

Phosphate Soluting *Actinomycetes* in the Rhizosphere of Corn (*Zea mays*) in Gorontalo

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ABSTRAK

Actinomycetes merupakan bakteri Gram-positif dengan karakteristik morfologi berupa filamen uniseluler yang berpotensi melarutkan fosfat terikat menjadi bentuk yang tersedia bagi tanaman. Penelitian ini bertujuan mengisolasi dan mengidentifikasi *Actinomycetes* pelarut fosfat dari rhizosfer tanaman jagung (*Zea mays*) di Gorontalo, Indonesia. Sampel tanah rhizosfer diambil pada kedalaman 15–30 cm dari tiga lokasi pertanian jagung di Desa Mohiyolo, Kecamatan Asparaga. Parameter yang dianalisis meliputi karakteristik lingkungan, morfologi isolat, indeks kelarutan fosfat, dan konsentrasi fosfat terlarut. Identifikasi dilakukan berdasarkan morfologi miselium aerial dan miselium substrat, serta uji fisiologi kemampuan pelarutan fosfat. Indeks kelarutan fosfat dihitung dari perbandingan diameter halo zone terhadap koloni pada media Pikovskaya agar, sementara konsentrasi fosfat terlarut diukur menggunakan spektrofotometri. Hasil menunjukkan isolat RFZm-Pg memiliki indeks kelarutan tertinggi sebesar 2,98 mm, sedangkan isolat RFZm-Pw sebesar 1,54 mm. Konsentrasi fosfat terlarut tertinggi pada media cair adalah 0,8048 ppm (RFZm-Pg) dan 0,4373 ppm (RFZm-Pw). Isolat *Actinomycetes* pelarut fosfat yang diperoleh memiliki potensi sebagai biofertilizer, sehingga diperlukan pengujian lebih lanjut.

ABSTRACT

Actinomycetes are Gram-positive bacteria with morphological characteristics in the form of unicellular filaments that have the potential to dissolve bound phosphate into a form available to plants. This study aims to isolate and identify phosphate-solubilizing *Actinomycetes* from the rhizosphere of corn (*Zea mays*) in Gorontalo, Indonesia. Rhizosphere soil samples were taken at a depth of 15-30 cm from three corn farming locations in Mohiyolo Village, Asparaga District. Parameters analyzed included environmental characteristics, isolate morphology, phosphate solubility index, and soluble phosphate concentration. Identification was based on the morphology of aerial mycelium and substrate mycelium, as well as physiological tests of phosphate solubilization ability. Phosphate solubility index was calculated from the ratio of halo zone diameter to colony on Pikovskaya agar medium, while soluble phosphate concentration was measured using spectrophotometry. The results showed that RFZm-Pg isolate had the highest solubility index of 2.98 mm, while RFZm-Pw isolate was 1.54 mm. The highest dissolved phosphate concentration in liquid media was 0,8048 ppm (RFZm-Pg) and 0,4373 ppm (RFZm-Pw). The phosphate-solubilizing *Actinomycetes* isolates obtained have potential as biofertilizers, so further testing is needed.

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1. INTRODUCTION

The decline in soil fertility caused by the continuous use of chemical fertilisers and lack of variety in cropping patterns has become a serious problem in the agricultural sector. The

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practice of using chemical fertilisers is often applied in intensive agriculture, but its long-term use causes a decline in soil quality due to the accumulation of fertiliser and pesticide residues (Ye *et al.*, 2020; Nasamsir *et al.*, 2022). This condition not only reduces soil quality but also eliminates important microorganisms that play a role in nutrient cycling, causing nutrient imbalances such as phosphate (Taher *et al.*, 2021). In acidic soils, phosphorus reacts with Aluminium (Al) and iron (Fe) to form complex compounds such as $AlPO_4$ and $FePO_4$, so it cannot be absorbed by plants (Sonia *et al.*, 2022).

Phosphate is an essential nutrient required for plant growth, in both organic and inorganic forms (Saif *et al.*, 2014). It plays an important role in various plant physiological processes, including cell division, photosynthesis and root system development (Nash *et al.*, 2014). However, phosphate availability is often hampered by marginal soil conditions with low pH and high aluminium levels (Urtati, 2021; Wahyumi *et al.*, 2023). One solution that can be applied is the use of Actinomycetes, which are unicellular Gram-positive bacteria that have the ability to produce secondary metabolites and act as Plant Growth Promoting Rhizobacteria (PGPR), including their ability to solubilise phosphate (Pan F. *et al.*, 2018; Rangseekaew, 2019; Selim, 2021; Walida *et al.*, 2019).

