

Isolation of Phosphate-Solubilizing Bacteria from Palm Oil Solid Decanter Waste

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Article Info	ABSTRACT
Article history: Received 06-02-2026 Revised 06-04-2026 Accepted 24-04-2026	This study aims to isolate and identify phosphate-solubilizing bacteria from palm oil decanter solid waste and explore its potential as a source of functional microbes that have not been widely utilized. The novelty of this study lies in the utilization of palm oil decanter solid waste as a source of phosphate-solubilizing bacterial isolates and more comprehensive characterization including morphological and physiological aspects as a basis for developing biofertilizers. The methods used include bacterial isolation by streak plate and pour plate methods on Pikovskaya selective media, and identification based on morphological characteristics through Gram staining and physiological characteristics through oxidase, catalase, and motility tests. The results showed that five bacterial isolates (DS1, DS2, DS3, DS4, and DS5) were obtained that had the ability to dissolve phosphate as indicated by the formation of a clear zone around the colony. Based on morphological and physiological analysis, the five isolates were identified as belonging to two bacterial genera: <i>Micrococcus</i> and <i>Pseudomonas</i>. This research contributes to the scientific study of the potential of palm oil industry waste as a source of phosphate-solubilizing bacteria, which play a role in increasing phosphorus availability in the soil and have the potential to be developed as a biological agent in environmentally friendly biofertilizer formulations.
Keywords: Phosphate-solubilizing bacteria Decanter solid Palm oil <i>Micrococcus</i> <i>Pseudomonas</i>	
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1. INTRODUCTION

A palm oil mill is an industry that produces residues during its processing. These residues or wastes in a palm oil mill include empty fruit bunches (FFBs), liquid waste, fiber, shells, and solid waste. The main products produced by a palm oil mill are CPO (Crude Palm Oil) or palm oil, at 20-23%, palm kernel oil at 5-7%, liquid waste at 50%, empty fruit bunch waste at 23%, fiber at 13%, shell waste at 6.5%, and decanter solids (palm sludge) at 4% (Haryanti et al., 2014). Palm oil decanter solids are a byproduct of palm oil milling, resembling black mud. Palm oil decanter solids are derived from the mesocarp, or loose fibers, of palm oil that have been milled or processed in palm oil mills. Palm oil decanter solids are the final solid product from the processing of fresh fruit bunches in palm oil mills that use the decanter system (Sarman et al., 2021). A decanter is used to separate the liquid phase (oil and water) from the solid phase down to the very last particles. The decanter can remove 90% of all solids from palm oil sludge and 20% of dissolved solids from palm oil (Maryani, 2018).

Solid palm oil decanter waste is generally used as fertilizer in palm oil plantations and this causes solid palm oil decanter waste to produce a foul odor, become a breeding ground for flies, produce leachate, thus hampering production activities. (Sari et al., 2022)

Palm oil solid decanter waste, rich in organic matter, is often used as organic fertilizer in agriculture. This organic fertilizer from palm oil solid decanter waste can increase soil nutrient levels, which are crucial for plant growth. Palm oil solid decanter waste is often applied to crops such as oil palm, chili peppers, mustard greens, corn, and other plants. The use of palm oil solid decanter waste also helps reduce the use of chemical fertilizers and supports more environmentally friendly agriculture. The use of palm oil solid decanter waste has been proven to improve agricultural yields. In line with research conducted by (Purwono, Langai, & Jumar, 2021), in general, the provision of solid palm oil decanter waste provides better growth compared to plants that are not given solid decanter waste. Solid palm oil decanter waste is waste with high levels of cellulose, nutrients, protein and fat, which makes it a trigger for bacteria to live (Imran & Mustaka, 2019).

Phosphate-solubilizing bacteria play a role in soil fertilization because they are able to dissolve phosphate by excreting a number of low-molecular-weight organic acids such as oxalate, succinate, fumarate, and malate. These organic acids will react with phosphate-binding materials, such as Al^{3+} , Fe^{3+} , Ca^{2+} , or Mg^{2+} , forming stable organic chelates that can release bound phosphate ions that can be utilized by plants (Simanungkalit in Saida & Edy, 2024). Phosphate in the soil cannot be completely absorbed by plants, only 25% can be absorbed by plants while 75% is bound by Al in the soil. Plants absorb P from the soil in the form of phosphate ions, especially in the form of $H_2PO_4^-$ and HPO_4^{2-} which are found in the soil solution. $H_2PO_4^-$ ions are more common in more acidic soils, while at higher pH (>7) the HPO_4^{2-} form is more dominant (Novriani in Saida & Edy, 2024). Phosphorus (P) is an essential nutrient that plays a role in plant metabolism. This function indicates that P plays a significant role in plant growth and production. However, its availability in the soil is very low, at less than 0.01% of total P. Therefore, the availability of P in the soil needs to be increased, one way being to increase the activity of soil microorganisms through the use of phosphate-solubilizing bacteria. (Lestari et al., 2019).

