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Capability of Rot Fungus Isolates from Oil Palm Empty Bunches in the Production of Indole Acetic Acid (IAA)

Sukriming Sapareng[1*], Ambo Ala[2], Tutik Kuswinanti[3] and Burhanuddin Rasyid[4]

[1]Post Graduate Program of Hasanuddin University, Faculty of Agriculture, Andi Djemma Palopo University, South Sulawesi, Makassar, Indonesia
[2]Department of Agronomy, [3]Department of Plant Pests and Diseases, [4]Departement of Soil Science, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia
**Corresponding author*

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ABSTRACT

This study focused on the growth rate and production of the Indole Acetic Acid (IAA) hormone and the ability of rot fungus to dissolve phosphate. A total of 15 rot fungi isolated from oil palm empty fruit bunches (EFB). The growth rate of isolates identified on Potato Dextrose Agar (PDA) which have been incubated for 7 days on a petri dish (ø 9 cm) with a temperature of 30 °C, measured by the diameter of the colony every day. Isolates TK-14 reaches maximum diameter on the 3rd day, while the other isolates achieve maximum growth in the 4th and 7th. Out of the 33 isolates of the fungus, 15 isolates were found to have the ability to produce hormones IAA. IAA production tested at Pikovskaya Broth media and measured using a spectrophotometer. Rot fungus has the ability to produce IAA with the concentration range of 0.048-8.429 mgl^{-1}. Isolates TK-8, TK-2, and TK-14 have the highest concentration of IAA. Similarly, the ability to dissolve the phosphate also varies between 5.272-10.620 mgl^{-1}. Three isolates were recommended to degrade the oil palm empty fruit bunches in future studies.

Introduction

Empty Fruit Bunch (EFB) are very abundant palm oil mill waste. Each processing of 1-ton fresh fruit bunches will produce EFB as much as 22 - 23% EFB or as much as 220 - 230 kg EFB (Ishani and Benjamin, 2014). EFB has characteristics with large size, dominated by materials that are difficult to decay the cellulose, hemicellulose, and lignin and has a high C/N ratio. EFB is a lignocellulosic material containing 25% lignin, 50% cellulose and 25% hemicellulose in the cell wall. In recent years, EFB has been used as fuel to produce steam in the palm oil industry. EFB burning causes serious and high environmental concerns. Currently, EFB is used as fertilizer on agricultural land to control weeds, maintain moisture and soil erosion (Thambirajah *et al.*, 1995; Umikalsom *et al.*, 1997; Molla *et al.*, 2004; Bari*et al.*, 2009; Misson *et al.*, 2009).

Indole acetic acid (IAA) is an important compound for the growth and development of roots and shoots, many microbes including Plant Growth Promoting Rhizobacteria (PGPR) produce IAA (Strobel, 2004). Auxin

was first isolated and characterized as plant growth and IAA is an auxin-type (Nakamura*et al.,* 2006). IAA was commonly known as the main hormone of plants that stimulate the development of various types of cells. Microorganisms, including bacteria and fungi, are also capable of simulating IAA (Tsavkelova *et al.,* 2006). Auxin, especially indole-3-acetic acid (IAA), is a growth hormone that has a profound effect on plant growth and development (Zhao, 2010).

Auxins affect almost all stages of plant development and these hormones are necessary for survival.

Auxins play the role of regulation in root growth (Casimiro *et al.,* 2001), apical dominance (Booker *et al.,* 2003), phyllotaxis (Reinhardt*et al.,* 2003), vascular differentiation (Aloni *et al.,* 2006), and fruit development (Gillaspy *et al.,* 1993). It has also been reported that auxin, along with gibberellic acid, is capable of increasing the size of the cell Chlamydomonas reinhardtii and the number of cells produced during cell division (Park*et al.,* 2013).

Most of the genus *Trichoderma* produces IAA, with or without L-tryptophan precursors. *Trichoderma* isolated from the rhizosphere is more efficient in producing IAA than *T. asperellum* T211 (Resende *et al.,* 2014). IAA produced by *Pseudomonas aeruginosa* MR-9 increases plant height, dry weight, number of nodules per plant, fresh weight nodules *Mucuna pruriens* of 184, 124, 139, 180% compared with controls (Deshwal*et al.,* 2011; Deshwal and Kumar, 2013).

The main purpose of this study was to obtain mushrooms from EFB that have the ability to produce IAA hormones and dissolved phosphorus.

