Production of Indol-3-Acetic Acid (IAA) by fungal isolates of taro (Colocasia esculenta var. antiquorum) rhizosphere

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Abstract. Many taros (Colocasia esculenta var. antiquorum) grow in Sulawesi due to their large scale acceptability and high return per unit area, but their productivity is still low. In this study, fungi producing indol-3-acetic acid (IAA) were selected and characterized from the taro rhizosphere with the aim that it can be applied in stimulating the growth and production of this plant. By analyzing the content of IAA in the medium of fungi isolated, 12 fungal isolates were found producing this compound. The highest level of IAA is provided by isolate ETR 33 (8.89 ppm), ETR 29 (8.21 ppm), and ETR 5 (7.82 ppm). These isolates were identified as Fusarium, Trichoderma, and Aspergillus, respectively. These data that three strains of fungi were identified and characterized as the producer of IAA and potentially be used to develop a biofertilizer to increase the productivity of taro.

1. Introduction
Taro (Colocasia esculenta var. antiquorum) is one of export commodities. Their large scale acceptability and high return per unit area but their productivity is still low. The effort to stimulate the growth and increase the production of the plant is highly depended on the use of chemicals e.g. pesticides, fungicides, and fertilizer. The continuous use of chemicals in a large quantity leave pollutant residue in the environment. Therefore, eco-friendly environmental technologies are starting to be developed to overcome that problem. An alternative technology to resolve this proposition situation is by using microbes that can produce phytohormone such as Indol-3-Acetic Acid (IAA) that serve as biofertilizer [1].

IAA is a growth hormone that is very important to encourage the growth of a plant, e.g. initiating the root, enlarge the cells, differentiating the vessels, and promoting the efflorescence. IAA is not just produced by plants, but also bacteria [2], yeast, and fungus [3-5]. Some fungi that can produce IAA include Trametes versicolor, Pleurotus ostreatus, Phanerochaete chrysosporium [6]. Trichoderma viride [7], Colletotrichum gloeosporioides [8], Aspergillus niger and Aspergillus flavus also have the ability to produce IAA [9].

Rizosphere is an ideal area for soil microorganism to grow and develop because it is affected by root exudation. The plant and this organism have a mutual interaction. The plants will attract beneficial microbes in the rhizosphere area by emitting root exudate, i.e. tryptophan [10, 11] that can provide nutrient and act as a precursor for microbes to produce IAA [11]. The IAA which is produced by the microbes is used by plants to regulate their biological growth and development [13, 14]. In this
study, fungi producing indol-3-acetic acid (IAA) were selected and characterized from the taro rhizosphere with the aimed that it can be applied in stimulating the growth and production of this plant.

2. Methods

2.1. The isolation of rhizosphere fungi

The samples of soil were taken randomly from the areas of healthy taro’s roots then mixed and put into sample paper. 10 gram of soil sample was taken and suspended with 10 ml aquades. Subsequently, 1 ml suspension was moved into 9 ml sterile aquades in the test tube, then shaken until it was homogenous (dilution $10^{-1}$). The same dilution was applied until $10^{-6}$. The results from each dilution ($10^{-1} - 10^{-6}$) were taken for 1 ml and put into a petri dish containing PDA (Potato Dextrosa Agar) medium and mixed. The mix was then incubated at room temperature for 2-3 days. The fungi colonies which grew in the dilutions $10^{-1} -10^{-6}$ were purified by moving them into new sterile PDA medium.

2.2. Indole-3-Acetic Acid (IAA) measurement

Seven days after inoculation, three samples of fungi isolate in the Potato Dextrosa Agar (PDA) medium was taken using cork borer and put into a bottle containing Potato Dextrosa Broth (PDB) medium which was added with 0.1 g l$^{-1}$ of L-Tryptophan. The suspension was then shaken with the speed of 150 rpm/minutes and incubated for 7 days at temperature room. The fungi suspension was centrifuged with the speed of 5000 rpm for 25 minutes. 5 ml of supernatant was added with 1 ml Salkowski reagent (12 gl$^{-1}$ FeCl$_3$ in 429 ml l$^{-1}$ H$_2$SO$_4$) [15]. The Supernatant was put for 24 hours at room temperature in the dark condition. Quantity test was performed to measure the compound absorbance using spectrophotometer with the wavelength of 530 nm.

The change on supernatant’s color to be pink indicated the production of auxin IAA. The concentration of the auxin was measured using a standard curve with regression $Y = 0.028 x + 0.0121$; $R^2 = 0.883$. The equation was taken from the series of IAA stock dilution around 0 – 5.0 mg$l^{-1}$. The concentration of culture filtrate is stated with mg$l^{-1}$ and compared with the standard curve.