Efforts to meet the availability of phosphorus in the soil can be carried out using biological methods, namely by providing phosphate-dissolving bacteria. (Sonia & Setiawati, 2022). Some genera of bacteria such as *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium*, and *Serratia* has been reported as a bacteria that has the potential to dissolve phosphate elements (Bhattacharyya & Jha, 2012 dalam Taisa et al., 2021). Phosphate-solubilizing bacteria are soil bacteria that can dissolve phosphate so it can be absorbed by plants. Besides increasing phosphate in the soil, they can also play a role in vitamin D metabolism, improving plant root growth and increasing nutrient uptake. (Wulandari in Sugianto, Shovitri, & Hidayat, 2019). Phosphate biologically functions as a structural element of nucleic acids and phospholipids, while in metabolism to produce energy and in the activation of metabolic intermediates as a component in signal transduction cascades, as well as enzyme regulation. (Rausch, Christine & Bucher in Sugianto, Shovitri, & Hidayat, 2019)

Bacteria play a significant role in the production of organic fertilizers. Organic fertilizers contain several beneficial microorganisms. Agricultural waste contains a large amount of lignocellulosic material, which can be broken down by cellulase. Lignocellulose consists of three polymers: hemicellulose, cellulose, and lignin. Lignocellulosic materials are abundant in agricultural waste such as corn stover, rice straw, and empty oil palm bunches (Setiawati et al., 2020). Research conducted by Imran & Mustaka (2019) found three types of bacteria in palm oil solid decanter waste, namely *Cellvibrio* sp., *Pseudomonas* sp., and *Micrococcus* sp. *Vibrio* bacteria are bacteria that are included in the gram-negative bacteria group, have rod-shaped cells, are catalase positive, motile, chemoorganotrophic, anaerobic, and have an optimum growth temperature of 20-30 degrees Celsius. The genus *Micrococcus* is a gram-positive bacterium, spherical in shape, measuring 0.5-0.2 μm , rarely motile, singly arranged, does not form endospores, tetrads, colonies are usually red or yellow, clustered, and produce catalase. *Micrococcus* lives aerobically, oxidase is often positive, although some are negative, does not produce acid from carbohydrates or produces little acid. The optimum growth temperature is 25-37 degrees Celsius, usually in mammalian skin, water, soil, and food

products (Holt et al, in Nofu et al., 2014). Research (Imran & Mustaka, 2019) shows the presence of phosphate-dissolving bacteria in palm oil solid decanter waste, namely from the genus *Pseudomonas* and *Micrococcus*.

Research on phosphate-solubilizing bacteria from palm oil solid waste has been previously conducted, as reported by Imran & Mustaka (2019) who identified the presence of several potential bacteria such as *Pseudomonas* sp., *Micrococcus* sp., and *Cellvibrio* sp. This research is still limited to identifying the presence of microorganisms without further examining the detailed morphological and physiological characteristics, as well as their potential application to plant growth. In addition, most previous studies have focused more on isolation from soil or rhizosphere, so that the exploration of microbes from industrial waste such as decanter solids is still relatively limited. Therefore, this research has novelty in several aspects, namely: (1) exploration of microbial sources from palm oil decanter solid waste which has not been optimally utilized as a source of functional bacteria, (2) more comprehensive characterization of bacteria including morphological and physiological aspects. Thus, this study not only confirms the existence of phosphate-solubilizing bacteria, but also clarifies their position and contribution in the development of biofertilizers based on palm oil industrial waste.

Although palm oil decanter solid waste has been widely used as an organic fertilizer, its use as a source of functional microbes, particularly phosphate-solubilizing bacteria, has not been explored in depth. Previous studies generally only reported the presence of microorganisms in this waste without comprehensive characterization, both in terms of morphology and physiology, and without linking them to their functional potential in increasing phosphorus availability in the soil. Furthermore, information on the quantitative ability of bacteria to dissolve phosphate, such as through measuring the phosphate solubilization index, is also limited. Therefore, there is a research gap in terms of exploring, characterizing, and evaluating the potential of phosphate-solubilizing bacteria from palm oil decanter solid waste. Based on this, this study aims to isolate and identify phosphate-solubilizing bacteria from palm oil decanter solid waste and analyze their morphological and physiological characteristics as a basis for determining their functional potential. This research is important because phosphorus is an essential nutrient whose availability in the soil is very limited due to binding to metal ions, so environmentally friendly biological alternatives are needed to increase its availability. Thus, this research is expected to provide a scientific contribution in the utilization of palm oil industry waste as a potential source of bacteria that can be developed into biological agents in biofertilizer formulations, while also supporting sustainable agricultural practices.

