Isolation of fungi producing hormone Indole Acetic Acid (IAA) on sugarcane bagasse and filter cake

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Abstract. Sugarcane bagasse and filter cake are the residual results of the process of extracting sugarcane stems into sugar which is widely used as an organic material that is beneficial to plants. The purpose of this study was to determine the ability of fungi that live in sugarcane bagasse and filter cake to produce Indole Acetic Acid (IAA) hormone. A bagasse sample of 1 gram was crushed and suspended in 99 ml of pure aquades water and a 10 gram sample of filter cake was suspended in 90 ml of pure aquades. 1 ml was taken and put into 9 ml of sterile distilled water and then mixed using vortex and diluted 10⁻⁵ to 10⁻⁶, 0.1 ml pipette then poured into a cup containing Potato Dextrose Agar (PDA) media. Purification was carried out by removing a fungi colony on a sterile PDA media and then morphologically characterized. Qualitatively testing IAA production capability by culturing 1 full loop on the PDA media, then put it into PDB media and added L-Triptophan and Salkowski reagents. Supernatant was stored for 24 hours at room temperature and dark. The change in color of the superoxide to pink indicates IAA and the quantity test was performed using a spectrophotometer λ 530 nm to measure absorbance. The results show that bagasse and filter cake isolates had morphological differences. All isolates tested had the ability to produce IAA. The average production of the hormone IAA of isolate from sugarcane bagasse was 1.063 – 3.469 ppm while filter cake was 0.891-1.547 ppm. The highest IAA production produced by T4 isolates of 3.469 ppm and fungi isolates which produced the lowest IAA hormone was B21 isolates of 0.047 ppm. T4 isolates from sugarcane bagasse have good potential to be developed for use in plants.

1. Introduction

Sugarcane in Indonesia is the highest contributor to national sugar production [1]. Bagasse is a by-product of sugarcane stems that have gone through the process of crushing and extracting juice [2]. Sugarcane bagasse and filter cake are widely used as organic material for plant growth media. Many fungi are found in organic matter from plant residues which have decayed and have the ability to produce secondary metabolites that are beneficial to plants including Indole Acetic Acid (IAA). IAA is the main hormone of almost all types of plants [3] and IAA is needed for plant growth because it plays a role in cell enlargement and elongation activities and tissue differentiation [4].

Microorganisms have the ability to produce IAA phytohormones [5]. According to Suciamith [6] IAA hormone can be produced by fungi. Fungi have different abilities in producing IAA hormones. This is influenced by the physiological nature of the host plant, nutrient intake, especially macro
molecules of proteins and carbohydrates. Fungi that produce IAA such as *Phanerochaete chrysosporium* [7] and *Aeschynomene* [8], *Trichoderma* sp., *Fusarium* sp. and *Penicilllium* sp. which is isolated from the rhizosphere [9].

The exploration of fungi found in bagasse and filter cake to find out the ability to produce IAA has never been done before and it is hoped that by conducting research, it is found superior isolates in producing the hormone Indole Acetic Acid (IAA).

2. **Research methods**

2.1. *Fungal isolation and macroscopic characterization*

A total of 100 grams of sample each bagasse and filter cake was taken at the Takalar Sugar Factory. 1 gram of sugarcane bagasse was then crushed and then suspended in a test tube containing 99 ml of sterile water then shook for 20 minutes while the sample was taken 10 gram and then suspended into 90 ml of sterile aquades. Suspension of sugarcane bagasse and filter cake was taken as much as 1 ml then mixed using vortex for several minutes then put in a test tube containing 9 ml of sterile distilled water and then shaken until homogeneous (dilution stage 1 / 10²), then carried out in the same way until dilution 10⁻⁶. Dilutions 10⁻⁵ and 10⁻⁶, taken 0.1 ml using a measuring pipette aseptically, then put in a sterile petri dish containing potato dextrose agar (PDA) media, then spread evenly over the petri dish using a spatula. After that it was incubated at room temperature for 8 days. Purification to obtain pure culture is done by transferring the fungus to a new sterile PDA medium [10]. Macroscopic observations were made at the age of eight days. Fungal identification refers to the Pictorial Atlas of Soil and Seed Fungi [11]. Macroscopic identification is done by observing the color, diameter and texture.

