Isolation And Characterization Of Phosphate Solubilizing Fungi From Fruit And Vegetable Peel Waste Compost Fertilizer

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Abstract.

Phosphate solubilizing fungi are fungi that can convert phosphate in soil from compound form (almost insoluble) into ionic form (easily soluble) so that it can be utilized by plants. The purpose of this study was to determine the isolation and characteristics of phosphate solubilizing fungi isolates found in fruit and vegetable peel waste compost. This research was conducted in January 2023 until completion in the Laboratory of the Faculty of Science and Technology, Labuhanbatu University. This research was carried out by isolating fungi from municipal waste compost, characterizing morphology and the ability to dissolve phosphate and molecular identification. Furthermore, the data were analyzed descriptively based on the research results obtained. There were 4 isolates of phosphate solubilizing fungi that were able to form clear zones on Pikovskaya media with different morphological characteristics and ability to dissolve phosphate. The largest clear zone diameter was shown in isolate A3 of 3 cm which is Aspergillus niger fungus.

Keywords: *Phosphate Solubilizing Fungi, and Compost.*

I. INTRODUCTION

The population growth is always directly proportional to the consumption and activities of the community, which also increases the amount of waste generated (solid waste). Based on data from the National Waste Management Information System (SIPSN), the average amount of landfill in North Sumatra is 5,587.50 tons of daily waste and 2,039,438.93 tons of annual waste, while 40 percent of the waste pile contains food waste[1]Municipal waste handling is currently limited to the Collection, Removal and Disposal (3P) system. Waste is collected at the source, then transported to temporary disposal sites (TPS) and finally disposed of in landfills [2]. One of the efforts to overcome the problem of urban waste is the recycling of organic waste, with an emphasis on the composting process [3].A number of studies have been conducted on municipal waste composting. The results of the nutrient content test of organic waste compost show that the nitrogen (0.64%), P2O5 (0.33%), K2O (1.32%) content exceeds the SNI 2004 standard, while the carbon content (5.29%) and C/N ratio are still below normal. Organic waste compost with the above content is usually within the standard values set by SNI 2004 and is safe to use for plants and the environment. Comparison with the nutrient content of manure shows that the K2O nutrient content of organic waste is better than manure (K2O 0.45%), but the N and P2O5 content of composted waste is lower than manure (N 0.75). (P2O5 0.5%). Composting organic waste can replace the use of chemical fertilizers up to 50% of the standard dose and can support crop productivity [4]. According to [5] Municipal waste compost can improve soil physical properties, namely soil texture and structure, forming more stable, loose aggregates and good soil aeration and drainage.

One of the properties of compost is that it contains microorganisms that can dissolve nutrients. One of the most important nutrients is phosphate. Phosphate is an important nutrient that plants need in their growth and development process. Phosphate is actually abundant in the soil, but about 95-99% is in the form of insoluble phosphate, so plants cannot use it[6]. To be utilized by plants, phosphate must be converted into a soluble form, which requires phosphate-solubilizing microorganisms, including fungi.Fungi are microorganisms that are widespread in Indonesia and affect the decomposition process of organic matter. The existence of fungi is very important in the life of microorganisms in nature. Degradation of organic matter is the basic improvement of organic matter by microorganisms under controlled conditions. The role of fungi as decomposers of organic matter can accelerate the decomposition of organic waste into simpler elements that are easily absorbed by plants [7].Phosphate solubilizing fungi are fungi that can convert phosphate in soil from the compound form (almost insoluble) to the ionic form (easily soluble) so that it can be utilized by plants [8]. Fungi that can dissolve phosphate usually come from the *Deuteromycetes* class, that is, *Aspergillus niger, Aspergillus awaori, Penicillium digitatum, Penicillium bilaji, Fusarium and Sclerotium* [9]. The dominant phosphate-solubilizing fungi in soil are *Penicillium* and *Aspergillus* [10].

