JUATIKA
JURNAL AGRONOMI TANAMAN TROPIKA
VOL. 7 NO. 1 January 2025

DOI :https://doi.org/10.36378/juatika.v7i1.3865
eissn 2656-1727
pissn 2684-785X
pages : 9 – 14

RESEARCH ARTICLE Open Access

Isolation and Potential Test of Phosphate-Solubilizing Bacteria in the Rhizosphere of Mangrove Plants (*Rhizophora mucronata* Poir) as Isolates for Biofertilizer

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Abstract

Mangrove forests play a significant role in environmental sustainability, particularly in their capacity to mitigate coastal erosion caused by seawater intrusion. Specifically, mangrove plants possess the ability to stabilize coastal land by retaining seawater, thereby preventing erosion along shorelines. This study aims to isolate phosphate-solubilizing bacteria and evaluate their potential from the rhizosphere of mangrove plants. The research was conducted at the Central Laboratory of the Stiper Agricultural Institute in Yogyakarta, located in Maguwoharjo, Depok District, Sleman Regency, Yogyakarta, with sampling carried out at Baros Beach in Kretek District, Bantul Regency, Yogyakarta. The study was conducted from January to February 2024. A descriptive research methodology was employed, including sterilizing tools and materials, media preparation, sampling, bacterial isolation, and identification. The analysis involved macroscopic and microscopic observations, measurement of the phosphate solubility index, and biochemical testing. The findings revealed a total of 16 bacterial isolates from five species of mangroves, specifically within the genera Streptococcus and Paracoccus. Notably, the isolate designated as SC 1 exhibited the highest phosphate solubility index, measuring 6.20 mm, indicating its potential for phosphate solubilization.

Keywords: Bacterial Isolation, Biofertilizer, Fertility, Mangrove, Phosphate Solubilizing Bacteria

1. Introduction

Mangrove plants play a significant role in the functioning of the environment. Mangrove vegetation, particularly the rhizosphere, serves as a biofilter to remove polluting compounds (Syah et al., 2018). The mangrove rhizosphere (root) area is home to a distinctive microbial ecosystem that is symbiotic with the mangrove roots.

The rhizosphere represents an optimal environment for the development of soil microorganisms. The numerous functions of bacteria in the soil rhizosphere include the capacity to solubilize phosphate and secrete the enzyme phosphatase, which facilitates the transformation of organic phosphate into inorganic phosphate and fix nitrogen (Maudy et al., 2020).

Phosphorus is an essential element for plants, yet despite its prevalence in soil, plants have limited access to it. This is because phosphorus is typically present in soil as an insoluble metal chelate. (Oksana et al. 2020).

Soil bacteria belonging to the genus Bacillus, which are capable of solubilizing phosphate, facilitate the dissolution of this nutrient, thereby enabling plants to absorb it. In addition to enhancing plant root development and elevating nutrient absorption, it elevates soil phosphate levels (Marista et al., 2013). The application of phosphate-solubilizing bacteria has been demonstrated to enhance plants' absorption of phosphorus and confer benefits to the environment. Furthermore, applying phosphate-solubilizing bacteria has been demonstrated to enhance plant growth and yield (Pane et al., 2022).

One source of rhizosphere isolates is mangrove plants. The bacterial breeding environment in mangrove forests, which are subject to extreme conditions, can produce robust bacteria capable of surviving in such an environment. Phosphate-solubilizing bacteria have been identified near mangrove roots and the rhizosphere (Anastia & Lubis, 2022). The phosphate-solubilizing bacteria found in mangrove areas demonstrate high environmental adaptability to a range of pollutants,

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including salinity, and have the potential to serve as a significant source of biofertilizer.

Biofertilizers, particularly those derived from mangrove rhizosphere phosphate-solubilizing bacteria, demonstrate a high degree of adaptability, making them suitable for utilization in a range of geographical locations. This study aimed to obtain phosphate-solubilizing bacterial isolates from the rhizosphere of plants and to ascertain the potential of phosphate-solubilizing bacteria derived from the rhizosphere of mangrove plants.