2.2. *Testing the ability of isolates to produce the hormone Indole Acetic Acid (IAA)*

Measurement of the production of the hormone Indole Acetic Acid (IAA) was done using a standard method by first culturing a full loop of fungus isolates on Potato Dextrose Agar (PDA) media, then extracting boletus fungus on 7-day-old PDA media as much as 5 cork borer and then inserted in bottles containing PDB liquid media added with L-Tryoptophan as much as 0.1 g l⁻¹ shaken at 150 rpm / min for 7 days at room temperature. The boletus suspension was centrifuged at 5000 rpm for 25 minutes. 5 ml supernatant was taken and 1 ml of Salkowski reagent was added (12 g l⁻¹ FeCl₃ in 429 ml 1⁻¹ H₂SO₄) [12]. Supernatant was stored for 24 hours at room temperature in dark conditions. Quantity test was carried out by measuring the absorbance of the mixture with a spectrophotometer with a wavelength of 535 nm. The change in color of the supernatant to pink indicates the production of IAA. Auxin concentration was measured using a standard curve with a regression equation \( Y = 0.064 x + 0.09 \), where \( R² = 0.995 \). The equation was obtained from the dilution series of IAA stock solutions ranging from 0-5.0 mg l⁻¹. Concentrations in culture filtrate are expressed in mg l⁻¹ and compared with standard curves. All experiments were repeated 3 replications. Observation parameters are qualitative observations, namely a change in the color of pink on isolates and quantitatively by calculating the production of IAA (ppm).

3. **Results**

3.1. *Morphological characteristics*

Morphological characterization of 10 bagasse isolates and 10 filter cake fungus isolates that grew on PDA media had diversity in shape, texture, diameter and color of the colony can be seen in Table 1 and the highest IAA production of T4 isolate is shown in Figure 1.
Table 1. Morphological characterization of IAA producing fungi

| Code Isolate (Bagasse) | Morphologically Observation | Code Isolates (Filter cake) | Morphologically Observation |
|------------------------|-----------------------------|-----------------------------|-----------------------------|
| T30                    | Round shape, stringy, flat, smooth surface, white, no pigment and bright, colony diameter of 9 cm. | B15 | Round, stringy, flat, green colonies with white and bright edges, colony diameter of 9 cm. |
| D14                    | Round, stringy, convex, rough surface, green colonies in the middle and white edges, no pigment and bright, colony diameter 4 cm. | B10 | Irregular shape, stringy, flat, rough surface, green in the middle of white edges, bright, colony diameter of 7 cm. |
| D19                    | Round shape, stringy, convex surface, smooth, green middle, white edge, no pigment and bright, colony diameter 8 cm. | B21 | Irregular shape, flat, flat surface, smooth, white colony, opaque, 4 cm diameter colony. |
| T20                    | Round, black, flat colony, no pigment, and bright, colony diameter of 6 cm. | B2  | Round, flat, smooth surface, white and opaque colonies, 5 cm diameter colony. |
| T4                     | Round, stringy, flat, rough surface, green colony, no pigment and bright, colony diameter of 8 cm. | B18 | Spread shape, flat, rough surface, colony, gray, brown and bright pigment, colony diameter of 9 cm. |
| D7                     | Round, stringy, flat, smooth, white colony, no pigment and bright, colony diameter 7.5 cm. | B4  | Round shape, spread, convex, rough, in the middle of green and white edges, bright, colony diameter of 6 cm. |
| D8                     | Round, stringy, convex, rough, green colonies in the middle, yellow and bright pigments, 5 cm diameter colony. | B1  | Round, flat shape, rough surface, green colony, white and opaque edge, colony diameter 9 cm. |
| A5                     | Round, stringy, convex, rough, gray colony in the middle, white on the edge, yellow and bright pigment, colony diameter 4 cm. | B13 | Round, stringy, flat, rough surface, green, bright colony, colony diameter of 9 cm. |
| T3                     | Round, stringy, flat, smooth, white colony, yellow and opaque pigment, 4 cm diameter colony. | B6  | Round, stringy, flat, smooth, white, bright colony, colony diameter of 6 cm. |
| T9                     | Irregular shape, flat, smooth, white and opaque colonies, colony diameter of 8 cm. | B12 | Round, stringy, flat, rough surface, slightly brown colony, yellow and bright pigment, 4 cm diameter colony. |
3.2. Qualitative testing

