Production of IAA of the rhizosphere fungus in the suren community forest stand

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Abstract. Plant growth can be influenced by the diversity of microbes that exist in the rhizosphere, e.g., fungi. Rhizosphere fungi found in plant roots can increase plant growth and protection against certain microbes. This study was aimed to identify rhizosphere fungi and evaluate the ability of IAA production. Research activities included isolating the fungi, identifying, and evaluating the ability of IAA production. Fungus identification observed five fungus genera (*Aspergillus*, *Trichoderma*, *Rhizopus*, *Penicillium*, and *Fusarium*). IAA production ability test showed *that Fusarium* has the highest concentration, which was 38,611 ppm. *Fusarium* isolates have the potency to be developed as biological fertilizers.

1. Introduction

Management of Suren community forest has been managed and utilized by the community. The type of suren wood is widely used as a construction material and is a source of the economic community. The increasing market demand resulted in a decrease of suren production. The way to find out good suren production results based on the quality and quantity, one of them is by knowing plant growth, which is determined by the presence of rhizosphere microbes. The good rhizosphere is colonized by an excellent microbial community [1]. Fungal microorganisms in the rhizosphere zone play a role in decomposing organic matter and helping plant growth [2]. The process of plant growth is influenced by auxin compounds, which are naturally produced by plants.

Auxin is one of the plant hormones that influence the process of forming plant tissues, namely growth, division, and cell differentiation and protein synthesis. The results showed that there is one type of auxin which has a considerable role, namely *Indole Acetic Acid* (IAA). IAA is naturally found in plants called endogenous IAA and which can be produced by microorganisms, the fungus obtained from the rhizosphere is called exogenous IAA. Endogenous IAA produced by plants has a limited amount and is not used directly by plants. While IAA obtained from fungus isolation can be applied in biological fertilizers as an addition that gives optimal results. Fungi that can produce auxin are *Phanerochaete chrysosporium*, *Colletotrichum gloeosporioides*, and Aeschynomene produced by fungi as secondary metabolites that act as ZPT. Based on research conducted by [3] one type of fungus that can produce IAA is *Penicillium* sp.

Several fungi used in biotechnological applications such as bioremediation and delignification [4]. Exogenous IAA production by *Phanerochaete chrysosporium* strain ME446, *Funalia trogii*, and *Lentinus sajor-caju*, respectively [5]. Suren stands that grow in community forests are expected to have good growth by utilizing the potential of rhizosphere fungi that produce IAA. This study aims to explore rhizosphere fungi and their ability to produce IAA. The fungus can be a collection of isolates

which can later be a formulation for biofertilizers that can support plant growth so that it can increase forest productivity and support increasing market demand.

2. Materials and methods

2.1. The materials used in this study were soil samples, jelly, distilled, PDA media (Potato Dextrose Agar), PDB media (Potato Dextrose Broth), glucose, L-Tryptophan, alcohol, methanol, synthetic IAA, H2SO4, FeCl36H2O, plastic wrap, aluminum foil, tissue, tips, and labels. The tools used in soil sampling are GPS, crowbars, plastic clips, plastic bags, cameras, and writing instruments. While the tools used in the laboratory (the stages of isolation and IAA test) are measuring cups, test tubes, ovens, analytical scales, drygalski, hot plate, magnetic stirrers, centrifuge tubes, Erlenmeyer, laminary airflow, autoclaves, Petri dishes, glass bottles, needle preparations, bunsen, glass objects, glass decks, cork borer, filter paper, microscope, micropipette, vortex, centrifuge, freezer, spectrophotometer, and camera.

2.2. Land sampling

Land sampling was carried out in a composition at a depth of 0-25 cm from the four corners of the wind (figure 1) in the rhizosphere of 10 plants that had been selected by purposive sampling.

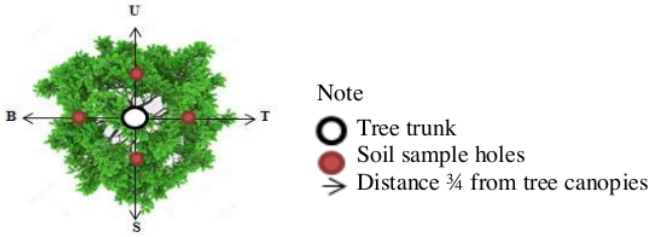

Figure 1. Sampling Pattern on Suren Stand

2.3. Isolation and isolation purification

Microbial isolation was carried out using a dilution method by making a series of dilutions. 10^{-2}, 10^{-3} dilutions were used to isolate the fungus. The incubation process was carried out at room temperature for 3-7 days. Purification was done by removing the fungus colonies on the new sterile PDA media.