2. RESEARCH METHOD

This study used an experimental method with a quantitative descriptive approach, which included the stages of bacterial isolation, selection of phosphate-solubilizing bacteria, and identification of bacterial morphology and physiology. The research design used was a Completely Randomized Design (CRD) with several replications for each treatment to ensure data reliability. Palm oil solid decanter waste was taken from 3 different locations in the palm oil mill area. At each location, 3 random sampling points (a total of 9 samples) were taken, with each sample taken as much as 500 grams. Environmental conditions during sampling were recorded, including temperature (28–32°C), waste pH (5–6), and humidity conditions. Each sample was analyzed in triplicate (3 replications) to increase the validity of the results. Bacterial isolation was carried out using the pour plate and streak plate methods. Samples were diluted in stages up to 10^{-4} , then inoculated on Pikovskaya agar media as a selective medium for phosphate-solubilizing bacteria. The culture conditions were set as follows: incubation temperature 28–30°C, incubation time: 48–72 hours, Pikovskaya media pH 7.0, and 3 replicates for each isolate. Colonies that show a clear zone (halo zone) around the colony are indicated as phosphate-solubilizing bacteria. The ability of bacteria to dissolve phosphate was measured quantitatively using the Phosphate Solubilization Index (PSI) with the formula $PSI = (\text{Diameter of the clear zone} + \text{colony diameter}) / \text{colony diameter}$. Measurements were made using a caliper on solid media after incubation. The best isolate was selected based on the highest PSI value, the largest clear zone diameter, growth consistency in replicates, and colony morphological stability. The isolate with the best performance then proceeded to the identification stage. Identification was carried out based on morphological characters, namely by observing the

shape of the colony, color, edges, elevation, then Gram staining to determine Gram properties and physiological or biochemical tests, including catalase tests, oxidase tests, and motility tests. Observations were made using a microscope with 1000x magnification (with immersion oil).

The method used in this study was an experimental method with a structured research design, including stages of bacterial isolation, morphological and physiological identification, and testing the effect of bacteria on plant growth. The initial stage of the study was the isolation of phosphate-solubilizing bacteria from palm oil decanter solid waste samples. Isolation was carried out using the streak plate and pour plate methods on selective Pikovskaya agar media containing an insoluble phosphate source ($\text{Ca}_3(\text{PO}_4)_2$). The main criteria for phosphate-solubilizing bacteria were determined based on the formation of a clear zone around the colony as an indicator of phosphate-solubilizing ability. The phosphate solubilization index can be calculated by comparing the diameter of the clear zone to the diameter of the colony. Bacterial colonies showing a clear zone were then purified to obtain a single isolate. Bacterial identification was carried out based on standard microbiology protocols referring to Bergey's Manual of Systematic Bacteriology and supporting literature. Morphological identification included observing the shape, color, edge, and elevation of the colony, as well as Gram staining to differentiate between gram-positive and gram-negative bacteria. Physiological identification was carried out through a series of biochemical tests, namely the catalase test, the oxidase test, and the motility test. The catalase test was performed by adding a 3% H_2O_2 solution and indicated by the formation of bubbles as a positive result. The oxidase test used an oxidase reagent, with a purple color change as a positive indicator. The motility test was performed using liquid media (nutrient broth) to observe the pattern of bacterial growth.

Bacterial isolation was performed to obtain pure cultures from solid palm oil decanter waste samples using a serial dilution method followed by cultivation on solid media. A 1-gram sample was placed in a sterile 0.85% physiological NaCl solution and then homogenized. Subsequently, serial dilutions were performed up to 10^{-6} to reduce the density of the microorganisms. The resulting dilution suspension was then inoculated onto Nutrient Agar media using the streak plate method and incubated at 28–30°C for 24–48 hours. Growing colonies were observed and selected based on morphological differences such as color, shape, size, and texture. Different colonies are then purified through repeated subcultures until a homogeneous, contamination-free bacterial isolate is obtained, characterized by uniform colony growth.