Actinomycetes are widely distributed in various soil ecosystems and are able to adapt to extreme conditions, especially in the rhizosphere region (Ratih *et al.*, 2020). *Actinomycetes* populations are higher in the rhizosphere compared to non-rhizosphere soils due to root exudates rich in organic compounds (Fitriana, 2021; Shirinbayan *et al.*, 2018). Research shows that some *Actinomycetes* genus, such as *Nocardia* sp., *Streptomyces*, and *Micromonospora* sp., are able to dissolve phosphate (Nurkanto, 2007; Putri *et al.*, 2021). In maize plants, the population of phosphate-solubilising microorganisms increases with plant age, especially in the early vegetative phase (Huang *et al.*, 2014; Niswati *et al.*, 2007).

The rhizosphere of maize (*Zea mays*) is an important area for investigating interactions between microorganisms and plant roots, where complex root morphology, such as fibrous root systems and actively dividing root tips, increases the production of exudates that attract microorganisms (Tai *et al.*, 2015; Chen *et al.*, 2024; Hems *et al.*, 2022). One of the important elements for maize growth is phosphorus, with the optimal dose of phosphate fertiliser being around 150 kg/ha (Puspitasari *et al.*, 2018). However, the availability of phosphorus for plants can be hampered under certain soil conditions. In acid soils, phosphorus can react with minerals such as Fe and Al, forming insoluble compounds, while in wet soils, phosphorus can react with Ca and Mg, which also produce insoluble compounds. As a result, plants are unable to absorb phosphorus bound in the soil (Niswati *et al.*, 2018; Solihin *et al.*, 2019; Edy *et al.*, 2022; Purba *et al.* 2015). Therefore, the interaction of maize plant roots and *Actinomycetes* in the rhizosphere is essential to release bound phosphorus and improve phosphorus use efficiency in agriculture.

Gorontalo is an area with many corn plantations that still have not been studied much about *Actinomycetes* associated with rhizosphere root systems that are able to dissolve phosphate, with the facts above and considering the enormous role of *Actinomycetes*, research on phosphate-solubilising *Actinomycetes* in the rhizosphere of corn plants in Gorontalo is needed.

2. METHOD

This research was conducted from June to October 2024. Rhizosphere soil sampling was carried out at the location of corn farming, namely the hills of mohiyolo village, asparaga sub-

district, Gorontalo district. Isolation and purification were carried out at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Gorontalo State University. The method used in this research is Quantitative descriptive, with sampling techniques using Purposive sampling technique, namely Determination of sampling at three points of corn farming areas with slopes based on the top, middle, and bottom of the hills and square plot size ± 10 metres long.

Research procedure

Actinomyces bacterial isolates were taken from soil samples around the roots of maize plants in Mohiyolo village, Asparaga sub-district. Furthermore, sample preparation was carried out by weighing 5 grams of soil samples each and dissolving them in sterile distilled water and homogenised using an incubator shaker at 225 rpm. The soil suspension was then heated in a water bath at 60°C for 15 minutes. Next, multistage dilutions were made up to 10^{-5} and incubated for 14 days at 37°C. Each colony that grew was used as a pure culture. Each isolate was then characterised by *Actinomyces* morphology marked by the presence of *substrate mycelium* and *areal mycelium*.

Furthermore, each isolate was tested for physiological characters with two tests, namely:

a. Qualitative Testing of Phosphate Solubiliser Ability of *Actinomyces* Isolates

Actinomyces isolates with a colony diameter of 1 cm aged 14 days were grown on Pikovskaya agar (PKA) media. The composition of PKA media was glucose 10 g/L; Ca_3PO_4 5 g/L; $(\text{NH}_4)_2\text{SO}_4$ 0,5 g/L; KCl 0,2 g/L; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0,1 g/L; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 0,01 g/L; ekstrak khamir 0,5 g/L; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 0,01 g/L; agar 18 g/L with pH 7. *Actinomyces* isolates that had been grown on PKA media were then incubated at 28°C for 14 days. Positive activity is indicated by the formation of a clear zone around the *Actinomyces* colony. Phosphate solubilising activity was determined by the ratio between the clear zone diameter and the colony diameter (Paul and Sinha 2017).

$$IP = \frac{\text{Halozone Diameter (mm)} + \text{Colony Diameter (mm)}}{\text{Colony Diameter (mm)}}$$

The phosphate solubility index is categorised based on the categories (Hindyahtulloh *et al.*,2022) in table 1.

