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Growth Rate and Indole Acetic Acid Production of Several Fungal Rot Isolates

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Abstract: ***This paper discuss about the growth rate and production of Indole Acetic Acid (IAA) by several fungal rot isolates collected from decayed cacao plants. Growth rate of isolates was revealed on media Malt Peptone Agar (MPA), on petridish (ø 9 cm), measured based on the colony diameter daily until 7 days.BSA isolate reaches a maximum diameter on day 3, while the other isolates reached the maximum growth on the day4th and 7th. The production of IAA was examined on Pikovskaya broth media and measured using spectrophotometer. The study showed that the fungal rot have ability to produce Indole-acetic acid (IAA). The IAA concentration range between 0.349-2.794 µg l^{-1}. The BSA, BPB, and BSF isolates had the highest concentration of IAA.***

Keywords: Growth, Indole Acetic Acid, Fungal Rot, Cacao Plant

1. Introduction

The white-rot and brown-rot fungi belong to the few organisms in nature that are capable of degrading wood (Heinzkill et al., 1998). White-rot fungi such as Trametes (Coriolus) versicolor (Wulf.: Fr.) Quél. andPhanerochaetechrysosporiumBurdsall are known producers of lignolytic enzymes that are involved in the natural delignification of wood (Call &Mücke, 1997; Poppius-Levlin et al., 1997).

Decomposer fungi help the availability of plats nutrients. The biological nutrient availability can occur through increased access to the crop nutrient for example by arbusculermycorrhiza, dissolving by microbial phosphate, as well as decompose by actinomycete, fungi, or earthworms.Provision of nutrient takes place through the connection simbiotis or nonsimbiotis. In a symbiosis with certain plant groups or with most plants, while nutrient absorption occurs through nonsimbiotis results by dissolving solvent by microbial phosphate groups, and the resulting shake-up of the organic material by groups of decomposer organisms.

Microbes have the ability to change something, especially in elaborate waste into compost. In addition to giving the intake of nutrient for plants, microbes also serves to produce the hormones to grow. The hormones produced, among others, are auxin and Gibberelin.Indoleacetic acid (IAA) is a molecule that is synthesized by plants and a few of microbes. In plants, IAA plays a key role in both root and shoot development(Reeta Prusty, 2004). GA was isolated from *Gibberellafujikuroi*by Yabuta and Sumiki (1938), and IAA was isolated from *Hizopussuinus*by Thinmann in 1935. GA, is the dominant component of the gibberellin complexes isolated from fungi (Hasan, 2002),Bozhkova et al, 1991, Baca and Elmerich, 2007).

2. Materials and Methods

2.1. Isolation of Fungal Rot

Fungal rotisolates was obtained from decayed cacao stems in the Bila village of PituRiase, Sidrap district, South Sulawesi, Indonesia. Aftersubculturing and purification, isolates are then coded according to the name of place of origin.

2.2. Growth Ability in MPA Media

Observation on growth rate of isolates was revealed on media Malt Peptone Agar (MPA), on petridish (ø 9 cm). The 2 mm mycelium disk of each isolates was cultured on MPA. (15 g of malt extract, 20 g glucose, 5 g of Peptoneand 16 g of agar/ L of destilled water). Isolates were incubated at room temperature. Growth rate ofeach isolates was measured based on the colony diameter daily until 7 days.

2.3. Extract preparations

Fungal rot isolates were sucultured on PDA (Potato Dextrose Agar) andincubated for 7 days. Five disks of fungal colony put on to liquid medium Potato Dextrose Broth (PDB) and incubated at 28 ^{o}C in a shaker with 150 rpm/min for 7 days, and centrifuged at 5000 rpm for 25 min. The supernatan transferred in to new flask and the pellet was removed.

2.4. Production of indole acetic acid (IAA)

Production of auxinindole -3-acetic acid (IAA) by bacteria was tested using nutrient brothand Salkowski reagent (Gutierrez *et al*, 2009). Fungal rot isolates cultured in PDB enriched with L-tryptophan (0,1g l^{-1}) and incubated at room temperature in the dark for five days. The supernatant was taken aftercentrifugation in 5000 rpm/min.One ml of the supernatant was added to one ml of Salkowski reagent (12 g l^{-1}$FeCl_3$ in 429 ml l^{-1}H_2SO_4) (Gintinget *al, 2006)* and incubated in dark for 24 hours at room temperature. Theintensity of developed pink colour was read at 535 nm using a UV-VIS spectrophotometer. From a standard curve prepared with known concentration of IAA, the quantity in the culture filtrate was determined and expressed as mg l^{-1}.