2. Material and Methods

The study employed a descriptive methodology. The descriptive method was conducted by identifying bacteria in a laboratory setting. The technical research employed the spread plate and four-way streak methods (Oksana et al., 2020).

The research was conducted from January to February 2024 at the Central Laboratory of the Stiper Agricultural Institute of Yogyakarta, located at (-7.761792644930246, 110.42543516998053) coordinates. The bacterial isolates were sourced from five distinct mangrove rhizospheres within the Baros Beach region of Kretek District, Bantul Regency, Yogyakarta.

The following tools were employed in this study: autoclave, laminar air flow cabinet, petri dish, beaker, stirrer, measuring cup, Erlenmeyer flask, test tube, Durham tube, ose needle, spreader, micropipette, dropper, Ohaus scale, Bunsen burner, Bunsen gauze, microscope, OptiLab, slide glass, cover glass, spray bottle, stove, matches, test tube rack, cooler box, ice pack, and vernier caliper.

The materials used were soil rhizosphere samples from 5 types of mangroves, namely Avicennia marina, Rhizopora mucronata, Rhizopora apiculata, Sonneratia caseolaris, and Sonneratia alba, selective pikovskaya media, NA media, NaCl, 96% alcohol, distilled water, spirits, gram staining sets, glucose media, lactose media, mannitol media, peptone, 3% H_2O_2 solution, kovac solution, 1 N NaOH solution, phenol red indicator, filter paper, plastic wrap, aluminium foil, tissue, plastic kilograms, HVS paper, blue tip, and fat cotton.

The next stage was a multilevel dilution up to 10-7 and incubated for 3 x 24 hours. Each colony was then purified and made into a pure culture.

All isolates were then observed macroscopically, and then the clear zone was observed for 7 x 24 hours (Mardyansah and Trimulyono 2021). Next, the isolate was observed microscopically using gram staining.

Furthermore, all isolates were tested biochemically with three tests, namely the catalase test to determine the nature of bacteria based on oxygen requirements using 3% H_2O_2. The presence of air bubbles or foam indicates positive test results. The oxidase test uses Kovac's solution and filter paper to determine the presence or absence of the oxidase enzyme in bacteria. Positive results if the bacteria turn purple and vice versa if there is no color change, then the result is negative (Farid and Setiadi 2019). The fermentation test determines whether bacterial isolates can ferment three different types of sugars, namely glucose, lactose and mannitol, with the addition of a phenol red indicator. Positive test results if the fermentation medium turns yellow and air bubbles are in the Durham tube (Nuryanti and Pratiwi 2021).

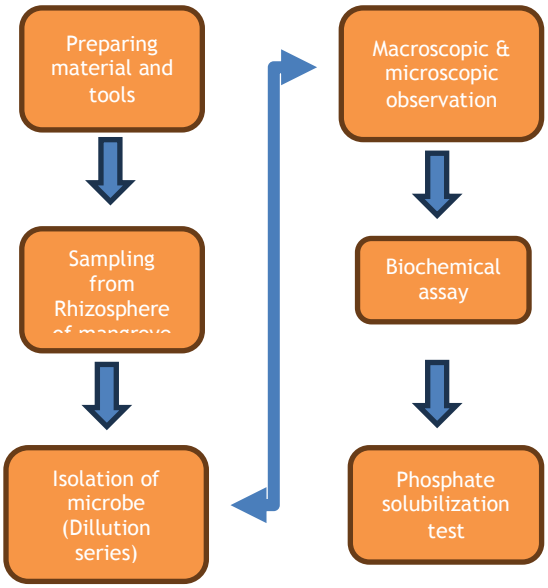

Figure 1. Research Flow Diagram

3. Results and Discussion

A common issue in plantations is a lack of nutrients, particularly phosphate. This is because phosphate is bound to Fe and Al, which causes the soil to become acidic. In order to make phosphate soluble and available to plants, phosphate-solubilizing bacteria are required. Phosphate-solubilizing bacteria benefit the environment and plant growth by producing organic acids, including acetic, lactic, and glycolic acids. These acids form stable compounds with Fe and Al cations, which bind P so that it is free from their bonds and can be absorbed by plants. As a result, phosphate-solubilizing bacteria can be considered environmentally friendly biofertilizers (Dewi, 2007).