2.4. IAA concentration measurement

IAA concentration measurement was carried out by inoculating the isolates at 45 ml of liquid GDP enriched 0.1 g / L L-tryptophan and incubated in the Water Bath Shaker for five days. Then take 1.5 ml of the soil (culture) and centrifuged it for 30 minutes at 8000 rpm. 1 ml of supernatant was taken then 1 ml of calcowski reagent was taken (125 ml of aquabidest, 75 ml of H_2SO_4 and 3.75 ml of $FeCl_3.6H_2O$ 0.5 M), left for 30 minutes in dark conditions. The absorbance is read at a wavelength of 520 nm using a spectrophotometer. IAA concentration in the media was calculated using the IAA standard curve.

2.5. The making IAA standard curvet

Prepare 50 ml of methanol, which has dissolved 2.5 mg of IAA synthesis (concentration of 50 ppm). The synthesis IAA solution was pipetted into test tubes 20 µl (1 ppm), 100 µl (5 ppm), 200 µl (10 ppm), 300 µl (15 ppm), 400 µl (20 ppm), 500 µl (25 ppm) respectively.) 600 µl (30 ppm), 700 µl (35

ppm), 800 µl (40 ppm) and 900 µl (45 ppm). Methanol was added, so the volume of each test tube became 1000 µl, then 4 ml of the Salkowski reagent was added to each test tube and then homogenized and incubated for 30 minutes at room temperature so that the solution would turn pink. The standard IAA solution measured the absorbance using a spectrophotometer at a wavelength of 530 nm. From the results of spectrophotometry, a standard IAA solution curve was made which showed the relationship between the standard IAA solution (x) and its absorbance (y), and will be obtained:

$Y = a + bx$
Note:
a = Intersep
b = Slope (Regression Coefficient)
Y = Absorbance
X = Concentration

3. Results and discussion

3.1. Exploration rhizosphere fungi

The isolation results and purification of fungi from ten trees in suren community forest stands obtained 33 isolates. The number of isolates in each tree varies in the number and type of fungi obtained. Several factors that influence the amount of fungus obtained in the isolation process are environmental factors such as the amount and type of nutrients, humidity, aeration level, temperature, pH, and organic matter applied to the soil.

3.2. The making of IAA standard curve

The standard curve is used to obtain equations in calculating the IAA concentration values produced by isolates. The process results of testing the standard solution obtained indicate its reaction by producing a pink color. Red density is directly proportional to the increase in IAA concentration produced can be seen in figure 2.

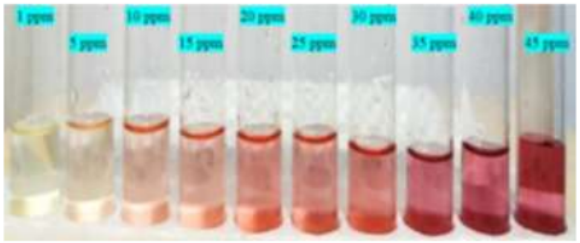

Figure 2. Solution concentration level for making IAA standard curve

L-tryptophan addition would show higher IAA concentrations. The IAA concentration in the sample was determined based on the results of measuring various concentrations of standard IAA (pure synthetic) with concentrations of 1 to 45 ppm. The results of the concentration obtained in the isolates were then put into the equation obtained from the making of the IAA standard curve (Figure 2). The ppm level in table 1 is obtained from the IAA standard curve equation Y = 0.018x - 0.001 can be seen in Figure 3.

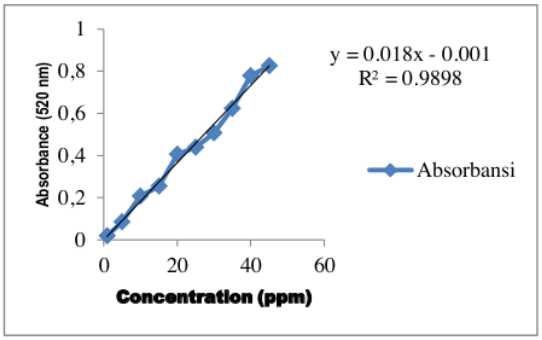

Figure 3. IAA Standard Curve

The IAA concentration produced by the fungus isolates was obtained by changing the variables in the standard curve equation, where y is the absorbance value produced by the spectrophotometer device. The results of the IAA standard curve will be obtained the value of x which is the concentration of IAA isolates of fungi expressed in units of ppm.

3.3. Production capability of fungi isolate IAA test

The color observations result carried out on fungus isolates obtained three isolates that had concentrated pink color having high IAA concentrations while the other three isolates did not have a color formation reaction which produced a deep yellow color which was compared with aqueduct color and calcowski solution as control can be seen in figure 4.

