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Phosphate Solubilisation Index and Antagonism Potential of Frangipani Tree Rhizosphere Bacterial Isolates from Cemetery

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ABSTRACT

Frangipani trees (*Plumeria acuminata*) are commonly found in cemeteries. Cemeteries are used as a location for interment so that the corpse actively decomposes to provide soil minerals that encourage the formation of microbes, including bacteria. Bacteria around the roots of frangipani trees are thought to have potential as plant growth-promoting rhizobacteria (PGPR). Based on reference searches, there has been no research on frangipani tree rhizosphere bacteria from burial grounds. The aim of this study was to investigate the phosphate-dissolving and blast disease-causing *Pyricularia oryzae* fungus-inhibiting properties of frangipani tree rhizosphere bacteria from Pracimaloyo cemetery, Surakarta. A total of 39 isolates of frangipani tree rhizosphere bacteria were tested for their ability to dissolve phosphates using PKA media (Pikovskaya), while the antagonism test against the fungal pathogen *Pyricularia oryzae* used the dual assay method. The results of the study showed that 15% of the rhizosphere bacteria of the frangipani tree were able to dissolve phosphate, and 33% of the isolates were able to inhibit the growth of the fungus *Pyricularia oryzae* with an inhibitory power of more than 40%. Based on the results, the rhizosphere bacterial isolates from Pracimaloyo TPU show the characteristics of a possible PGPR.

1. INTRODUCTION

The frangipani tree, which has the Latin name *Plumeria acuminata*, is included in the gummy plant and grows at an altitude of 1–1000 meters above sea level. This plant comes from South America and is spread throughout Indonesia (Yuliani et al. 2021) where it is commonly found in public burial places (TPU) (Bihani et al. 2021). The presence of trees in the cemetery area is beneficial to the environment. The presence of frangipani trees in the cemetery area that have sweet-smelling flowers can make the atmosphere more sacred (Zain et al. 2020). Its habitus, in the form of a large tree, can also be used as a protector and shade so as to create a beautiful atmosphere. Plant roots secrete root exudate that can affect the activity of surrounding microorganisms (Prayudyaningsih et al. 2015). In addition, root exudates such as sugars, amino acids, organic acids, phenolic compounds, enzymes, phytohormones, and vitamins can attract some rhizosphere bacteria, and others microorganisms (Olanrewaju et al. 2019).

Rhizosphere bacteria are bacteria found around plant roots. Its existence around the roots is one form of specific interaction between plants and microorganisms (Yang et al. 2017) so the type of rhizosphere bacteria will be different for other types of plants. Rhizosphere bacteria are referred to as PGPR (Plant Growth Promoting Rhizobacteria) because they can trigger plant growth by producing growth hormones, dissolving phosphates, producing siderophores to chelate Fe, and being biocontrol agents against plant pathogens (Nuraini et al. 2020; Pathania et al. 2020). Soil moisture and the state of plant roots affect the bacterial population of the rhizosphere. High

bacterial populations indicate that energy requirements, temperature suitability, and water availability are sufficient for bacteria to grow (Kadeawi et al. 2020).

In previous studies, a population of frangipani tree rhizosphere bacteria from TPU Pracimaloyo was calculated, and it was detected that the population was abundant, ranging from 1.9–10.4 x 106 CFU/g. These rhizosphere bacteria isolates are known to act as biostimulants because they are able to produce IAA growth hormone up to 113.58 ppm (Putra et al, 2023), whereas rhizosphere bacteria isolates from other plants, such as rice plants, only range from 64.03–84.12 ppm (Prihatiningsih et al. 2019). Therefore, it is necessary to characterize its capabilities as another PGPR. In this study, the character to be tested is its ability to dissolve phosphate, determined through the phosphate solubilization index, and its potential antagonism to pathogenic fungi.