A biofertilizer is defined as a biological fertilizer comprising a variety of living microorganisms that are beneficial to plants. These microorganisms can provide nutrients that enhance soil fertility and the quality of crop yields (Surtiningsih 2015).

A total of 16 bacterial isolates from 5 different types of mangroves, namely Avicennia marina, Rhizopora mucronata, Rhizopora apiculata, Sonneratia caseolaris, and Sonneratia alba have been found as phosphate-solubilizing bacteria based on the results of bacterial isolation from the mangrove rhizosphere area taken from Baros Beach, Kretek District, Bantul Regency, Yogyakarta.

The characteristics of phosphate-solubilizing bacteria

originating from the mangrove rhizosphere can be seen in Table 1 along with the observation parameters of colonies growing on selective pikovskaya media.

Table 1. Characteristics of Phosphate Solubilizing Bacteria

isolate	Edge	Type	Elevation	Size	Color
AM 1	*Undulate*	*Circulair*	*Flat*	Medium	Cream
AM 2	*Undulate*	*Circulair*	*Flat*	Medium	Cream
AM 3	*Undulate*	*Circulair*	*Flat*	Medium	Cream
RM 1	*Entire*	*Circulair*	*Flat*	Small	white
RM 2	*Entire*	*Circulair*	*Flat*	Small	white
RM 3	*Entire*	*Circulair*	*Flat*	Small	white
RM 4	*Entire*	*Circulair*	*Flat*	Small	white
RA 1	*Entire*	*Circulair*	*Flat*	Small	white
RA 2	*Entire*	*Circulair*	*Flat*	Small	white
RA 3	*Entire*	*Circulair*	*Flat*	Small	white
RA 4	*Entire*	*Circulair*	*Flat*	Small	white
SC 1	*Entire*	*Circulair*	*Flat*	Medium	white
SC 2	*Entire*	*Circulair*	*Flat*	Medium	white
SC 3	*Entire*	*Circulair*	*Flat*	Medium	white
SA 1	*Entire*	*Circulair*	*Flat*	Medium	white
SA 2	*Entire*	*Circulair*	*Flat*	Medium	white

Notes: AM (Avicennia marina), RM (Rhizopora mucronata), RA (Rhizopora apiculata), SC (Sonneratia caseolaris), SA (Sonneratia alba), Undulate (Wavy), Entire (Flat), Circulair (Round), Flat (Flat).

The isolates that have been obtained are then observed macroscopically, including the edges, shape, elevation, size and color of the bacteria (Kristinanda 2018). Based on the observations made, the characteristics of the bacterial isolates obtained vary with undulate (wavy) and entire (flat) colony edges, the shape of the colony is circular (round), elevation variations are flat (flat) with small and medium colony sizes, the color of the colony is cream and white. Different environmental conditions and gene expression originating from bacteria cause changes in bacteria's morphological properties, so each bacteria has a different morphology (Lestari et al. 2011).

Figure 2. Results of isolation of phosphate solubilizing bacteria isolate SC 1.

Table 2. Phosphate Solubility Index (PSI) Measurement

Isolate	Colony Diameter (mm)	Clear zone (mm)	IKF (mm)
AM 1	5.45	18.45	4.38
AM 2	5.45	8.65	2.58
AM 3	7.45	26,4	4.54
RM 1	4.5	6,8	2.51
RM 2	8.5	13.7	2.61
RM 3	5.45	16,65	4.05
RM 4	9.55	14.5	2.51
RA 1	16,55	23.4	2.41
RA 2	6,55	9.4	2.45
RA 3	15.45	17,45	2.12
RA 4	18.6	21,4	2.15
SC 1	7,4	38.5	6,20
SC 2	4.7	21,6	5.5
SC 3	4.55	13.6	3.9
SA 1	4.35	10.35	3.37
SA 2	3.45	10.55	4.05

Notes: AM (Avicennia marina), RM (Rhizopora mucronata), RA (Rhizopora apiculata), SC (Sonneratia caseolaris), SA (Sonneratia alba).