Table 1: Phosphate Solubility Index Category

Phosphate Solubility Index	Category
<1,00	Very low
1,00-2,00	Low
2,00-3,00	Medium
>3,00	High

b. Quantitative Testing of Phosphate Solubilisation Ability of *Actinomyces* Isolates

The phosphate solubilisation test on *Actinomyces* isolates was conducted by adding *Actinomyces* isolates into a 50 mL Erlenmeyer flask filled with liquid Pikovskaya media. After that, the mixture was incubated for 7 to 35 days at 26°C at 150 rpm in a shaker incubator. Afterwards, the pellet and supernatant were separated by centrifuging 10 mL of culture for 15 min at 1000 rpm. Next, up to 1 mL of the resulting supernatant was taken and added to 2.5 mL of 2.5% sodium molybdate solution and 1 mL of 0.3% hydrazine sulfate to initiate the reaction. After that, the mixture was cooked for ten minutes and then cooled. At a wavelength of 830 nm, a spectrophotometer was used to detect the amount of dissolved phosphate.

KH_2PO_4 solution with a concentration of KH_2PO_4 was used to make a standard curve. 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9, and 1.0 mg/L (Hartanto *et al.*, 2023; Mardiyansah, 2021).

3. RESULT AND DISCUSSION

The research was conducted in mohiyolo village, gorontalo district. Soil sampling from the rhizosphere was carried out at one location in the hilly area of corn crops, which includes three points of corn farming areas based on the top, middle and bottom of the hills with each coordinate point (Figure 1).



Figure 1. Sampling location

Sampling locations to obtain rhizosphere samples of maize plants show environmental characteristics including soil pH and soil moisture. soil pH at three location points tends to be acidic. While the highest humidity is found at point A (Table 2).

Table 2 Environmental Parameter Table

No.	Location	Environmental parameters	
		Rerata pH	Average Soil moisture
1.	Mohiyolo Village, Asparaga Sub-district (A)	5,1	4,8%
2.	Mohiyolo Village, Asparaga Sub-district (B)	5,2	4,5%
3.	Mohiyolo Village, Asparaga Sub-district (C)	5,1	4,7%

Measurements of the soil environment of the maize rhizosphere showed that the three points tended to have an acidic pH. This condition can have a positive effect on microbial growth, especially the *Actinomycetes* group. Although *Actinomycetes* generally prefer neutral to slightly alkaline pH (optimal between 6.5-8.0) they can still be found in environments with lower pH although their population may decrease significantly (Kanti, 2005). While the humidity level of the rhizosphere area of corn is said to be low but very good for the growth of *Actinomycetes* (Widyanti *et al.*, 2016).

Observation of Colony Morphology

Seven purified *Actinomycetes* isolates were obtained, including RFZm-Pg, RFZm-Pw, RFZm-Plg, RFZm-Po, RFZm-Pb, RFZm-Py, and RFZm-Pr (Figure 2). The purified isolates were observed for the morphology of the colonies formed, ranging from the colour of the areal mycelium, and substrate mycelium. Based on the results, it shows that the *areal mycelium* has a colour type of grey, yellow, orange, red, white, and the *substrate mycelium* is black, brown, white yellow, orange, grey and yellow. According to Astuty (2019), the diversity of the shape, colour of *areal mycelium*, and *substrate mycelium* depends on the type of *Actinomycetes* bacteria and rhizosphere environmental conditions.

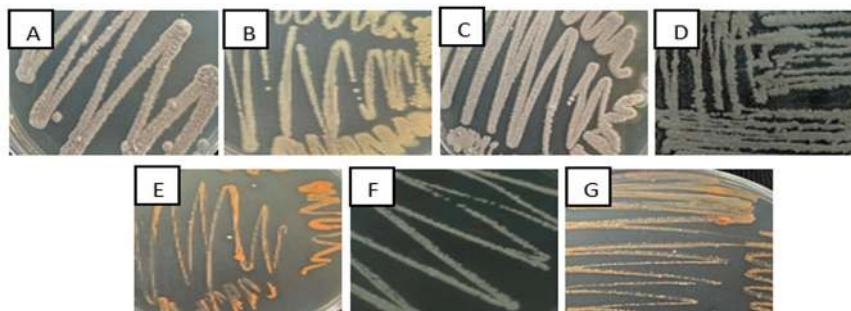


Figure 2. Colony Morphology of *Actinomycetes* codes RFZm-Pg (A), RFZm-Py (B), RFZm-Pb (C), RFZm-Plg (D), RFZm-Po (E), RFZm-Pw (F), RFZm-Pr (G)

Ability of phosphate-solubilising *Actinomycetes*

The ability of phosphate-solubilising *Actinomycetes* is done by growing *Actinomycetes* from each of the rhizosphere soil samples found at the three location points using Pikovskaya media. Each isolate was inoculated on Pikovskaya media with 3 repetitions. From the 3 sampling points of rhizosphere soil, 7 pure isolates of *Actinomycetes* were obtained which showed clear zones on Pikovskaya media, only 2 isolates were potential phosphate solvents (Table 3).