II. MATERIALS AND METHODS

3.1 Place and Time of Research

This research was conducted in January 2023 until completion in the Laboratory of the Faculty of Science and Technology, Labuhanbatu University.

3.2 Tools and Materials

The tools used for this research include test tubes, test tube racks, petri dishes, erlenmeyer, 1 ml micropipette, bunsen, microscope, object glass, autoclave, measuring pipette 10 ml, analytical scales, incubator, hot plate, refrigerator and stationery**.**The materials used for this research include municipal waste compost, distilled water, alcohol, aluminum foil, cotton, cling wrap, spirtus, PDA, Pikovskaya media and label paper.

3.3 Research Methods

The method used in this research is descriptive method.

3.4 Research Implementation

3.4.1 Source of Mushroom Isolation

Fungi were isolated from composted fruit and vegetable peel waste fermented in Tanjung Harapan Village, Labuhanbatu Regency.

3.4.2 Tool Preparation Stage

The research tools needed are glass such as Petri dishes, Erlenmeyer, measuring pipettes and test tubes. Then the tools are wrapped in HVS paper, then arranged in an autoclave. Materials that need to be sterilized are also put into the autoclave. The autoclave was run at 120◦C for 15 minutes. All forms of activity are carried out in a sterile manner to avoid bacterial growth.

3.4.3 Media Preparation

Potato Dextrose Agar (PDA) powder as much as 19.5 grams was dissolved in an erlenyemer tube containing 500 ml of sterile distilled water, stirred until dissolved and then heated until boiling. The PDA solution was cooled and put into Petri dishes that had been sterilized by autoclaving. Then the media was closed and tied with cling wrap and labeled, then the media was cooled until it solidified.

3.4.4 Fungi isolation from fruit and vegetable peel waste compost fertilizer

A total of 1 g of the city waste compost fertilizer sample was dissolved in 9 ml of distilled water, then 1 ml of suspension was added to 9 ml of sterile distilled water to obtain a suspension with a dilution level of 10-2. Dilution is done in the same way until the 10-4 level suspension. Next, 1 ml of the 10-4 dilution was spread onto a Petri dish containing solid PDA media. The dishes were then incubated at 250C in an incubator for 3-6 days.

3.4.5 Phosphate Solubilizer Test

The phosphate test is carried out to see the ability of phosphate solubilizing fungi to live. The fungal isolate to be tested is then taken a little and scratched into *Pikovskaya* media in a zig zag manner and then observed for 48 hours at 25oC. The ability of phosphate solubilizing fungi to live on pikovskaya media is characterized by the formation of a clear zone around the fungal colony.

3.4.6 Identification of Phosphate Solubilizing Fungi

a. Macroscopic

The identification of phosphate solubilizing fungi is done macroscopically by looking at the color of the colony, the shape of the colony, and the diameter of the fungal colony.

b. Molecular Identification

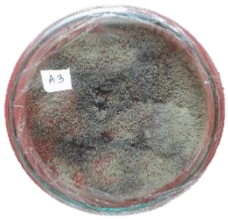
Molecular identification of phosphate solubilizing fungi was carried out by sending samples to PT Genetika Science Indonesia in Tanggerang, Banten. using the Genomic DNA Extraction method with Quick-

DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005), PCR Amplication with MyTaq HS Red Mix, 2x (Bioline, BIO-25048), and Bidirectional Sequencing.

III. RESULTS AND DISCUSSION

Based on Table 4.1, it can be seen that from the results of bacterial isolation from vegetable and fruit waste compost, 4 fungal isolates were found with different morphological characteristics. Fungi have unique characteristics such as a true nucleus, absence of chlorophyll, thallus-shaped filamentous somatic bodies, and reproduction by spores. Fungi grow optimally in places that do not require direct sunlight or shade. Fungi can grow faster in these conditions compared to excess sunlight [11].