Measurements were carried out using a caliper to measure the colony and clear zone diameters. The results obtained were calculated using the phosphate solubility index formula (Sharon et al. 2016), which states that isolate SC 1 has the highest phosphate solubility index (IKF) while RM 3 is the isolate with the lowest phosphate solubility index.

Figure 3. The results of the clear zone potential test of phosphate-solubilizing bacteria isolate SC 1

Table 3. Gram Staining of Bacteria

Isolate	Staining	Cell Shape	Genus
AM 1	+	*coccus*	*Streptococcus*
AM 2	+	*coccus*	*Streptococcus*
AM 3	+	*coccus*	*Streptococcus*
RM 1	+	*coccus*	*Streptococcus*
RM 2	+	*coccus*	*Streptococcus*
RM 3	-	*coccus*	*Paracoccus*
RM 4	-	*coccus*	*Paracoccus*
RA 1	-	*coccus*	*Paracoccus*
RA 2	-	*coccus*	*Paracoccus*
RA 3	-	*coccus*	*Paracoccus*
RA 4	-	*coccus*	*Paracoccus*
SC 1	-	*coccus*	*Paracoccus*
SC 2	-	*coccus*	*Paracoccus*
SC 3	+	*coccus*	*Streptococcus*
SA 1	-	*coccus*	*Paracoccus*
SA 2	-	*coccus*	*Paracoccus*

Notes: AM (Avicennia marina), RM (Rhizopora mucronata), RA (Rhizopora apiculata), SC (Sonneratia caseolaris), SA (Sonneratia alba), (+) = positive, (-) = negative.

Microscopic observation was carried out using gram staining, the materials used in gram staining are crystal violet, lugol's iodine, ethanol, and safranin. Crystal violet is the main dye in gram staining; crystal violet is basic and will react with acidic bacteria, making transparent bacteria look purple (Hidayanti et al. 2021). Gram-positive bacteria contain protein, while gram-negative bacteria have a high fat percentage (Amin et al., 2023).

The results of microscopic observations that have been carried out show that isolates AM 1, AM 2. AM 3. RM 1, RM 2. and SC 3 are purple, which means they are gram-positive, and then they isolate RM 3. RM 4. RA 1, RA 2. RA 3. RA 4. SC 1, SC 2. SA 1, and SA 2 are pink, which means they are gram-negative. Isolates with gram-positive and coccus shapes are classified as the genus Streptococcus (Ariyani 2020). Meanwhile, isolates that are gram-negative and coccus-shaped are classified as the genus Paracoccus. (Febriansah and Meiliza, 2020).

The genus Streptococcus is one of the bacteria that can dissolve phosphate (Syarwani et al., 2022). Paracoccus is also a bacteria that can dissolve phosphate in the soil (Marista et al., 2013). Both genera of bacteria have the potential to become plant biofertilizers.

The catalase test measures the catalase activity of the bacteria being tested. Most bacteria produce the enzyme catalase, which converts H_2O_2 into water and oxygen. Because the catalase enzyme can degrade H_2O_2 which is harmful to microbial organisms, this enzyme is believed to be important for aerobic growth (Sianipar et al. 2020). The catalase test results showed that all isolates could not produce the catalase enzyme or were negative. The oxidase test aims to determine the presence of the oxidase enzyme in bacteria using Kovac's solution and filter paper. Kovac's solution functions as a detector of indole which results from oxidation (Rifai 2021), a positive oxidase test is indicated by the appearance of a purple color on the filter paper while a negative result on the filter paper does not change color. Based on the oxidase test, all isolates showed positive results.