Bacterial morphological identification is performed through macroscopic and microscopic observations. Macroscopic observations are made of colonies growing on agar media, observing morphological characteristics of the colonies, including shape (round or irregular), edges (flat, wavy, or serrated), elevation (flat, convex, or raised), color, size, and surface texture. Microscopic observations are performed using the Gram staining method to differentiate between Gram-positive and Gram-negative bacteria. The Gram staining procedure involves the application of crystal violet, Lugol's solution, washing with alcohol, and counterstaining with safranin. The results are used to determine the shape of the bacterial cells, such as cocci, bacilli, or spirals, and their cell arrangement, such as singly, in pairs, in chains, or in clusters. This identification process adheres to standard microbiological procedures commonly used to ensure the accuracy of bacterial isolate characterization. Singleton dan Sainsbury (in Sabbathini, Pujiyanto, Wijanarka, & Lisdiyanti, 2017), states that the isolation of microorganisms from the natural environment involves taking microbes from diverse populations, consisting of various types that can be found in soil, water, air, food, and in the bodies of animals and plants. Separating bacteria is necessary to identify their types and study their growth properties, morphology, physiological functions, and other characteristics. Isolation is a method for separating bacteria that is carried out simultaneously with the purification process. This process involves taking bacteria from their natural environment, then growing them on artificial media to obtain pure cultures..

The pour plate method is a method for isolating bacteria after serial dilution. The purpose of using the pour plate method is to ensure that all bacterial cells are evenly distributed throughout the medium and not just on the surface. The advantage of the pour plate method is that it allows for the creation of pure cultures in Petri dishes. However, the disadvantage is that the count results do not

reflect the actual number of microbial cells, as several cells can form a single colony (Novitasari et al., 2021).

To identify bacteria morphologically, Gram staining is performed to distinguish between gram-positive (purple) and gram-negative (red) bacteria. Staining is performed on a 48-hour-old bacterial culture grown on Pikovskaya agar medium. The staining consists of Gram A (Crystal violet), Gram B (Lugol's solution), Gram C (Alcohol), and Gram D (Safranin). The staining results are observed under a microscope at 1000x magnification. Through Gram staining, the shape, arrangement, and color of bacterial cells, as well as whether or not endospores are present, can be determined (Giyatno & Retnaningrum, 2021). The oxidase test is performed by preparing a slide on a tissue or filter paper, then adding a drop of oxidase reagent solution. A positive result is indicated by a color change to violet, while a negative result shows no color change (Akihary & Kolondam, 2020). The catalase test aims to test the ability of bacteria to produce the enzyme catalase. This experiment uses a 3% hydrogen peroxide (H_2O_2) solution. Isolates from bacterial cultures are placed on a glass slide, then one or two drops of 3% (H_2O_2) 3%. are added. If bubbles appear, the bacteria are catalase-positive; if no bubbles appear, the bacteria are catalase-negative (Akihary & Kolondam, 2020). The bacterial motility test aims to observe the movement of bacteria in a growth medium. The bacterial motility test is performed aseptically using a straight loop needle. Then, one loop of bacterial isolate is inserted into Nutrient Broth medium and incubated for 24 hours. The motility test results are motile if the bacterial growth is spread out, and non-motile if the bacterial growth is only in the form of lines (Akihary & Kolondam, 2020). The initial stage of the research was the isolation of phosphate-solubilizing bacteria from palm oil decanter solid waste samples. Isolation was carried out using the streak plate and pour plate methods on selective Pikovskaya agar media containing an insoluble phosphate source ($Ca_3(PO_4)_2$). The main criterion for phosphate-solubilizing bacteria was determined based on the formation of a clear zone around the colony as an indicator of phosphate-solubilizing ability. The phosphate solubilization index can be calculated by comparing the diameter of the clear zone to the diameter of the colony (Larasati et al., 2018; Sonia & Setiawati, 2022).

3. RESULTS AND ANALYSIS

Based on the results of the research that has been carried out from the results of the isolation and characterization of phosphate-solubilizing bacteria from palm oil solid decanter waste, the isolates with the largest clear zones were taken and 5 isolates were obtained, namely with the isolate codes DS1, DS2, DS3, DS4, and DS5.



Figure 1. Image Of Isolation of Phosphate-Solubilizing Bacteria from Palm Oil Solid Decanter Waste

The results of macroscopic morphological observations of 5 isolates of palm oil solid decanter waste bacteria coded DS (decanter solid) are presented in the following table:

Table 1. Macroscopic Morphological Observation Results of Phosphate-Solubilizing

Isolate Code	Form	Elevation	Edge	Color	Colony Diameter (mm)	Clear Zone (mm)	Phosphate Solubility Index
DS1	Round with embossed edges	flat	slippery	white	6	4	1,6
DS2	Round	flat	slippery	white	8	4	1,5
DS3	Round with embossed edges	flat	choppy	white	6	6	2
DS4	Round	arise	slippery	white	14	6	1,4
DS5	Round with embossed edges	arise	slippery	white	6	4	1,6

Table 1 shows that the five isolates (DS1 to DS5) exhibited generally circular colony shapes, with some variations. Three isolates (DS1, DS3, and DS5) have a circular shape with a raised edge, while DS2 and DS4 have a regular circular shape. In terms of elevation, three isolates (DS1, DS2, and DS3) have a flat surface, while DS4 and DS5 have a raised elevation. In terms of colony edge characteristics, most isolates (DS1, DS2, DS4, and DS5) showed a smooth edge, while only DS3 had a wavy edge. The color of all colonies in all isolates was white. These data indicate that despite similarities in color and basic colony shape, there are variations in elevation and edge shape that can be used as a basis for distinguishing the morphological characteristics of each isolate.