Isolates that are able to dissolve phosphate can be applied as biofertilizer because most of the phosphate present in the soil is present in the form of bound metal elements. The presence of rhizosphere bacteria can help break the bond between phosphate and metal elements so that they can be used by plants (Pratika et al. 2020). Physiological status and bacterial growth as well as soil nutrient diversity are influences for rhizosphere bacteria in dissolving phosphates (Alori et al. 2017). Phosphate itself has an important role for plants, especially in the metabolic process, namely at the time of photosynthesis (Sharma et al. 2013).

The ability of rhizosphere bacteria to inhibit the growth of plant pathogenic fungi is actually a supporting character of plant growth indirectly by forming biocontrol agents (Ahemad and Kibret 2014). This ability can be used as a biopesticide (Walida et al. 2019) because some strains of rhizosphere bacteria are effective in inhibiting the growth of pathogenic fungi (Kadeawi et al. 2020). One of the pathogenic fungi is *Pyricularia oryzae*, which causes blast disease in rice plants. This disease causes rhomboid-shaped spots with tapered tips on the leaves, and in the middle of the spots are gray spots with brown surroundings (Kadeawi et al. 2020). Based on data obtained from the Central Statistics Agency (BPS 2020) in 2018–2019, there was a decrease of 7.76 % in national rice production. Then in 2020, the area of harvested land decreased by 6.15 % compared to 2018. As a result of this disease, farmers lose their crops by around 50 – 90 % (Kadeawi et al. 2020).

This study aims to test the ability of frangipani tree rhizosphere bacteria from Pracimaloyo Cemeteries, Surakarta, to dissolve phosphate and inhibit the growth of the Pyricularia oryzae fungus that causes blast disease in rice plants. Data from this study will complement the character of Cambodian tree rhizosphere bacteria isolated as PGPR, so that its application to plants will be more appropriate.

2. MATERIALS AND METHODS

2.1. Materials

The tools used in this study were Erlenmeyer 125 ml and 500 ml (Iwaki), test tubes (Iwaki), test tube racks, 100 ml measuring cups (Iwaki), beakers, petri dishes, analytical balances (Durascale DAB-E223), spatulas, micropipettes (Socorex 10-100), yellow tips, deck glass, object glass, scissors, plastic, cotton buds, aluminum foil, magnetic hot plate (Ciramex +), oven (Maspion), incubator (Memmert), autoclave (GEA LS-35LJ), laminar air flow (LAF), microscope (Olympix CX2 Binocular), spirtus burner, lighter, spray bottle (sprayer), refrigerator (Sharp), jar glass, ose, latex gloves, stationery, and documentation tools.

The materials needed in this study are 39 isolates of frangipani tree rhizosphere bacteria from TPU Pracimaloyo, which is a collection of the Biological Laboratory of FKIP Universitas Muhammadiyah Surakarta, nutrient agar (Merck), nutrient broth (NB) media, Pikovskaya media (PKA), bacterialogical agar (Himedia), potato dextrose agar (PDA) media, 70% alcohol, aquades, tissue, cotton, and paper.

2.2. Methods

2.2.1. Rejuvenation of Bacterial Isolate

Rejuvenation is carried out by isolating rhizosphere bacteria subcultured using ose to oblique nutrient agar media (NA) in test tubes to be grown by incubating at 37 °C for 48 hours.

2.2.2. Phospate Dissolving Ability Test

This study was conducted in the Biological Laboratory of FKIP Universitas Muhammadiyah Surakarta. To determine the solubility of phosphate tested using solid Pikovskaya media (PKA) with a composition of ingredients per 1 liter of media used, namely glucose 10 g; $(NH_4)_2SO_4$ 0.5 g ; NaCl 0.2 g ; $MgSO_4.7H_2O$ 0.1 g ; KCl 0.2 g ; $MnSO_4.H_2O$ 0.002 g ; $FeSO_4.7H_2O$ 0.002 g ; yeast extract 0.5 g ; and bacteriological agar 20 g, follows the procedure (Rahayu 2022). PKA (Pikovskaya) media is made into a hole with a diameter of 6 mm using a cork borer, and then 10µL suspension of rizosphere bacteria that has been shaken for 24 hours on NB media is inserted into the hole. Then incubated for 72 hours at room temperature. Measure the clear zone (halo) as well as the diameter of the bacterial colony and calculate the phosphate solubility index (PSI) using the following formula (Sharon et al. 2016):

$$\text{PSI} = \frac{CD + HZ}{CD}$$

where *PSI* is the phosphate solubility index, *CD* is the colony diameter, and *HZ* is the halo zone.