Table 3 Qualitatively phosphate-solubilising *Actinomycetes* isolates

Kode Isolat	IKF	Categories
RFZm-Pg	2,98 mm	Medium
RFZm-Py	-	-
RFZm-Pb	-	-
RFZm-Po	-	-
RFZm-Plg	-	-
RFZm-Pw	1,54 mm	Low
RFZm-Pr	-	-

The results showed that *Actinomycetes* were found in the rhizosphere of maize plants in Mohiyolo village, Asparaga sub-district, at points A and B with isolate codes RFZm-Pg and RFZm-Pw. The presence of phosphate solubilising *Actinomycetes* is considered positive if a clear zone (Halazone) is formed on Pikovskaya media, as seen in Figure 3 below.

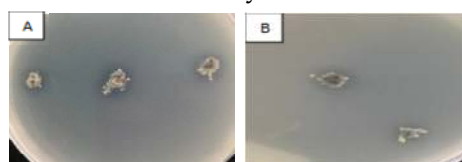


Figure 3 A. Colonies of phosphate-solubilising *Actinomycetes* Code RFZm-Pg, B. Colonies of phosphate-solubilising *Actinomycetes* Code RFZm-Pw

Actinomyces isolates obtained were tested for phosphate solubilisation using Pikovskaya agar medium. The ability to dissolve phosphate is indicated by the clear zone formed around the colony. The results showed that the isolates had the ability to dissolve phosphate with a Dissolution Index (Ip) of 2.98 mm (Medium) and 1.54 mm (Low). Even so, according to Larasati et al. (2018), the ability of bacterial isolates to dissolve phosphate cannot always be assessed only based on the width of the clear zone formed. The results of the qualitative test are not yet fully effective to assess the extent to which the bacteria are able to dissolve phosphate. Therefore, further quantitative tests are needed to gain a deeper understanding.

Isolates that showed qualitative phosphate solubilisation were then measured quantitatively using a spectrophotometer with a wavelength of 830 nm. A standard curve was made using KH_2PO_4 with concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 1.0 ppm. The test results of the ability of *Actinomyces* bacteria to dissolve phosphate in liquid pikovskaya media are shown in figure 4. This data illustrates the level of phosphate release by each isolate during the incubation period. This quantitative test is very important to assess the extent of the effectiveness of *Actinomyces* in increasing phosphate availability in the environment, especially for practical applications in supporting sustainable agriculture.

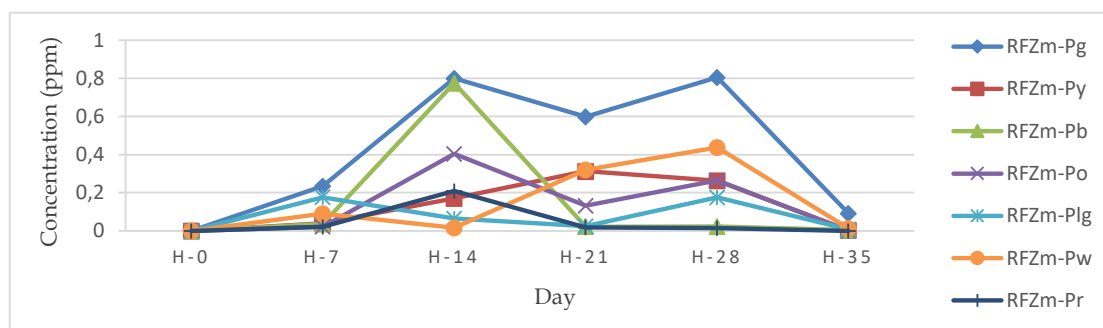


Figure 4 Phosphate solubilisation ability by *Actinomyces* isolates in liquid picovskaya medium

Based on the Figure, the measurement results show that the phosphate dissolution of all isolates increased until day 28 except RFZm-Pr and all isolates also decreased after day 28. Isolates RFZm-Pg and RFZm-Pw can dissolve the highest phosphate on day 28 with a value of 0,8048 ppm and 0,4373 ppm respectively. While on day 35 it decreased with the value of RFZm-Pg of 0,0915 ppm and RFZm-Pw 0,0134 ppm. The longer the activity of phosphate solubilising bacteria will decrease due to limited energy sources and cause a decrease in population so that cell metabolism also decreases (Sonia *et al.*, 2022).