Based on colony diameter, isolate DS4 had the largest size at 14 mm, followed by DS2 at 8 mm, while DS1, DS3, and DS5 had the same diameter at 6 mm. However, colony size is not always directly proportional to phosphate-dissolving ability. Phosphate-dissolving ability is indicated by the formation of a clear zone around the colony. Isolates DS3 and DS4 produced the largest clear zone at 6 mm, while the other isolates (DS1, DS2, and DS5) only produced a clear zone of 4 mm. This indicates that DS3 and DS4 have higher phosphate-dissolving activity than the other isolates. The Phosphate Solubility Index (PSI) value shows that isolate DS3 has the highest value at 2, which means it has the best ability to dissolve phosphate. Isolates DS1 and DS5 have the same PSI value at 1.6, slightly higher than DS2 (1.5) and DS4 (1.4). Although DS4 had a large colony diameter and clear zone, its index value was relatively lower due to the ratio between the clear zone diameter and the colony. Overall, isolate DS3 was the best isolate in solubilizing phosphate because it had the highest index value, followed by DS1 and DS5. Meanwhile, DS4, despite having the largest colony growth, did not show optimal phosphate solubilization efficiency. This indicates that the phosphate solubilization ability is more influenced by bacterial metabolic activity than by colony size.

Following morphological observations, macroscopic observations were also performed on streak plates to obtain a more complete picture of the characteristics of the colonies formed. The results of macroscopic morphological observations using streak plates on solid palm oil decanter waste are presented in the Tabel 2.

Table 2. Plate Results of Phosphate-Solubilizing Bacteria Isolates from Palm Oil Solid Decanter Waste

Isolate Code	Bacterial Colony Morphology			
	Form	Elevation	Edge	Color
DS1	Irregular	Flat	choppy	white
DS2	round	Flat	slippery	white
DS3	Irregular	Flat	choppy	white
DS4	round	Flat	slippery	white
DS5	Round with smooth edges	round	slippery	white

Table 2 shows the colony morphology of five bacterial isolates coded DS1 to DS5. Based on observations, all isolates had white colonies, but showed variations in colony shape, elevation, and edges. Isolates DS1 and DS3 had irregular colony shapes, flat elevations, and wavy edges. Meanwhile, isolates DS2 and DS4 showed round colony shapes with smooth edges and flat elevations. Isolate DS5 had round colonies with smooth edges, which in its description also included information that the shape and edges tended to be smoother and more defined than the other isolates. The uniformity of white color in all isolates indicates the possibility of similar pigments or uniform growth media conditions, while differences in colony shape and edges may reflect the diversity of the isolated bacterial species or strains.

Based on the macroscopic morphological observations in Tables 1 and 2, the five bacterial isolates (DS1–DS5) showed variations in colony characteristics, although they generally had a uniform white color. This uniformity in colony color indicates that the isolates likely belong to the non-pigmented bacterial group, commonly found in soil bacteria or organic waste, such as the genera *Bacillus*, *Pseudomonas*, or *Enterobacter*. However, variations in the shape, elevation, and edge of the colonies indicate differences in physiological and genetic characteristics between the isolates. In Table 1, most isolates had round colonies, but three isolates (DS1, DS3, and DS5) showed round colonies with raised/embossed edges, which may indicate more active growth activity at the periphery of the colony. Meanwhile, differences in elevation between flat colonies (DS1, DS2, DS3) and raised colonies (DS4, DS5) reflect variations in extracellular matrix production or biofilm formation ability. Isolates with raised ridges generally have a higher mucoid production capacity, which is often associated with phosphate-solubilizing bacteria due to their ability to secrete organic acids to dissolve unavailable phosphate. Differences in colony edges also provide important indications for initial identification. Most isolates have smooth edges (entire), while DS3 exhibits irregular (choppy/undulate) edges, which may indicate a more diffuse growth pattern or higher motility. This character is often found in bacteria that have flagella and the ability to actively move, such as some *Pseudomonas* species. The results of observations in Table 2 (streak plate method) show changes or variations in morphology compared to Table 1. Isolates DS1 and DS3, which were previously round with raised edges, changed to irregular with jagged edges, which could be caused by differences in inoculation methods or nutrient distribution in the media. This suggests that environmental factors such as inoculum density and cultivation techniques can influence the expression of colony morphology. Meanwhile, isolates DS2 and DS4 maintained a consistent round shape with smooth edges, indicating higher morphological character stability, thus possibly originating from a more homogeneous bacterial group. Isolate DS5 showed a unique character with a round shape and smooth edges but more prominent elevations, which could be a specific characteristic that distinguishes it from other isolates. Overall, the morphological variations observed