2.2.3. Antagonism Test

This study was conducted in the Biological Laboratory of FKIP Universitas Muhammadiyah Surakarta. To see the ability of rhizosphere bacteria to inhibit pathogenic fungi, *Pyricularia oryzae* was tested using solid potato dextrose agar (PDA) media to make pure cultures. Then the fungi culture is inoculated into NA media by the agar block method using cork punches and incubated for 3 days at room temperature. Cultures of frangipani tree rhizosphere bacteria were etched on NA media containing *Pyricularia oryzae* colonies at a distance of 2-3 cm and reincubated for 4 days at room temperature. Measuring the inhibitory power of frangipani tree rhizosphere bacteria against *Pyricularia oryzae* fungi using the formula (Ningsih et al. 2016):

$$\text{IP} = \frac{(r1 - r2)}{r1} \times 100$$

where *IP* is the inhibitory power (%), *r1* is the radius of the fungal colony away from the bacteria, and *r2* is the maximum radius of the fungus.

3. RESULTS AND DISCUSSION

The test results of the ability of frangipani tree rhizosphere bacteria isolates to dissolve phosphate and their antagonism against *Pyricularia oryzae* are fully presented in (**Table 1**).

Table 1. Results of phosphate dissolving test and antagonism test

Isolate Code	Antagonism towards *P.oryzae* (%)		Phosphate Dissolving	
	Day 2	Day 4	Ability	PSI for 72 hours
P1	24,1	40.5	+	4.4
P2	37.9	51.4	+	5.3
P3	31.0	48.6	+	3.8
P4	27.5	45.9	+	5.6
P5	27.5	40.5	-	-
P6	31.0	40.5	-	-
P7	34.4	37.8	-	-
P8	37.9	35.1	-	-
P9	37.9	37.8	-	-
P10	31.0	39.2	-	-
P11	27.5	41.9	-	-
P12	20.6	32.4	-	-
P14	27.5	37.8	-	-
P15	24.1	35.1	-	-
P16	24.1	37.8	-	-
P17	27.5	39.2	-	-
P18	25.8	35.1	-	-
P19	31.0	37.8	-	-
P20	27.5	43.2	-	-
P21	31.0	40.5	-	-
P24	34.4	39.2	-	-
P25	27.5	37.8	-	-
P26	24.1	37.8	-	-
P27	31.0	41.9	-	-
P28	29.3	40.5	-	-
P29	17.2	32.4	-	-
P31	24.1	37.8	-	-
P32	27.5	40.5	-	-
P33	24.1	37.8	-	-
P34	22.4	32.4	-	-
P35	25.8	29.7	-	-
P36	20.6	32.4	-	-
P37	24.1	28.3	-	-
P38	25.8	40.5	+	4.5
P39	20.6	35.1	-	-
P40	24.1	39.2	-	-
P41	25.8	37.8	+	3.5
P42	24.1	35.1	-	-
P43	20.6	32.4	-	-

Note :
(-) does not have the ability to dissolve phosphates and (+) has the ability to dissolve phosphates.