Materials and Methods

Isolation and Growth of Fungi

The fungus was isolated on EFB at the Luwu 1 Palm Plantation Plant, Lagego Village, Burau Subdistrict, East Luwu Regency, South Sulawesi Province, Indonesia. Examples of fungi obtained are stored in paper bags, and then taken to the laboratory for isolation. Isolates were further purified and reproduced on Potato Dextro Agar (PDA).

The first stage is sterilization, both tools and materials used. The scissors are cleaned with 70% alcohol, and then some hymenium fungus (where the basidiospores are formed) is cut in size 1 x 1 cm.

The piece was immersed in sterile water, then immersed in 70% alcohol, then immersed in sterile water, then placed on sterile filter paper in the petri dish that had been cured, and then the pieces were incubated at room temperature (28-30 °C) for 7 days.

The growing mushroom colony was cut on the side of the colony and transferred to a petri dish containing PDA media. The growth rate of isolates was seen in PDA media incubated for 7 days in a Petri dish (ø 9 cm) with a temperature of 30 °C, measured daily colony diameter.

Production of indole acetic acid (IAA)

The ability to rot fungi in producing IAA hormones is done using the methods used by Bhagobathi and Joshi (2009). The supernatant of the moldy fungus mixed with Salkowsky reagents was then incubated for 20 minutes. Observations of discoloration before absorbance were measured using a spectrophotometer with a wavelength of 535 nm. The IAA concentrations of each isolate were compared with the standard curve.

The ability of phosphate solvent

Isolates were tested for their quantitative ability in phosphate solvent by using Pikovskaya media broth with $Ca_3(PO_4)_2$ as a source of phosphate. The ingredients of the Pikovskaya broth medium are 10 g glucose; $Ca_3(PO_4)_2$ 5 g; $(NH_4)_2$ SO_4 0,5 g; $MgSO.2H_2O$ 0,1 g; $MnSO_4$ 25 mg; $FeSO_4$ 25 mg; KCl 0.2 g; yeast extract 0.5 g; and agar 15 g, dissolved in sterile water until volume 1 l. Pipette 30 ml suspension and put into Erlenmeyer, Pikovskaya broth medium, and incubated in 150 rpm rotary shaker for 7 days.

Filtered 20 ml with Whatman no. 42. The filtrate was centrifuged at 1000 rpm for 15 min, 5.0 ml of supernatant then poured into the test tube, added with 0.5 ml of concentrated P reagent (12 g of ammonium molybdate, 0.277 g potassium toxoltartate) and concentrated dyeing Reagents (0.53 g ascorbic acid), shake for a few minutes, and let stood for 30 minutes. The absorbance of the solution was measured by a spectrophotometer at a wavelength of 693 nm. In the same way, it is done in Erlenmeyer flask containing mushroom and Pikovskaya broth media inoculated as the control.

Results and Discussion

Growth of fungus

A total of 15 fungi were isolated from EFB. The isolate growth rate was seen in PDA media incubated for 7 days in a petri dish (ø 9 cm) with a temperature of 30 °C, measured by daily colony diameter. The TK-14 isolate reached maximum diameter on day 3, while the other isolates achieved maximum growth on day 4 to 7 (Fig. 1).

Isolates grown on a PDA medium have different viability. On the second day, the fastest growth was seen in isolate TK14 with colony diameter of 6.80 cm, while the slowest growing capacity occurred in TK4 isolate, which was 1.82 cm (Figure 1). Differences in the growth period in each isolate is thought to occur due to differences in adaptation processes required by each isolate as well as various other factors such as environmental conditions. Different isolates will cause different growth rates, virulence, and colonization (Zhao, 2010; Deshwal *et al.*, 2011) and will determine the success of storage of isolates (Nagaiet *et al.*, 2000).

Production of IAA and phosphate solvent

Isolate fungal mushroom produces IAA plant growth hormone at tryptophan concentration of 0.1 mgl^{-1} and absorbance λ= 535 nm. The molding fungus has the ability to produce IAA with a concentration range of 0.048-8.429 mgl^{-1}. The TK-8, TK-2, and TK-14 isolates had the highest IAA concentrations with values of 1.175 mgl^{-1}, 3.063 mgl^{-1} and 8.429 mgl^{-1} (Table 1). The endophytic fungi isolated from aromatic rice also yielded IAA of 0.635 to 2,651 mgl^{-1} (Syamsia *et al.*, 2015). In addition, previous studies reported that there were 10 fungal isolates from cocoa skin waste resulting in IAA varying between 0.349 .794 µg l^{-1} (Iradhatullah *et al.*, 2015). IAA has many different effects, such as inducing elongation and cell division of plant growth and development.