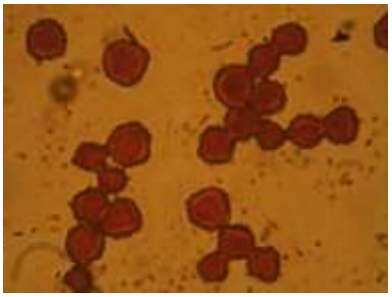

Figure 4. Results of microscopic observations of the shape of coccus cells with a magnification of 100 x

Table 4. Bacterial Catalase and Oxidase Test

Isolate	Catalase test	Oxidase Test
AM 1	-	+
AM 2	-	+
AM 3	-	+
RM 1	-	+
RM 2	-	+
RM 3	-	+
RM 4	-	+
RA 1	-	+
RA 2	-	+
RA 3	-	+
RA 4	-	+
SC 1	-	+
SC 2	-	+
SC 3	-	+
SA 1	-	+
SA 2	-	+

Notes: AM (Avicennia marina), RM (Rhizopora mucronata), RA (Rhizopora apiculata), SC (Sonneratia caseolaris), SA (Sonneratia alba), (+) = positive, (-) = negative.

Table 5. Fermentation Test

Isolate	Fermentation Test		
	Glucose	Lactose	Mannitol
AM 1	+	+	+
RM 1	+	+	+
RA 1	+	+	+
SC 1	+	+	+
SA 1	-	-	-

Notes: AM (Avicennia marina), RM (Rhizopora mucronata), RA (Rhizopora apiculata), SC (Sonneratia caseolaris), SA (Sonneratia alba), (+) = positive, (-) = negative.

Fermentation tests were conducted using glucose, lactose, and mannitol media to ascertain the capacity of the bacteria to ferment carbohydrates. The outcome of carbohydrate fermentation by bacteria is the generation of acid, which causes the phenol red indicator to transition from red to yellow. The presence of gas during fermentation results in the formation of air bubbles within the Durham tube. Conversely, in the absence of fermentation, the medium remains colorless, and no air bubbles are observed (Anggraini et al., 2019). The four isolates (AM 1, RM 1, RA 1, and SC 1) demonstrated positive fermentation results in all three media, while isolate SA 1 exhibited negative results.

4. Conclusion

The research yielded 16 bacterial isolates from five types of mangrove, belonging to the genera Streptococcus and Paracoccus. Of these, isolate SC 1 exhibited the most significant potential for phosphate dissolution, with a phosphate solubility index of 6.20 mm.

References

Amin, S. S., Ghozali, T. Z., & Efendi, M. R. S. (2023). Identifikasi bakteri dari telapak tangan dengan pewarnaan Gram *Identification of bacteria from palms with Gram stain*. *Jurnal Kimia dan Ilmu Lingkungan*, 1(1), 30–35.

Anastia, N., & Lubis, S. S. (2022). Potential of phosphate solubilizing bacteria from mangrove rhizosphere effect plant growth corn (*Zea mays*). *KENANGA Journal of Biological Science and Applied Biology*, 2(1), 1–14. https://doi.org/10.22373/kenanga.v2i1.1919

Anggraini, I., Ferniah, R. S., & Kusdiyantini, E. (2019). Isolasi khamir fermentatif dari batang tanaman tebu (*Saccharum officinarum* L.) dan hasil identifikasinya berdasarkan sekuens internal transcribed spacer. *Jurnal Berkala Bioteknologi*, 2(2), 12-22.

Ariyani, A. N. (2020). Gambaran hasil uji hemolisis bakteri *Streptococcus pyogenes* yang diinokulasi pada media agar darah manusia kadaluarsa dan agar darah domba. (pp. 7–26).

Farid, E., & Setiadi. (2019). Teknik isolasi, karakterisasi, dan identifikasi bakteri pada ikan toman (*Channa micropeltes*).

Jurnal Perikanan Indonesia, 17(2), 155–159.

Febriansah, R. E., & Meiliza, D. R. (2020). Buku ajar mata kuliah.

Hidayanti, A. S. N., Sulfiani, S., & Taufiq, N. (2021). Pemanfaatan ekstrak kulit ubi jalar ungu sebagai pengganti crystal violet pada pewarnaan Gram. *Jurnal Sehat Mandiri*, 16(2), 46–56. https://doi.org/10.33761/jsm.v16i2.364

Kristinanda, A. D. (2018). Isolasi bakteri pendegredasi senyawa hidrokarbon pada tanah tercemar di PT. KAI (Persero) UPT Balai Yasa Yogyakarta. *Universitas Islam Indonesia*.