3.1. Phosphate dissolving ability

Based on the results shown in Table 1, it can be known that 6 out of 39 isolates of rhizosphere bacteria from frangipani trees (15 %) have the ability to dissolve phosphate. This can be seen from the formation of a halo zone (clear zone) on a petri dish containing Pikovskaya media that has

been suspended from rhizosphere bacterial isolates. The clear zone formed is only used as an early indication that bacteria can dissolve phosphate or not, not showing how much phosphate is dissolved by bacteria (Silaen 2015). Of the 6 isolates, the phosphate solubility index (PSI) was highest at P4 with a value of 5.6, while the phosphate solubility index (PSI) was lowest at P41 with a value of 3.5. The difference in the solubility index value of phosphate in each isolate occurs due to the ability of genetically varying bacteria. Bacteria produce organic acids and phosphatase enzymes needed for phosphate dissolution (Sembiring et al. 2020). Phosphatase enzymes themselves are a group of enzymes that catalyze hydrolytic mineral reactions by releasing insoluble phosphates into enzymatic solutes (Ranjan et al. 2013).

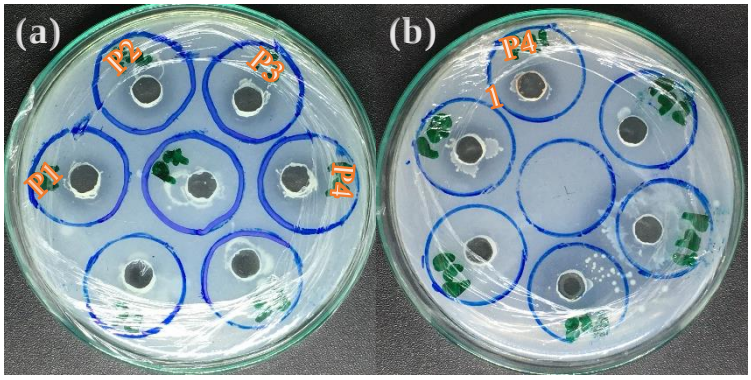

Figure 1. Test results for phosphate dissolving ability (a) The highest phosphate solubility index at P4 is 5.6; (b) The lowest phosphate solubility index at P41 is 3.5.

The Solubility Index (IKF) value of rhizosphere bacteria of frangipani trees at Pracimaloyo Cemeteries is higher than rhizosphere bacteria from rubber plantations (Wulandari et al. 2020), bamboo plants (Lengkong et al. 2022) and acacia plants (Rini et al. 2020) each ranging from 1.0 to 1.62, 1,0 to 1,29, and 0.1 to 1.1 cm. These results prove that in the rhizosphere area of TPU, which is known as the place where the decomposition of bodies rich in nutrients occurs, it can help bacteria dissolve phosphate. Burial soils that are thousands of years old have high calcium and phosphorus content due to bone decomposition processes in the soil, which are a source of metal elements for plants (Asare et al. 2020). This is in accordance with research (Żychowski 2021) which suggests that the soil used as a burial place during World War II was dominated by phosphorus, which is a transitional stage of bone decomposition. Soil composition, such as soil pH, can affect bacteria's ability to dissolve phosphates. Phosphate-solvent bacteria tend to be more effective at neutral soil pH. The activity of phosphate solvent bacteria in the mineralization of organic matter and nitrification will be disrupted in soils that are too acidic (Marista et al. 2013). Phosphate solvent bacteria have thermophilic properties that can withstand hot temperatures in a certain range, so enzymes and organic acids produced by bacteria to dissolve phosphate are not easily damaged (Respati et al. 2017).

3.2. Antagonism towards Pyricularia oryzae

Table 1 shows that all isolates of rhizosphere bacteria can inhibit the growth of the pathogenic fungus *Pyricularia oryzae* with different inhibitory powers. On day 2, the observations that had the highest resistance were P2, P8, and P9 with 37.9 %, and the lowest resistance was P29 with 17.2 %. Then on the 4th day, the observation that had the highest