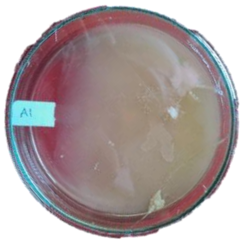
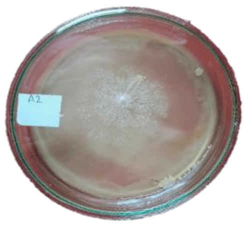
Table 4.1. Macroscopic Characteristics of Phosphate-Solubilizing Fungi from Vegetable and Fruit Waste Compost Samples

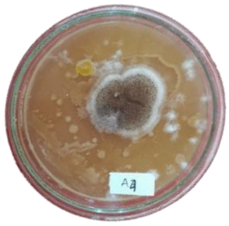
Isolate Code	Parameter			Image of isolate
	Color	Shape	Spore Color	
A1	White	Round	White	 Picture 1 Isolate A1
A2	White	Spreading	White	 Picture 2 Isolate A2
A3	Grayish white	Round	Black	 Picture 3 Isolate A3
A4	Orange	Round	Black	 Picture 4 Isolate A4

Phosphate solubilizing fungi are fungi that are able to convert phosphate in the soil from compound form (difficult to dissolve) into ion form (easily dissolved) so that it can be utilized by plants [8]. Phosphate-soluble fungi are capable of producing organic acids, but they can also secrete the enzyme phosphatase, which plays an important role in hydrolyzing organic P into inorganic P for plant use [12].*Pikovskaya* medium is a selective medium used for isolation and testing of phosphate-solubilizing bacteria and fungi. Phosphate-solubilizing microbial colonies growing on this substrate form a clear zone around it. The clear zone occurs because phosphate from phosphate sources is poorly soluble in the environment and dissolves in organic acids produced by microbial colonies. Growth time, color, colony size and clear zone vary with the type of phosphate solubilizing microbes. Qualitatively, wider and brighter clear zones indicate higher phosphate solubility in the medium, so the colonies can be isolated as MPF isolates/strains that can be cultured further. Pikovskaya medium can be modified for isolation purposes. For example, to isolate phosphate-solubilizing microbes capable of dissolving phosphate from Al-P, ALP04 can be used as a source of phosphate in the environment. In this way, phosphate-soluble microbial isolates can be developed on acidic soils with relatively high Al content.

Likewise, if the phosphate source is FePO4, Ca3(PO4)2, or other phosphate rocks, then the phosphate-solubilizing microbial colonies that grow are colonies that can utilize phosphate from these phosphate sources [13].The formation of a clear zone around the colony indicates that the isolate is able to dissolve complex phosphate. Clear zones can appear on Pikovskaya agar due to the dissolution of tricalcium phosphate (Ca3[PO4]2) suspension. The formation of clear zones on *Pikovskaya* medium indicates that microorganisms can dissolve phosphate [14]. The four isolates were then incubated on Pikovskaya media for 27 days and obtained different clear zone diameters. The largest clear zone was shown in A3 fungal isolate at 3 cm, while the lowest clear zone was shown in A1 fungal isolate at 1.5 cm. Observations were made on the 27th day because on the previous day the clear zone formed was not optimal and could not be observed. Observations were made by observing the colonies on Petri dishes and then looking at the clear zone and measuring its diameter using a ruler. The clear zone formed on day 27 of the four isolates is presented in Table 4.2 below.

Table 4.2. Diameter of Clear Zone of Phosphate Solubilizing Fungi Isolate

No	Isolate Code	Diameter of Clear Zone (cm)	Picture of clear zone
1	A1	1,5	 Picture5 Isolate A1
2	A2	2	 Picture6 Isolate A2