Quantitative phosphate solubility test results are directly proportional to qualitative results. The ability of phosphate solubilising bacteria can vary, caused by many factors found in the soil, such as soil nutrients, pH, moisture, organic matter, and enzyme activity (Dewi *et al.*, 2023). Lovitna *et al.* (2021) stated that there is a relationship between the population of phosphate solubilising bacteria, then the P-available content in the soil will also increase. According to Niswati *et al.* (2008), differences in the population of phosphate-solubilising bacteria cause differences in the availability of P in the soil. The difference in population causes differences in the amount of organic acids produced by the phosphate-solubilising bacteria. Taniwan *et al.* (2016) stated that phosphate solubilising bacteria are known to reduce the pH of the substrate by secreting a number of organic acids such as formic, acetic, propionic, lactic, glycolic, fumaric and succinic acids. These acids may form chelates with cations such as Ca and Fe resulting in effective phosphate solubilisation. Adequate P nutrients are associated with increased plant root growth (Triadiawarman *et al.*, 2022).

Actinomyces are gram-positive bacteria that can survive in extreme environments. In this study, three phosphate-solubilising *Actinomyces* isolates were obtained from the rhizosphere of maize plants in three research locations. *Actinomyces* population density is strongly influenced by environmental physicochemical factors, including soil pH. Measurements of pH in the rhizosphere of maize plants in

Mohiyolo Village, Asparaga Sub-district, Gorontalo Regency showed acidic conditions with pH ranging from 5.1 to 5.2, which is in accordance with the findings of Dewi (2024) who explained that *Actinomyces* tend not to develop in environments with pH below 5.0, while the optimal pH for their growth is 6.5 to 8.0. Besides pH, soil moisture is also an important factor in the growth of *Actinomyces*. The measurement results showed that the humidity at points A, B, and C was 4.8%, 4.5%, and 4.7%, respectively. Although this value is relatively low, *Actinomyces* can still grow well. Solihin (2017) stated that the *Actinomyces* population varies in the humidity range of 0.67% to optimal at 0.98%.

The study showed that of the seven *Actinomyces* isolates tested, only two isolates, RFZm-Pg and RFZm-Pw, were able to dissolve phosphate. This finding is consistent with the study of Utami, Lidar, and Rizal (2021), who found that only a small proportion of *Actinomyces* isolates could form clear zones on Pikovskaya media with varying diameters. Dewi (2017) explained that the higher the phosphatase enzyme activity produced by phosphate-solubilising bacteria, the larger the clear zone formed, because the bacteria are able to convert insoluble phosphate into a soluble form.

In this study, *Actinomyces* isolates obtained from the rhizosphere of corn plants in Gorontalo showed specific results. Isolate RFZm-Pg produced a clear zone with a diameter of 2.98 mm and a dissolution index (Ip) of 0,8048 ppm, while isolate RFZm-Pw produced a clear zone with a diameter of 1.54 mm and Ip of 0,4373 ppm.

The formation of clear zones on Pikovskaya media indicates that the bacteria are capable of producing extracellular phosphatase enzymes. Pikovskaya media contains insoluble phosphate in the form of $\text{Ca}_3(\text{PO}_4)_2$, which cannot be directly utilised by bacteria as a source of nutrients (Rahmayuni *et al.*, 2018). Therefore, bacteria produce the enzyme phosphatase to dissolve insoluble phosphate, so that the phosphate can be utilised as a source of nutrients.

4. CONCLUSION

The research that has been done, obtained the results of 7 pure isolates of *Actinomyces* bacteria (RFZm-Pg, RFZm-Pw, RFZm-Plg, RFZm-Po, RFZm-Pb, RFZm-Py, and RFZm-Pr). Qualitatively, there are 2 *Actinomyces* isolates that are able to dissolve phosphate having a phosphate solubility index value in the medium category, namely RFZm-Pg and RFZm-Pw with a low category. While quantitatively, RFZm-Pg and RFZm-Pw isolates dissolved the highest phosphate on day 28 with values of 0,8048 ppm and 0,4373 ppm, respectively. Based on the results indicate that *Actinomyces* isolates from the rhizosphere of corn plants have the potential to be used as biofertilisers to increase the availability of phosphate in the soil.

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