in these two tables indicate that phosphate-solubilizing bacterial isolates from palm oil solid decanter waste have quite high character diversity. These differences can be used as a basis for selecting potential isolates and as an initial step in the further identification process. However, identification to the species level cannot be determined solely based on colony morphology, so further tests such as biochemical tests and molecular analysis are needed to ensure more accurate bacterial classification. Microscopic observations will be conducted using microscopic observations covering cell morphology, namely cell shape and gram-positive properties. Microscopic observations were conducted under a microscope at 100x magnification using immersion oil. The results of microscopic observations of the phosphate-solubilizing bacterial isolates obtained from solid palm oil decanter waste are presented in the following table:

Table 3. Results of Microscopic Observations of Phosphate-Solubilizing Bacterial Isolates from Solid Decanter Waste from Palm Oil Plants

Isolate Code	Cell Shape	Gram Properties
DS1	Coccus	Gram Positive
DS2	Coccus	Gram Positive
DS3	Coccus	Gram Positive
DS4	Basil	Gram Negative
DS5	Basil	Gram Negative

Table 3 shows that the four bacterial isolates, namely DS1, DS2, and DS3, have a coccus cell shape, which means they are round with Gram-positive, while isolates DS4 and DS5 have a bacillus shape, which means they are rod-shaped with Gram-negative. Observations of physiological characteristics were carried out by conducting a series of biochemical tests including motility tests, catalase tests, and oxidase tests. The results of observations of the physiological characteristics of the phosphate-solubilizing bacterial isolates obtained from palm oil solid decanter waste are presented in the following table:

Table 4. Results of physiological observations of phosphate-solubilizing bacterial isolates from palm oil solid decanter waste

Character	Isolate Code				
	DS1	DS2	DS3	DS4	DS5
Motility test	–	–	–	+	+
Biochemistry Catalase Test	+	+	+	+	+
Oxidase Test	+	+	+	+	+

Information: (-) Negative, (+) Positive

Table 4 shows that the motility test of three negative isolates, namely isolates DS1, DS2, and DS3, which means that the three isolates do not have the ability to move or are non-motile, while DS4 and DS5 are positive for motility, which means that the four isolates have the ability to move or are motile. The catalase test of the five isolates (DS1, DS2, DS3, DS4, and DS5) showed positive results, indicating their ability to produce the catalase enzyme which can decompose

hydrogen peroxide into water and oxygen. All five isolates (DS1 to DS5) were positive in the oxidase test, which indicates the presence of the oxidase enzyme in the bacteria.