3	A3	3	 Picture 7 Isolate A3
4	A4	2,6	 Picture8 Isolate A4

The results obtained are reinforced by the statement [15] which explains that each microbe has a different ability to break down available phosphate depending on its type and adaptability to the environment. It forms transparent areas or clear bands of various sizes, and some cannot even break down phosphate. The degradation mechanism of phosphate-solubilizing fungi can be carried out by biological and chemical means. Fungi that dissolve phosphate in a biological way have phosphatase enzymes that can break down insoluble phosphate directly into usable phosphate. Fungi that dissolve phosphate chemically can produce several organic acids, such as citric, oxalic, succinic and glutamic acids. This process takes longer and the organic acids produced increase, causing the pH to drop. In addition, the organic acids produced react with phosphate-binding materials such as Al3, Mg2, Ca2, and Fe3 to form stable organic chelates that can release the bound phosphate ions and can be used by plants [9]. Furthermore, the fungi with the greatest potential in dissolving phosphate (isolates A3 and A4) were identified molecularly. Based on the results, it is known that isolate A3 is *Aspergillus niger* strain SG1 and isolate A4 is *Aspergillus niger* strain MM1. Based on the literature, it is known that *Aspergillus niger* has a white or yellow surface and there are blackish brown conidiospores.

A typical part of the *Aspergillus niger* fungus is the hyphae berseptat, asexual spores and has aerobic properties so that during growth requires sufficient oxygen. *Aspergillus niger* grows in the temperature range of 35 ° C - 37 ° C (optimum), 6 - 8 ° C (minimum), 45 - 47 ° C (maximum). pH required ranges from 2.8-8.8 and humidity 80-90% [16]. [17] indicates that *Aspergillus* species are widespread and can grow on almost any type of substrate. According to [18] the genus Aspergillus is a group of phosphate solubilizing fungi that are dominant in acidic soils in Indonesia, where the genus Aspergillus can dissolve bound P so that P is available to the soil. In accordance with the opinion of [10] stated that phosphate solubility becomes bioavailable to plants because *Aspergillus niger* produces enzymes, including the enzyme phosphatase.Some research using *Aspergillus niger* as a phosphate solubilizing microbe was shown by [19], that *Aspergillus niger* gave a significant effect on P uptake at doses of 20 and 25 mL. The results of research [20] showed that the application of *Aspergillus niger* can increase the availability and uptake of P and corn growth on Andisol Cangar. This shows that the higher the dose of *Aspergillus niger* given, the higher the P uptake. The use of phosphate-soluble fungi such as *Penicillium*, *Mucor* and *Aspergillus* can increase plant growth by 5 to 20 percent while reducing the use of chemical fertilizers [21].

From the research [22], it was found that the chitinase enzyme from *Aspergillus niger* from crab shell waste had an optimum pH of 6 and an optimum temperature of 40◦C. [23] *Aspergillus niger* is better at increasing phosphate availability in Andisol soil. Research results [24] found that the application of phosphate solubilizing microorganisms increased the number and weight of seeds and significantly increased the vegetative growth of corn plants. Similarly, the application of phosphate solubilizing fungi as much as 20 ml/polybag on Andisol soil was able to increase the wet weight and P uptake of chili plants and decreased with increasing application doses [25].The application of *Trichoderma sp.* and *Aspergillus sp.* both singly and in combination on Anjasmoro soybean plants can extend the incubation period and suppress the intensity of CPMMV. The decrease in CPMMV intensity in Anjasmoro soybean plants ranged from 29.40% to 60.42% [26]. In this research, *Aspergillus niger* fungi provide the best benefits, which can increase the availability of nutrients that can be absorbed by plants through the restructuring process carried out by these fungi for mangrove growth. The treatment of *Rhizophora apiculata* seedlings with Aspergillus niger has a significant effect on stem height, stem diameter and leaf width [27].

IV. CONCLUSIONS

1. There are 4 isolates of phosphate solubilizing fungi that are able to form clear zones on *Pikovskaya* media with different morphological characteristics and ability to dissolve phosphate.

2. The largest clear zone diameter is shown in isolate A3 of 3 cm which is *Aspergillus niger* fungi.

V. SUGGESTION

Phosphate solubilizing fungi isolates that have been obtained in *Pikovskakya* media should be tested in the field to determine their effect in increasing available P on plant growth.

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