inhibitory power was P2 with 51.6 %, and the lowest inhibitory power was P35 with 29.7%. The percentage of inhibitory power produced by rhizosphere bacteria from frangipani trees at Pracimaloyo Cemeteries is higher than that produced by rhizosphere bacteria from rice plants in Bali (Quintao et al. 2015) and rhizosphere bacteria from oil palm plants (Widiantini et al. 2018) which each have a percentage of inhibition ranging from 39.47 – 46.67 % and 7.67 – 22.89 %, respectively. According to (Pitasari and Ali 2018) this difference in inhibition occurs because each isolate releases different types and amounts of secondary metabolites to inhibit the growth of pathogenic fungi. Physiological differences in bacteria in the use of nutrients from the media are also the cause of differences in inhibitory power (Saputra et al. 2015) because some bacteria use nutrients to support growth and some use them to produce secondary metabolite compounds (Flori et al. 2020).

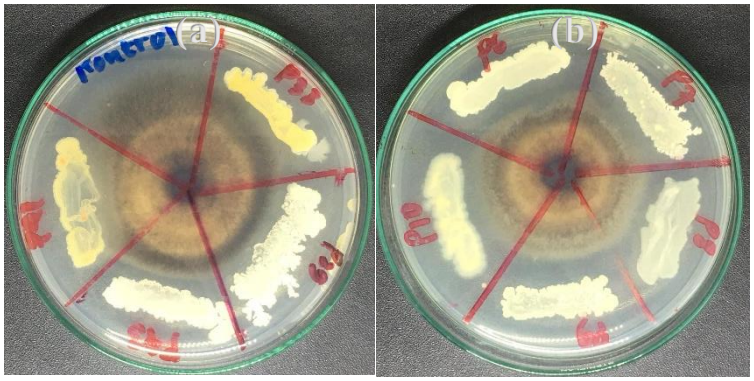

Figure 2. Antagonism Test Results (a) Control treatment; (b) P6 rhizosphere bacterial isolate with 40.5% inhibitory power

In fact, PGPR bacteria have the ability to secrete secondary metabolite compounds when facing pathogens. This is in accordance with the statement (‘Aini et al. 2013) that the antifungal effect of metabolite compounds can be produced by bacteria that have a role as biological agents. According to (Widiantini et al. 2018) compounds released by antagonistic bacteria are able to inhibit the growth of pathogenic fungi by causing swelling and shortening of hyphae. The mechanism of antibiosis that causes changes in hyphal structure can also occur due to antagonistic activity (Ainy et al. 2015).

Inhibition of bacterial isolates in antagonism tests against pathogenic fungi can also occur because bacterial isolates are able to secrete chitinase enzymes. It is known that the chitinase enzyme can degrade cell walls in several types of fungi (Banna and Hartati 2017). This statement is in accordance with what was stated by (Gómez-Godínez et al. 2023) that some enzymes produced by rhizosphere bacteria, such as the chitinase enzyme, can destroy pathogenic cell walls. In addition, rhizosphere bacteria can also produce antimicrobial peptides that control infection through induced systemic resistance (ISR). Other mechanisms used by rhizosphere bacteria to protect themselves from pathogenic fungi are competing for nutrients, producing antibiotics (Wang et al. 2021) and colonizing root surfaces to prevent pathogenic fungi (Ali et al. 2022).

4. CONCLUSION

As many as 15% of frangipani tree rhizosphere bacteria isolates from Pracimaloyo Cemeteries have the character of potential PGPR, which is able to dissolve phosphate, and as many

as 33 % of rhizosphere isolates are able to inhibit the growth of *Pyricularia oryzae* fungi, which cause blast disease in rice plants, with inhibitory power above 40 %. From this study, it can be concluded that the isolates of frangipani tree rhizosphere bacteria from Pracimaloyo Cemeteries have the characteristics of potential PGPR. The study obtained a potential bacterial isolate as PGPR, but the identity of the species is not known, so further testing is needed to identify species of rhizospheric bacteria from the frangipani trees present in the cemetery and need to conduct direct experiments on plants to prove that the bacteria isolate can help the growth of plants.

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