Isolation And Characterization Of Nitrogen Fixing Fungi From Fruit And Vegetable Waste Compost

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Abstract.

Nitrogen fixing fungi are fungi capable of fixing free nitrogen into ammonium or nitrate, so that it can be absorbed by plants. Many species of microbes/fungi have the ability to fix nitrogen, but very few are able to excrete nitrogen in the form of ammonia so that their contribution in providing nitrogen for plants is also still low. This study aims to determine the characteristics and type of nitrogen fixing fungi in fruit and vegetable waste compost. The method used in this research was descriptive method. Identification of nitrogen-fixing fungi was carried out macroscopically by looking at the color of the colonies, the shape of the colonies, and the diameter of the fungal colonies, an then identified by molecularly. There were 4 isolates of nitrogen-fixing fungi capable of forming clear zones on Jensen's media with different morphological characteristics and nitrogen-fixing abilities. The largest diameter of the clear zone was shown in isolate A3 of 3.2 cm which is the fungus Aspergillus niger strain SG1.

Keywords : *Compost, Nitrogen fixing fungi, and Aspergillus niger strain SG1.*

I. INTRODUCTION

Compost is organic materials (organic waste) that have undergone a weathering process due to interactions between putrefactive microorganisms working in it, organic materials that can be composted can be in the form of leaves, grass, straw, remaining twigs and branches, animal waste, fallen flowers, animal urine, animal feces and others [1]. The level of nutrient content of compost is largely determined by the basic material, composting method, and storage method. In general, compost contains organic C of 4.38 - 8.00%, N of 0.10 - 0.51%, P2O5 of 0.35 - 1.21%, K2O of 0.32 - 0.80%, Ca of 1.00 - 2.09%, Mg of 0.10 - 0.19%, Fe of 0.50 - 0.64%, Al of 0.50 - 0.92% and Mn of 0.02 - 0.04% [2]. In addition to nutrient content, compost also contains organic compounds, such as humic acid, and sulfuric acid which are useful for spurring plant growth [3].Compost is like a multivitamin for agricultural soil. Compost will increase soil fertility and stimulate healthy roots. Soil microbial activity will also increase with the addition of compost. This microbial activity will help plants to absorb nutrients from the soil and can also produce compounds that can stimulate plant growth [3].One source of organic matter that can be composted is fruit and vegetable waste. Food waste is the most common waste in the world. The proportion reaches 44% of all types of waste. In 2017, fruits and vegetables were the largest contributors in the food waste category.

The contribution reaches 38% of the total food waste. Cereals followed by contributing 24%, root foods also produced 19% food waste. Meanwhile, milk and eggs contribute 9%, followed by meat as much as 5% [4].One that can succeed the process of making compost is microorganisms. Fungi are one of the microorganisms that are widespread in soil and water and have the potential to decompose organic matter, The role of fungi as an organic matter remodel can accelerate the process of remodeling organic waste into simpler elements, so that it is easily absorbed by plants [5]. Some fungi have the ability to hydrolyze cellulose naturally through their cellulase activity. Fungi that are able to produce cellulase components can be used as composting microorganisms. The fungi that help in the composting process are *Trichoderma sp.* and *Penicillium Sp.* [2].In addition to containingmicroorganisms that have cellulase abilities, compost also requires fungi as a producer of one of the nutrients needed by plants, namely nitrogen.Nitrogen-fixing fungi are fungi that are able to fix free nitrogen into ammonium or nitrate, so that it can be absorbed by plants.

Many species of microbes/fungi have the ability to fix nitrogen, but very few are able to excrete nitrogen in the form of ammonia so that their contribution in providing nitrogen for plants is also still low [6]. Based on this, it is necessary to know the characteristics of nitrogen-fixing isolates in the compost used.

II. METHODS

2.1 Place and Time of Research

This research was carried out from December 2022 to February 2023 at the Laboratory of the Faculty of Science and Technology, University of Labuhanbatu And PT. Genetics Science Indonesia.

2.2 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, 10 ml meausuring pipette, analytical scales, incubators, hotplates, HVS paper, refrigerators, ose needles and stationery.

The materials used for this study include fruit and vegetable waste, aquades, alcohol, aluminum foil, cotton, cling wrap, spirtus, PDA, and label paper, sucrose, K2HPO4, MgSo4, NaCl, FeSo4, CaCo3 and agar.

2.3 Research Methods

The method used in this study is the descriptive method.

2.4 Research Implementation

2.4.1 Sources of Fungal Isolation

The fungus was isolated from fermented fruit and vegetable waste waste in Tanjung Harapan Village, Labuhanbatu Regency.

2.4.2 Tool Preparation Stage

Research tools made of glass such as petri dishes, erlenmeyer, measuring pipettes, and test tubes. Then the tools are wrapped in HVS paper, then arranged into an autoclave. The materials that need to be sterilized are also included in the autoclave. The autoclave was run at 121°C for 15 minutes. All forms of activities are carried out sterilely to avoid bacterial growth.