Based on the results of biochemical tests and morphological observations, as well as comparisons with descriptions in Bergey's Manual of Systematic Bacteriology, it can be concluded that several phosphate-solubilizing bacterial isolates that have been analyzed belong to the genus *Micrococcus* and *Pseudomonas*. This identification is important as a basis for determining the potential of each isolate in agricultural biotechnology applications, especially as a phosphate-solubilizing agent to increase the availability of phosphorus in the soil. Based on the results of the identification of morphological and physiological characters compared with references in Bergey's Manual of Systematic Bacteriology, isolates DS1, DS2, and DS3 show characteristics of the genus *Micrococcus*. Isolates DS4 and DS5 were identified as the genus *Pseudomonas*. Based on the morphological observations in Tables 1 and 2, it can be seen that although all isolates had uniform colony color (white), there were significant variations in colony shape, elevation, and margins. These variations indicate differences in physiological characteristics and metabolic potential between isolates. Isolates DS1, DS3, and DS5, which had uneven (undulate/choppy) colony margins and were not completely symmetrical, tended to exhibit more active and diffuse growth patterns. This characteristic is often associated with bacteria that have a high capacity to produce extracellular metabolites, including organic acids that play a role in phosphate solubilization. In contrast, isolates DS2 and DS4, which had round colonies with smooth (entire) and consistent margins, showed more stable growth patterns, which may indicate different metabolic efficiencies in phosphate solubilization. Differences in colony elevation also provide important indications of bacterial physiological activity. Isolates with raised elevations, such as DS4 and DS5, are thought to have a higher capacity to produce extracellular compounds such as exopolysaccharides (EPS). EPS production plays a role in increasing bacterial adhesion to the substrate and assisting the diffusion of organic acids into the surrounding environment, thereby accelerating the dissolution of insoluble phosphate. Meanwhile, isolates with flat elevations tend to have more limited surface activity, but do not rule out the possibility of still having phosphate solubilization ability through other mechanisms. Furthermore, the morphological changes seen between the pour plate and streak plate methods indicate that the expression of colony characters is strongly influenced by environmental conditions and inoculation techniques. Isolates DS1 and DS3, which changed to an irregular shape in the streak method, indicate adaptive properties to nutrient distribution and growth space. This can be attributed to the bacteria's ability to compete and colonize, which are important factors in their effectiveness as phosphate-solubilizing bacteria in soil environments. Microbiologically, the ability of bacteria to dissolve phosphate is primarily based on the mechanism of organic acid production and enzymatic activity. Phosphate-solubilizing bacteria such as the genera *Pseudomonas* and *Micrococcus* are known to secrete various organic acids such as citric, oxalic, malic, and gluconic acids, which play a role in lowering environmental pH. This decrease in pH causes the dissolution of insoluble phosphate compounds, such as $\text{Ca}_3(\text{PO}_4)_2$, AlPO_4 , and FePO_4 , into phosphate ions available to plants. Furthermore, some bacteria also produce phosphatase enzymes capable of hydrolyzing organic phosphate compounds into inorganic phosphates that can be absorbed by plants. Another mechanism that plays a role is the chelation process, in which organic acids produced by bacteria bind metal ions such as Ca^{2+} , Fe^{3+} , and Al^{3+} that bind phosphate in the soil. This bond releases phosphate ions, increasing their availability. Isolates that exhibit morphological characteristics such as uneven edges, raised elevations, and diffuse growth are thought to have higher metabolic activity, thus potentially being more effective in the phosphate solubilization process. This is supported by the results of physiological tests, where the motile and Gram-negative isolates DS4 and DS5 (suspected to be of the genus *Pseudomonas*) are generally known to have higher phosphate solubilization capabilities than non-motile bacteria. Thus, colony morphological variations not only serve as a basis for initial identification but can also provide an indication of the bacteria's functional potential, particularly in phosphate solubilization. However, to confirm this relationship quantitatively, further analysis, such as measuring the phosphate solubilization index and specific organic acid production tests, is required. The results of the isolation of phosphate-solubilizing bacteria from palm oil solid decanter waste indicate that the isolated bacteria are phosphate-

solubilizing bacteria. This is evidenced by the presence of a clear zone around the bacterial colonies on Pikovskaya agar media. The growth of phosphate-solubilizing bacteria is characterized by the presence of a clear zone around the colonies. (Larasati et al., 2018). This finding is reinforced by research (Imran & Mustaka, 2019), which indicates the presence of phosphate-solubilizing bacteria in palm oil solid decanter waste, namely from the genus *Pseudomonas* and *Micrococcus*. The dilution factor used was a dilution of 10⁻⁴ because the number of colonies was the highest. The distribution of the number of colonies per sample and each dilution factor showed a uniform diversity of data and was in accordance with the principle of the dilution factor, where the higher the dilution factor, the lower the number of microbial colonies or the dilution factor was inversely proportional to the number of microbial colonies. (Sukmawati & Hardianti, 2018). Based on the results of the isolation and identification of phosphate-solubilizing bacteria from palm oil solid decanter waste, five bacterial isolates were obtained, namely DS1, DS2, DS3, DS4, and DS5. The five isolates obtained from palm oil solid decanter waste have varying colony and cell characteristics. Using the streak plate method, you can see the morphological characteristics of the bacterial colonies resulting from isolation and purification (Wulandhani et al., 2024).

Isolate DS1 has morphological characteristics, namely a round colony shape with raised edges, flat elevation, smooth edges, and white colony color. Isolate DS2 has morphological characteristics, namely a round colony shape, flat elevation, smooth edges, and white colony color. Isolate DS3 has morphological characteristics, namely a round colony shape with raised edges, flat elevation, wavy edges, and white colony color. Isolate DS4 has morphological characteristics, namely a round colony shape, raised elevation, smooth edges, and white colony color. Isolate DS5 has morphological characteristics, namely a round colony shape with raised edges, raised elevation, smooth edges, and white colony color. The streak plate results of phosphate-solubilizing bacteria from palm oil solid decanter waste showed that isolate DS1 had morphological characteristics, namely irregular shape, flat elevation, wavy edges, and white colony color. Isolate DS2 had morphological characteristics, namely round colony shape, flat elevation, smooth edges, and white colony color. Isolate DS3 had morphological characteristics, namely irregular colony shape, flat elevation, wavy edges, and white colony color. Isolate DS4 had morphological characteristics, namely round colony shape, flat elevation, smooth edges, and white colony color. Isolate DS5 had morphological characteristics, namely round colony shape with raised edges, raised elevation, smooth edges, and white colony color. Using the streak plate method, the morphological characteristics of isolated and purified bacterial colonies can be seen. (Wulandhani et al., 2024).