3. Result and Discussion

3.2. Growth of Fungal Rot Isolates on Malt Peptone Media

Previous studies conducted by growing the tenth such isolates on 3 different types of media, namely Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Malt Peptone Agar (MPA)(Rahim et al., 2015). All of the three tested media were rich in essential nutrients needed for growth and development of fungal rot. PDA medium have a carbohydrate content of nutrients, water, and protein derived from potato, glucose, substrates and in order. MEA medium has a composition of nitrogen, carbohydrates, sodium chloride, and so on. While the media MPA has nutritional nitrogen, carbohydrates, sodium chloride, agar, and pepton. Carbon compounds have two functions, the first for the metabolism of other heterotrophic organisms as mushrooms (Cochrane, 1958). Optimal growth of the fungal rot isolates obtained by using MPA. BSA isolate reaches a maximum diameter, or fillful the petridish (9 cm) on day 3, while the other isolates reached the maximum growth on the day 4th and 7th (Fig.1)

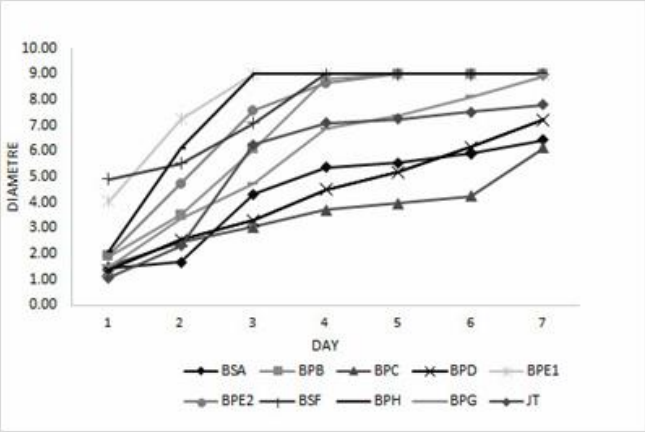

Figure 1: Colony diameter of ten fungal rot isolates on Malt Peptone Agar (MPA) 7 days after incubation

Peptones are the most widely used source of nitrogen in microbial media. Some are made by cooking milk or meat products in acid, but most are made by incubating milk or meat with trypsin, pepsin, or other proteolytic enzymes to digest the protein to a mixture of amino acids, peptides, and polypeptides. Many microbes, called proteolytic, can digest proteins, but most can't. The choice of peptone is sometimes of importance (Eddleman, 1999).

3.3. Production of Indole-Acetic Acid (IAA)

The fungal rot isolates were screened for their ability to produce plant growth regulator, IAA. IAA production was recorded with concentrations of tryptophan 0, 1 mg l^{-1}. All of fungal rot isolates have ability to produce IAA in absorbanceλ =535 nm.

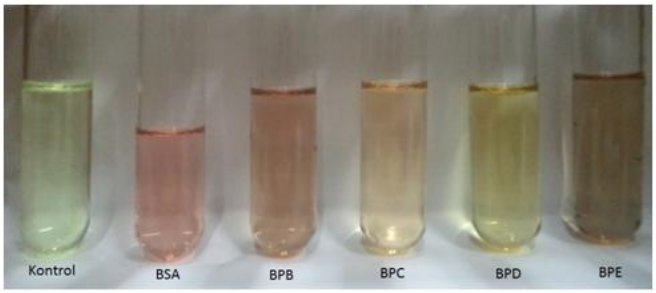

Figure 2: The ability to produce IAA by fungal rot isolates from cocoa cultivation with precursorL-Tryptopan 0.1 mgl^{-1} in Salkowski reagent. Changes colorsupernant to pink indicatesabilityto produce IAA

According to Herter (1908), the reactant salkowski can be used to detect the hormones of IAA on an organism; it also can be used to know the behaviour of acidity of organism. Crozier et al (1988), adding that salkowski reagent used for calculating the IAA after medium culture centrifuged. Gordon and Weber (1950), high stability and density of color after addition of reactant will determine the high degree of concentration of the IAA. The sensitivity and stability of reaching balance, through an increase in the reaction of some other indole compounds. This indicates that the presence of hydrogen peroxide oxidation reaction enlarge and speed up the formation of the colors and the possibility of increasing the intensity (Suriaman, 2010).

The production of IAA by fungsl rot have concentration range between 0.349-2.794 µg l^{-1}(Table 1).The higher of IAA concentration were produce by BSA isolate, followed by BPB and BSF isolates. The lowest concentration of IAA by BPE2 isolate.

Table 1: Production of IAA by of fungal rot isolated from cacao cultivation

Isolates	*Absorbance*	*Concentration (µg/l)*
Blanko	0.000	-0.206
BSA	0.189	2.794
BPB	0.171	2.508
BPC	0.081	1.079
BPD	0.062	0.778
BPE1	0.151	2.190
BPE2	0.035	0.349
BSF	0.167	2.444
BPG	0.106	1.476
BPH	0.127	1.810
JT	0.126	1.794

IAA represents one of the most important plant hormones, regulating many aspects of plant growth and development throughout the plant cell cycle, from cell division, cell elongation and differentiation to root initiation, apical dominance, tropistic responses, flowering, fruit ripening and senescence. Regulation of these processes by auxin is believed to involve auxin-induced changes in gene expression (Guilfoyle*et al.*, 1998, B.E. Baca And C. Elmerich, 2007).

The results of the test in calorimetric using a spectrophotometer, suggests that culturing the fungal rot on supernatan tested the hormone producing IAA different absorbance value of each isolate. Gosh and Basu (2002) in the Kumari et al (2008), stated that the production of hormones IAA can be known on supernatan culture and the presence of carbon on the medium affect hormone production.

4. Conclusion

Present study showed that the fungal rot have ability to produce Indole-acetic acid (IAA). The IAA concentration range between 0.349-2.794 µg l^{-1}. The fastest isolate reaches a maximum diameter is BSA. BSA, BPB, and BSF isolates had the highest concentration of IAA.

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