2.4.3 Media creation

Potato Dextrose Agar (PDA) powder as much as 9.75 grams is dissolved into an erlenyemer tube containing 250 ml of sterile aquades, stir until dissolved and then heated waiting for boiling. The PDA solution is cooled and fed into a petri dish that has been autoclaved. Next, the substrate is closed, then tied with cling wrap and labeled. Then the substrate is cooled to solidify.

2.4.4 Isolation of fungi from fruit and vegetable waste compost

A total of 1 gr of fruit and vegetable waste compost samples was dissolved into 9 ml of aquades, then 1 ml of suspension was added to 9 ml of sterile aquades to obtain a suspension with a dilution level of 10^{-2}. Dilution is carried out in the same way up to a suspension of 10^{-4} degree. Next, 1 ml of 10^{-4} dilution is spread into a petri dish that has contained solid PDA media. The cup is then incubated at 25°C in the incubator for 3-7 days.

2.4.5 Nitrogen Fixing Test

The nitrogen fixing test is carried out by taking as much as 1 ose and inoculating it on Jensen media. The isolates were then incubated at 25°C for 3-7 days. Isolates that form clear zones suggest fungi can fixing nitrogen.

2.4.6 Identification of nitrogen-fixing fungi

a. Macroscopic

Identification of nitrogen-fixing fungi is done macroscopically by looking at the color of the colony, the shape of the colony, and the diameter of the fungal colony

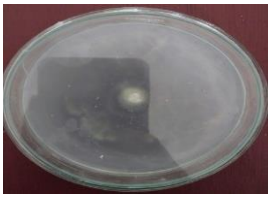
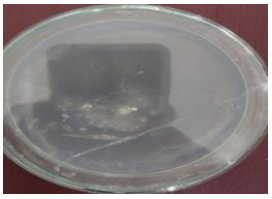
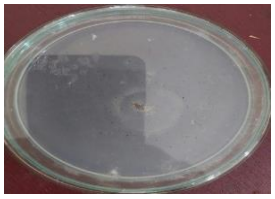
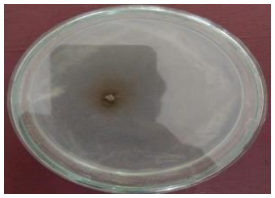
b. Molecular

Molecular identification using Genomic DNA Extraction with Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005), PCR amplification with MyTaq HS Red Mix, 2X (Bioline, BIO-25048) and Bidirectional Sorting.

III. RESULT AND DISCUSSION

Fungi are organisms that can survive in various environments with different media, and obtain their food from the media where the fungus grows. Fungi can also live on plant residues or live attached to other organisms. Mushrooms have the ability to and different functions according to the environment they live in [7]. Based on the results of isolation from vegetable and fruit waste compost, 4 fungal isolates with different morphological characteristics were found. Based on Table 4.1 above, it can be seen that three of the four isolates have the same colony shape, namely round (A1, A3 and A4), and white colony color (A1, A2) while A3 and A4 have different colony colors, namely grayish white and orange. The color of the spores, namely A1 and A2, is white, while the isolates of A3 and A4 are black.

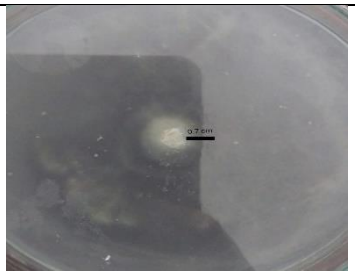
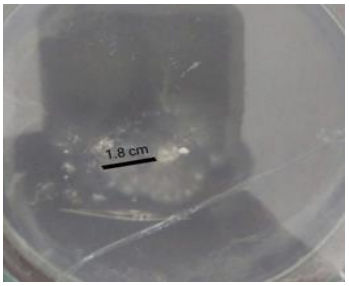
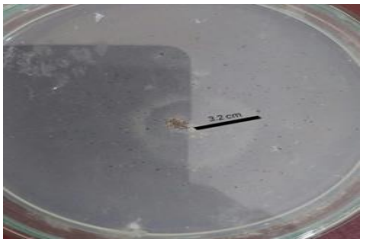
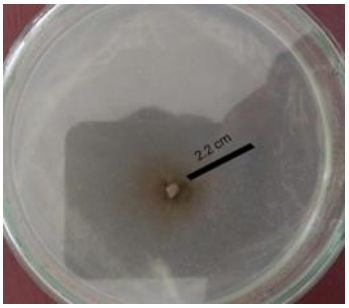
Table 1. Macroscopic characteristics of nitrogen-fixing fungi.