Gram staining is carried out using a special dye to observe the shape of bacterial cells and classify them into gram-positive and gram-negative bacterial groups. The Gram staining method is an effective technique for identifying bacteria, and is a very important differential staining and is often used in microbiology laboratories (Hidayanti, Sulfiani, & Taufiq, 2021). Based on the Gram staining that has been done, the gram-positive bacteria were isolates DS1, DS2, and DS3, while the gram-negative bacteria were isolates DS4 and DS5. The cell wall of Gram-positive bacteria is composed of a peptidoglycan layer that forms a thick and rigid structure. This thick peptidoglycan layer makes Gram-positive bacteria resistant to rinsing with alcohol, so they are able to maintain the purple color of crystal violet. (Fitri & Yasmin in Reimena et al., 2017). Gram-negative bacteria can appear pink because gram-negative bacteria have a high lipopolysaccharide content in their cell walls, so that when staining is carried out in the decolorizing stage using 95% alcohol, the lipopolysaccharide layer becomes colorless because the first staining with crystal violet solution adheres to the lipopolysaccharide layer and when the second staining is carried out with safranin, it produces a red color. (Amiruddin et al., 2017).

Motility tests on isolates DS1, DS2, and DS3 were negative (no spreading). This indicates that these isolates lack flagella and are unable to move (Fallo et al., 2022). Meanwhile, the motility test results for isolates DS4 and DS5 were positive, indicating that these isolates could spread. The catalase test results were positive for isolates DS1, DS2, DS3, DS4, and DS5. A positive test result was indicated by the appearance of gas bubbles, indicating the presence of the catalase enzyme. This enzyme is considered to play a crucial role in the growth of aerobic bacteria because it can break down H₂O₂, a compound that is toxic to microbial cells. According to Wahid (in Zebua et al.,

2020) bacteria that do not show oxygen bubbles are classified as catalase negative, which means that H_2O_2 cannot be broken down because these bacteria do not have the catalase enzyme. Wahid (in Zebua et al., 2020). The oxidase test results for all isolates DS1, DS2, DS3, DS4, and DS5 showed positive results, producing a purple color. The oxidase test was performed by making a smear on a glass slide covered with tissue or filter paper, then dripping with an oxidase reagent solution. A positive reaction is indicated by a color change to purple (violet), while a negative reaction is indicated by no color change. (Damayanti et al., 2018).

Micrococcus is a phosphate-dissolving bacteria in the soil. Holt et al., (in Yanti, Rahmawati, & Kurniatuhadi, 2022) states that the characteristic of the genus *Micrococcus* is the coccus cell shape, which is arranged in pairs, tetrads, or irregular clusters in chains. In addition, the genus *Micrococcus* is gram-positive. (Yanti et al., 2022). This type of bacteria lives in abundance in the surrounding environment and plays a role in the environment. The role of *Pseudomonas* bacteria in the environment is as a P binder, thus increasing the role of phosphate in plants. It produces intercellular antibiotic enzymes in the root cortex tissue, thus inhibiting pathogen invasion, making this bacteria beneficial for plants. (Imran & Mustaka, 2019).

4. CONCLUSION

Based on the research conducted, five isolates of phosphate-solubilizing bacteria were obtained from solid palm oil decanter waste, which have been characterized based on their morphological and physiological properties. The identification results indicate that the isolates belong to the genera *Micrococcus* and *Pseudomonas*, which are known to play an important role in the nutrient cycle, particularly in phosphate solubilization. The main scientific contribution of this study is to demonstrate that solid palm oil decanter waste is a potential source of functional microbes that have the ability to dissolve phosphate, thereby contributing to the development of sustainable agricultural biotechnology. These findings add scientific insight by highlighting the use of previously underutilized waste as a source of beneficial microbes. Among all isolates, DS3 showed the best performance with the highest phosphate solubility index (PSI) value of 2, which indicates the most superior ability to dissolve phosphate compared to other isolates. This indicates that the DS3 isolate has great potential for further development as a biofertilizer agent. The use of these bacteria, especially the DS3 isolate, has the potential to improve soil fertility by increasing phosphorus availability for plants, while supporting more environmentally friendly management of palm oil industrial waste. For further research, it is recommended to conduct molecular identification to confirm the taxonomy of the isolate, testing the effectiveness on plant growth at the greenhouse and field scale, and optimizing the formulation for mass production of biofertilizer.

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