Fungi isolated from bagasse and filter cake were able to produce IAA, this was seen from the change in pink (pink) to dark red compared to control (yellow) after administration of the Salkowski reagent (Table 2 and Figure 2).

Table 2. Qualitative tests of bagasse and filter cake fungi isolate isolates by Salkowski reagents

| Number | Isolate Code (Baggase) | Color Change | Isolate Code (Filter cake) | Color Change |
|--------|------------------------|--------------|----------------------------|--------------|
| 1      | T30                    | Solid red    | B13                        | Solid red    |
| 2      | D14                    | Solid red    | B10                        | Solid red    |
| 3      | D19                    | Solid red    | B21                        | Pink         |
| 4      | T20                    | Pink         | B2                         | Pink         |
| 5      | T4                     | Pink         | B18                        | Pink         |
| 6      | D7                     | Pink         | B4                         | Pink         |
| 7      | D8                     | Pink         | B1                         | Pink         |
| 8      | A5                     | Pink         | B13                        | Pink         |
| 9      | T3                     | Pink         | B6                         | Pink         |
| 10     | T9                     | Pink         | B12                        | Pink         |

Figure 2. Best fungal isolate test results from Lees Cane (T4) and filter cake (B13) in producing IAA. Color changes into pink observed in isolates containing IAA while control showed yellow color.
3.3. Quantitative testing
The test results (Table 3), show the production of Indole Acetic Acid (IAA) in fungi isolated from sugarcane bagasse has an IAA value range of 0.391-3.469 ppm. Isolate T4 showed the highest IAA production of 3.469 ppm while the lowest DA isolate produced IAA of 1.063 ppm. Whereas the fungus isolates isolated from filter cake had an IAA value range of 0.047-1.547 ppm. Isolate B13 had the highest IAA production at 1.547 ppm while B21 isolates produced the lowest IAA production at 0.047 ppm. Based on table 3 it can be seen that T4 isolate has a higher IAA production value compared to other isolates.

Table 3. Measurement results and calculation of IAA production (ppm) fungi isolates from bagasse and filter cake.

| Number | Isolate Code (Bagasse) | Absorbance Value | IAA Production (ppm) | Isolate Code (Filter Cake) | Absorbance Value | IAA Production (ppm) |
|--------|------------------------|------------------|----------------------|---------------------------|------------------|----------------------|
| 1      | T4                     | 0.312            | 3.469                | B13                       | 0.189            | 1.547                |
| 2      | A1                     | 0.202            | 1.750                | B6                        | 0.173            | 1.297                |
| 3      | D7                     | 0.186            | 1.500                | B12                       | 0.168            | 1.219                |
| 4      | T30                    | 0.152            | 0.969                | B2                        | 0.156            | 1.031                |
| 5      | D19                    | 0.148            | 0.906                | B10                       | 0.152            | 0.969                |
| 6      | A5                     | 0.147            | 0.891                | B1                        | 0.147            | 0.891                |
| 7      | T3                     | 0.138            | 0.750                | B4                        | 0.143            | 0.828                |
| 8      | D14                    | 0.125            | 0.547                | B18                       | 0.135            | 0.703                |
| 9      | T20                    | 0.115            | 0.391                | B15                       | 0.125            | 0.547                |
| 10     | D8                     | 0.115            | 0.391                | B21                       | 0.093            | 0.047                |

4. Discussion
Morphological observations of boletus isolates (table 1) were carried out after purification and were observed during the 8th day. Some isolates have a fast growth and some are slow. Fast growth is characterized by isolates filling the 3rd day cup and some are slow growing until the 8th day.