Isolate code	Parameter			Image of isolate
	Color	Shape	Spore color	
A1	White	Round	White	
A2	White	Spreading	White	
A3	Grayish white	Round	Black	
A4	Orange	Round	Black	

The four isolates also showed clear zones in *Jensen* media, meaning that they had the potential to tether nitrogen (Table 4.2). [8] States that where the most effective medium that can be used to isolate nitrogen-fixing microbes is Jensen media. The presence of nitrogen-fixing fungi is said to be positive if there is a clear zone in the jensen media. This media contains nutrients needed by nitrogen-fixing microbes, namely sucrose, dipotassium phosphate, magnesium sulfate, sodium chloride, ferrous sulfate, sodium molybdate, and calcium carbonate. Jensen media is formulated according to Jensen and is recommended for detecting and growing nitrogen-fixing microbes. Jensen Agar media is selective because it does not contain nitrogen elements so that only bacteria that have the ability to tether nitrogen can grow in the medium. In general, isolated nitrogen-fixing bacteria can grow on Jensen media So that the presence of a clear zone (holo zone) around the colony shows that bacterial isolates have the ability to tether nitrogen [9].Table 4.2 shows the large diameter of the clear zone produced in the nitrogen-fixing isolate test on Jensen media. The

results showed that the isolates that had the greatest nitrogen-fixing ability were A3 isolates of 3.2 cm, followed by A4 isolates of 2.2 cm and the smallest were A1 isolates of 0.7 cm.

Table 2. Clear Zone Diameter of Nitrogen Fixing Fungi Isolate.

No	Isolate code	Diameter of Clear Zone (cm)	Picture of Clear Zone
1	A1	0,7	
2	A2	1,8	
3	A3	3,2	
4	A4	2,2	

Furthermore, the fungi with the greatest potential toincrease nitrogen (A3 and A4 isolates) were identified molecularly. Based on the results, it is known that A3 isolate is a fungus species *Aspergillus niger* strain SG1 and isolate A4 is a fungus species *Aspergillus niger* strain MM1. Imran,.et al (2020) [10] stated that *Aspergillus* has the ability in the soil to decompose cellulose content into simple carbon compounds, this fungus is also able to dissolve phosphate rock in the soil so that it becomes organic phosphate for plants to absorb easily. Nitrogen (N) is a macroelement needed by plants in large quantities. Vegetative, stem, and leaf growth are major factors in nitrogen metabolism. If the supply of nitrogen to the plant is sufficient, then vegetative growth will be dark green. However, excess nitrogen will cause delays in flowering and fruit determination, and symptoms of yellowing leaves, stunted growth, and crop failure due to lack of nitrogen supply [11].The decomposition of organic matter carried out by the fungus comes from the results of the process of remodeling organic matter carried out by the fungus A. niger. *A. niger* is one of the fungi classified as a type of fungus that acts as a remodel of organic matter in the form of N nutrients that are broken down into the soil. The function of N nutrients itself is used by plants to stimulate vegetative growth, namely the addition of plant height [12].

Nitrogen can be biologically bound by symbiotic tethering in legume and non-legume plants. Types of plants that can tether nitrogen, for example through symbiosis with *rhizobium*, namely in soybean and peanut plants; symbiosis with Actinomicetes in *Alder* and *Casuarinaequisetifolia*; and symbiosis with blue algae, namely in kiambang and lichen plants [11]. *Aspergillus sp.* also has the ability to produce urea reductase and phosphatase enzymes that play a role in the tethering of free N from air and P solvent from insoluble compounds. In addition, the fungus is able to produce organic acids, solvents, P and/or polysaccharides that function as adhesives in the formation of micro-aggregates. The role of soil microbes in the cycle of various nutrients in the soil is very important, so that if one type of microbe does not function, there will be an imbalance in the nutrient cycle in the soil. The availability of nutrients is closely related to the microbial activity involved in them [12].

Nitrogen is gaining attention because it is small in the soil, but at each harvest the amount taken by plants is quite large; the easy loss of inorganic N is very soluble when evaporated or in drainage water; and the clear and rapid growth affected by N, resulting in increased production in efficient use of N. Organisms with phosphate solvent potential increase the availability of dissolved phosphates and can promote plant growth by increasing the efficiency of biological nitrogen fixation or increasing the availability of other trace elements such as iron, zinc and by the production of plant growth promoting regulators [11]. N-fixing microbes and P solvents are a synergistic relationship, meaning that the two microsymbionts support each other in their lives, fungal inoculation results in increased absorption of P elements available in the soil, so as to help the effectiveness of N-tethering microbes in tethering N free from air. Therefore, the presence of element N is a very essential part in increasing plant vegetative growth, microorganisms have an important role and function in supporting the implementation of environmentally friendly agriculture, microorganisms are positioned as nutrient producers whose work functions as the main supply of nutrients in supporting plant growth [13].

IV. CONCLUSION

4.1 Conclusion

1. There are 4 isolates of nitrogen-fixing fungi that are able to form clear zones in *Jensen* media with different morphological characteristics and nitrogen-fixing abilities.
2. The largest clear zone diameter is shown in A3 isolate of 3.2 cm which is *Aspergillus niger* strain SG1..

4.2 Advice

There needs to be further research on nitrogen-fixing fungi, namely the types of bacteria and their chemical content, as well as their application to plant growth**.**

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