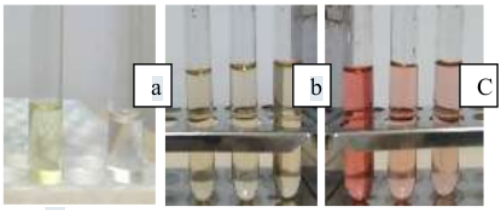

Figure 4. (a) Control of Aquades (left) and Salkowski (right), (b) The lowest IAA concentration and (c) Highest IAA concentration

The absorbance measurements results were included in the formula obtained from the IAA standard curve, and different results were obtained with the lowest concentration value of 1.888 ppm and the highest 38.611 ppm after growing on GDP media with the addition of L-tryptophan. Based on the test results obtained 3 isolates that had the highest IAA concentration of 10.278 ppm (S7.2), 13.889 ppm (S1.3) and 38.611 ppm (S7.1) were marked with thick pink, while 3 isolates had low IAA concentrations included 1.888 ppm (S2.1), 2,055 ppm (S1.1) and 2.5 ppm (S9.2) which were marked by the color of the solution which did not turn red but was thick yellow.

The IAA concentration test results showed differences in the concentration produced by each fungus Isolate. The fungus collection tested was able to produce IAA hormones with varying levels in the range of 1,888 ppm to 38,611 ppm can be seen in table 1.

Table 1. IAA production capability test results on 33 isolates of suren surge rhizosphere

Isolate Code	Absorbance (nm)	IAA Concentration (ppm)
S2.1	0,033	1,888
S1.1	0,036	2,055
S9.2	0,044	2,500
S5.1	0,05	2,833
S8.1	0,054	3,055
S4.2	0,055	3,111
S4.3	0,057	3,222
S5.2	0,06	3,388
S6.2	0,06	3,388
S1.2	0,062	3,500
S6.1	0,065	3,666
S2.2	0,066	3,722
S2.4	0,066	3,722
S6.3	0,066	3,722
S9.1	0,069	3,888
S8.2	0,071	4,000
S10.1	0,072	4,055
S9.3	0,075	4,222
S5.3	0,077	4,333
S10.2	0,087	4,888
S1.4	0,088	4,944
S9.4	0,09	5,055
S7.3	0,093	5,222
S4.1	0,103	5,777
S2.5	0,108	6,055
S2.3	0,138	7,722
S7.2	0,184	10,278
S1.3	0,249	13,889
S7.1	0,694	38,611

The IAA production ability test results on 33 rhizosphere isolates of suren stands showed that the IAA concentration produced was different even though with the same genus, this happened because in one genus had a different species so that the IAA content produced by each isolate could be different. The difference in IAA concentration produced by each fungus isolate can also be affected by the ability of the fungus to synthesize tryptophan as a precursor.

The growth speed of the isolates also influences IAA production. The faster the cell divides, the higher IAA production, the slower the cell divides, the lower the amount of IAA produced, this is evidenced by the growth rate at the stage of identification of isolates that produce high IAA having a diameter above 7 cm on the seventh day of incubation period in the PDA media. IAA production is also influenced by the incubation period, where the longer the incubation period can cause a reduction in the number of nutrients present in the growth media, on the other hand, the IAA produced is consumed again for growth. The results of the research conducted by Sugiharto [6] suggested that the production of IAA tended to increase during the two-day incubation period and decreased during the six-day incubation period. The difference can also influence different results in the tryptophan concentration added to the culture media, the higher the feed tryptophan concentration, the higher the IAA concentration produced. In addition to the factors above the IAA, concentration is also influenced by

environmental factors or the conditions of each sampling location, namely carbon conditions, pH and oxygen conditions [7].

The found fungus isolates in suren community forests can produce IAA with different concentrations. Fusarium had the highest IAA concentration, but only four isolates were found in the study location. Therefore, it can be done multiplication of isolates, which will later be used in biological fertilizer formulations. IAA has a role in increasing plant growth and producing longer shoots and roots, besides IAA can prevent the possibility of diseases caused by soil pathogens. The microbial ability to produce IAA is an important parameter that must be possessed by bio-fertilizer microbes. Isolates that can produce IAA that have been found in suren community forest plants can be applied as biological fertilizers and can be applied directly to the soil around the plants.

4. Conclusion

The isolation results of rhizosphere fungi in suren stands obtained 33 isolates belonging to five genera, namely Aspergillus, Trichoderma, Penicillium, Rhizopus, and Fusarium. The isolate of rhizosphere fungi suren stands can produce IAA hormones. The fungus produced the lowest IAA concentration was from the Aspergillus genus and fungi, which produced the highest IAA concentration was the Fusarium genus.

Acknowledgment

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