Lestari, W., Linda, M., & Martina, A. (2011). Kemampuan bakteri pelarut fosfat isolat asal Sei Garo dalam penyediaan fosfat terlarut dan serapannya pada tanaman kedelai (*Capability of phosphate solubilizing bacteria from Sei Garo in soluble phosphate and its uptake by soybean*). *Biospecies*, 4(2), 15.

Mardyansah, D., & Trimulyono, G. (2021). Isolasi, karakterisasi, dan uji potensi bakteri pelarut fosfat dari rhizosfer di Pegunungan Kapur Selatan, Tulungagung. *LenteraBio: Berkala Ilmiah Biologi*, 10(2), 188–198. https://doi.org/10.26740/lenterabio.v10n2.p188-198

Marista, E., Khotimah, S., & Linda, R. (2013). Bakteri pelarut fosfat hasil isolasi dari tiga jenis tanah rizosfer tanaman pisang nipah (*Musa paradisiaca* var. nipah) di Kota Singkawang. *Protobiont*, 2(2), 93–101.

Maudy, R. N., Zulaika, E., & Shovitri, M. (2020). Karakter isolat bakteri P1 dari rhizosfer tanaman tebu (*Saccharum officinarum*). *Jurnal Sains dan Seni ITS*, 8(2), 2–3. https://doi.org/10.12962/j23373520.v8i2.43550

Nuryanti, S., & Pratiwi, A. R. (2021). Karakterisasi isolat bakteri penghasil selulosa dari buah naga merah (*Hylocereus polyrhizus*). *Jurnal Teknologi Pangan Indonesia*, 13(1), 71–79.

Oksana, O., Irfan, M., Fianiray, A. R., & Zam, S. I. (2020). Isolasi dan identifikasi bakteri pelarut fosfat pada tanah ultisol di Kecamatan Rumbai, Pekanbaru. *Agrotechnology Research Journal*, 4(1), 22–25. https://doi.org/10.20961/agrotechresj.v4i1.36063

Pane, R. D. P., Ginting, E. N., & Hidayat, F. (2022). Mikroba pelarut fosfat dan potensinya dalam meningkatkan pertumbuhan tanaman. *Warta PPKS*, 27(1), 51–59.

Rifai, K. R. (2021). Uji indole sebagai kegiatan penjaminan mutu tambahan pada hasil pengujian coliform dalam sampel air mineral. *Jurnal Teknologi Proses dan Inovasi Industri*, 6(1), 1–6.

Sharon, J. A., Hathwaik, L. T., Glenn, G. M., Imam, S. H., & Lee, C. C. (2016). Isolation of efficient phosphate solubilizing bacteria capable of enhancing tomato plant growth. *Journal of Soil Science and Plant Nutrition*, 16(2), 525–536. https://doi.org/10.4067/S0718-95162016005000043

Sianipar, G. W. S., Sartini, S., & Riyanto, R. (2020). Isolasi dan karakteristik bakteri endofit pada akar pepaya (*Carica papaya* L.). *Jurnal Ilmiah Biologi UMA*, 2(2), 83–92. https://doi.org/10.31289/jibioma.v2i2.312

Surtiningsih, T. (2015). Peran biofertilizer dari campuran mikroorganisme sebagai upaya untuk meningkatkan produktivitas tanaman pangan nasional. *Perpustakaan Universitas Airlangga*, 3(2), 123–129.

Syah, R. F., Irianto, A., & Ratnaningtyas, N. I. (2018). Biodegradation of diesel oil by yeast isolated from mangrove's rhizosphere. *Scripta Biologica*, 5(2), 79–82.

Syarwani, A., Aisyah, S., Hadija, & Nirawati. (2022). Isolasi dan identifikasi bakteri pelarut fosfat dari rhizosfer tanaman aren (*Arenga pinnata* (Wurmb) Merr) di Desa Bonto Somba Kabupaten Maros. *Eboni*, 4(2), 63–70.