2.3. The characterization of isolates

Isolate that indicates the highest concentration of IAA was macroscopically and microscopically characterized. The macroscopic characterization was based on the morphological observation on colonies in Potato Dextrosa Agar (PDA) medium after incubated for 7 days. Microscopic observation with 40 X magnification was to see the sporangium, sporangiophore, and the spore of fungi isolates [16].

3. Results and discussion

Naturally, a plant can produce IAA (endogen hormone) for its growth. However, it is not sufficient so that is needed exogenous auxin hormone which can be obtained from microorganism like bacterium, fungi around the roots. According to Bose [6], IAA is not only produced by plants but also by bacterium, yeast, and fungi. The sample isolation of soil taken from the rhizosphere of taro (Colocasia esculenta var. antiquorum) had 12 fungi isolates. Isolates were analyzed for IAA which have varied produced (Figure 1).

The test conducted using Salkowski reagent to detect the ability of isolates to produce IAA showed a color change to be various pink colors (Figure 2). This was resulted by the ability of microbes to produce IAA which qualitatively is pink because of the interaction between IAA and Fe$^{3+}$ to form a complex compound $[Fe_2(OH)_2(IAA)]_4$. IAA refers to indole-3-acetate which interaction occurred in the acid environment. There were two reactions, namely complex and redox reactions [17].
The result test using Salkowski colorimetric technique show that the darkest pink isolate was ETR33. According to Kovacs [17], The darker the pink color is the higher the IAA produced by the fungi. Level of IAA concentration will be determined by highly stability and density colour of sample after have been added salkowski reagent [18]. Salkowski reagent is frequently used in detecting indolic substance. The addition of this reagent into the isolate medium will change it into red and pink. Red microbes actively change the Tryptophan (TRF) to be tryptophol (TOL), and some of them produce indole-3-acetic (IAA) acid resulted from the production of tryptophol [19].

Quantitative analysis for the IAA concentration using spectrophotometry based on the absorbance value at the wavelength of 530 nm. The wavelength of 530 nm was selected because of visually detectable colour reaction by Salkowski reagent which produce pink colour for IAA [15]. The result of spectrophotometer indicated that the concentration of IAA which was produced in each isolate was
very varied (figure 1). The highest IAA concentration was 8.89 ppm, at the ETR 33 isolate. This was similar to the qualitative test indicating that ETR33 which produced the darkest pink color with Salkowski colorimetric techniques. It was followed by ETR29 isolate with 8.21 ppm producing IAA and ETR5 producing 7.82 ppm of IAA.

Three isolates indicating the highest IAA concentration (ETR 33, ETR 29 and ETR5) were characterized macroscopically and microscopically (Table 1). The results showed that isolates that were obtained including Fusarium sp, Trichoderma sp, and Aspergillus sp. A similar species found by Kumar [7] was Trichoderma viride and Usha [8] stated that Aspergillus niger and Aspergillus flavus have the ability to produce IAA. The production of IAA was very varied among species and strains in the same genera and it is affected by the condition of the environment, the growth rate, and the availability of substrates like amino acid.

| Isolate Code | Macroscopic | Microscopic |
|--------------|-------------|-------------|
| ETR33        | ![Macroscopic Image of ETR33](image1.png) | ![Microscopic Image of ETR33](image2.png) |
| ETR29        | ![Macroscopic Image of ETR29](image3.png) | ![Microscopic Image of ETR29](image4.png) |
| ETR5         | ![Macroscopic Image of ETR5](image5.png) | ![Microscopic Image of ETR5](image6.png) |

Table 1. Characterization macroscopically and microscopically of fungal isolates
4. Conclusions
Based on the qualitative and quantitative tests that were performed, ETR33 isolate (*Fusarium* sp) can produce the highest IAA (8.89 ppm), following ETR29 isolate (*Trichoderma* sp) can generate 8.21 ppm IAA and ETR5 (*Aspergillus* sp) can produce IAA with concentration of 7.82 ppm. ETR33, ETR29 and ETR5 isolates were potential to be developed as bio-fertilizer.

Acknowledgment
This research was funded by Indonesian Lecturer’s Scholarship (BUDI), Ministry of Research, Technology and Higher Education in collaboration with Institute of Fund Management for Education (LPDP). Therefore, I would like to thank them for the funding and their trust to carry out this study.

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