table I. The isolates of NG1, NG2, and Forest show the form of filamentous or entire colonies, while MG2 and Bush have lobate or undulate edges. The filamentous form is often associated with the genus *Streptomyces* or *Actinobacteria*, which are known to produce extracellular enzymes such as phosphatase and organic acids to dissolve phosphates (Boubekri et al., 2021; Chouyia et al., 2022). The undulate edges of CG2 and Forest isolates show adaptability to nutrient fluctuations, common in soil-dwelling bacteria with unstable organic inputs (Zhang et al., 2023). Meanwhile, lobate margins (MG2 and Bush) are often found in bacteria with surfactant or biofilm production, which may aid colonization in marginal soils (Bhattacharyya & Furtak, 2022).

The results of the study show that there are three colors of colonies, including beige, yellow, and white. The beige color is found in the nutmeg orchard land use type (NG1, NG2), where the beige pigment is often produced by Gram-positive bacteria such as *Bacillus* or *Paenibacillus*, which are associated with the synthesis of siderophores or antimicrobial compounds for nutrient competition (Goswami et al., 2024). Yellow in color, there are clove dusks (CG1), mixed gardens (MG2), and shrubs. The yellow pigments (e.g. carotenoids) in CG1 and MG2 isolates may play a role in protection against oxidative stress, which is commonly found in the genus *Pseudomonas* or *Flavobacterium* (Zolfaghari et al., 2020). While the white color in CG2 and MG1 isolates is often associated with exopolysaccharide-producing bacteria (EPS) to protect themselves from drought, such as *Rhizobium* or *Azotobacter* (Ahmed et al., 2025; Hu et al., 2023).

Bacteria that have a flat colony elevation (NG1, NG2, CG2, MG2, bush) are generally bacteria with a high motility rate (e.g. *Pseudomonas*) that have the ability to spread rapidly in agar media, which reflects the colonization strategy in porous soils (Liu et al., 2019). Meanwhile, colonies with convex elevations (CG1, MG1) are often found in spore-forming bacteria (e.g. *Bacillus*), which are resistant to extreme environmental conditions such as low pH or high temperatures (Moghimian et al., 2017).

NG1 and NG2 isolates are gram-positive bacteria with coccus cell forms, possibly belonging to the genus *Staphylococcus* or *Micrococcus*, which are reported to have phosphate dissolving capabilities through the production of citric acid (Meena et al., 2020). Gram-positive bacteria in the form of bacilli cells (CG1, CG2, Forest, and Bush) are generally dominated by the genus *Bacillus* or *Paenibacillus*, which is known to produce organic acids (gluconic acid) that effectively dissolve phosphates (Ren et al., 2018) and enzyme phosphatase (Kour et al., 2023). Meanwhile,

gram-negative bacteria in the form of bacilli cells (MGI) can be categorized into the genus *Pseudomonas* or *Entrobacter*, which is a group of bacteria with high phosphate dissolving activity through the secretion of 2-ketogluconic acid (Kumar & Rai, 2015), producing weak acids and siderophorics to dissolve  $\text{FePO}_4$  or  $\text{AlPO}_4$  (Goswami et al., 2017).

## CONCLUSION

This research reveals that the dusk system, as a traditional agroforestry in Maluku, plays a significant role in maintaining the population and diversity of phosphate-solubilizing bacteria (PSB). Forest lands exhibit the highest PSB population ( $8.3 \times 10^4$  CFU/ml) due to stable availability of organic matter and minimal anthropogenic disturbances, while shrubs have the lowest population ( $7.1 \times 10^3$  CFU/ml) due to acidic soil conditions and low organic inputs. PSB characterization identified the predominance of Gram-positive bacteria in the form of *bacillus* (genus *Bacillus*) and *coccus* (genus *Staphylococcus*) capable of producing organic acids and phosphatase enzymes. These results emphasize the importance of maintaining the *dusing* system to support natural soil fertility. Recommendations include: (1) increasing the use of organic fertilizers, (2) reducing dependence on chemical fertilizers, and (3) restoration of degraded land through revegetation based on local wisdom. The integration of traditional practices with ecology-based management is expected to strengthen the resilience of sustainable agriculture in the tropics.

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