The isolates obtained from isolation on bagasse and filter cake have different diversity, this is likely due to the nutrient content of the place where the fungus lives. Diversity is largely determined by the nutrient content of the substrate where the fungus grows [13], the type of soil, plants will affect the different number and types of isolates from various locations [14].

The isolates obtained from bagasse and filter cake all have the ability to produce IAA in very small concentrations. This can be seen from the change in color from red to easy red (pink) from each isolate compared to controls (yellow). This is consistent with the results of Abri, et al. [15] research in which the rhizosphere fungi isolates tested showed a change in pink color after administration of the Salkowski reagent. The change in color occurred after the Salkowski reagent was given a reaction with IAA and IAA-forming compounds [4]. The reactions that occur Indole Acetic Acid (IAA) with salkowski reagents give a reddish to red color. This color change indicates the ability of fungi to produce IAA.

The results of quantitative testing both isolates from bagasse and filter cake showed the ability to produce IAA is different for each isolate. This difference in ability is probably caused by the type of isolate and nutrient content that exists in bagasse and filter cake. The ability to produce IAA from fungi is due to nutrient content [13].

IAA production measurements (table 3), were carried out after the isolates were incubated for 72 hours, during which hours the highest IAA production was produced in the logarithmic phase and the beginning of the stationary phase [15,16]. In this phase, the fungus will experience the stress of the medium environment, where the amount of carbon decreases, the medium becomes more acidic, so that the fungus experiences slow growth. This situation will activate certain genes in the fungus to
produce intermediate compounds into IAA. Quantitative T4 test results (table 3) produced the highest IAA production of 3.469 ppm compared to other isolates. The results of this measurement are in line with the results of qualitative measurements, where T4 isolates provide dark red changes. Changing the color of pink to dark red indicates the higher IAA content produced [18].

The highest IAA production from the results of this study is lower when compared to the results of the study of [18] which produced 93.4 ± 1.9 ppm in the fungus *Penicillium* sp and Astriani et al. [20] which produced 71.00 ± 0.70 in the RPL3-10T4 isolate isolated from peat soil, but was higher than the yield Research by Abri et al. [15] which produced 2,190 mg / L from the isolation of aromatic rice rhizosphere fungi.

The low IAA production produced in this study is probably caused by fungal species factors, nutrient content and environmental factors. This is consistent with what was said by Frankenberger and Arshad. [20] that the production of IAA by microorganisms varies greatly depending on species, growth rates, environment and availability of substrates such as amino acids. According to Bose et al. [22], IAA production produced by microorganisms is strongly influenced by growth rate, tryptophan concentration, carbon source, incubation time, supernatant culture, agitation and dissolved oxygen, while Retnowati and Wiraningsi [23] stated that different IAA production is influenced by the physiological characteristics of host plants and the available nutrient content can be used by fungi such as protein and carbohydrates. In addition, IAA production by fungi is caused by the presence of tryptophan compounds used. Tryptophan is a source of nitrogen, energy and carbon in the process of carbohydrate metabolism in microorganisms to promote growth [24].

5. Conclusions

The results of the isolation of fungi from bagasse and filter cake have morphological diversity. Qualitative testing of all isolates tested both from bagasse and filter cake were able to produce IAA hormone. Quantitative testing on all bagasse isolates produced an average IAA hormone that is 0.115-3.469 ppm and on all isolates that produced IAA hormone production was 0.047-1.547 ppm. The highest IAA production produced T4 isolates of 3.469 ppm and fungi isolates which produced the lowest IAA hormone B21 isolates of 0.047 ppm. T4 isolates from sugarcane bagasse have good potential to be developed for use in plants.

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