The addition of IAA (10^{-10} M) to the nutrient solution can relieve Zn and Pb stress on sunflower. In particular, IAA reduce the influence of negative metals on roots, shoot dry weight, root length, root volu and root surface area (Erika *et al.*, 2010). Inoculation of plants with PGP and producing IAA can be a way to reduce salt stress, stimulate plant growth and prote crops from root rot disease in cotton plants under saline soil conditions (Egamberdieva *et al.*, 2015).

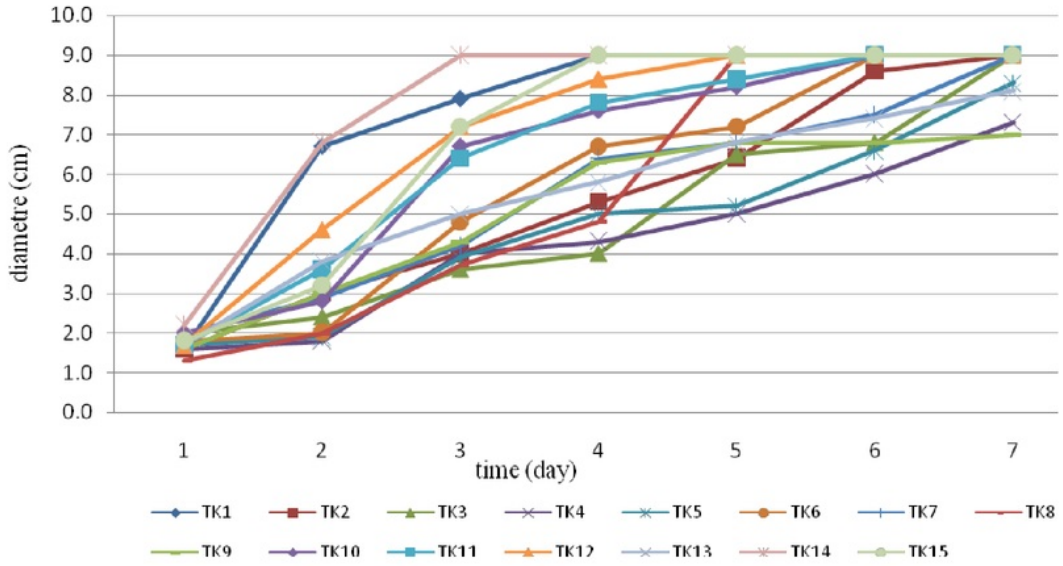
Table.1 Production of IAA from rotting fungus isolated from EFB

Isolates	Absorbance (l)	IAA Concentration (mgl^{-1})
KS1	0.048	0.556
KS2	0.206	3.063
KS3	0.016	0.048
KS4	0.029	0.254
KS5	0.082	1.095
KS6	0.035	0.349
KS7	0.032	0.302
KS8	0.087	1.175
KS9	0.034	0.333
KS10	0.029	0.254
KS11	0.033	0.317
KS12	0.017	0.063
KS13	0.026	0.206
KS14	0.544	8.429
KS15	0.028	0.238

Table.2 The ability of fungal isolates dissolves phosphate

Isolates	Absorbance (l)	Concentration (mgl^{-1})
KS1	1.059	5.271
KS2	1.665	8.427
KS3	1.745	8.844
KS4	1.464	7.380
KS5	2.007	10.208
KS6	1.927	9.792
KS7	1.854	9.411
KS8	2.078	10.578
KS9	2.045	10.406
KS10	1.995	10.146
KS11	1.811	9.188
KS12	1.673	8.469
KS13	1.708	8.651
KS14	1.986	10.099

Fig.1 Clonic diameter of fifteen fungal isolates on PDA media, 7 days after incubation



Quantitative measurements using a spectrophotometer at a wavelength of 693 nm indicate that the fungal isolates have the ability to dissolve phosphates varying from 5,271 - 10,620 mgl^{-1} (Table 2).

Fungal isolates have the ability to produce IAA with concentration ranges from 0,048 – 8,429 mgl^{-1}. TK-8, TK-2, and TK-14 isolates have the highest IAA concentrations. The ability to dissolve phosphates also varies from 5,271 to 10,620 mgl^{-1}. The three isolates are recommended to decompose empty palm oil bunches in subsequent research.

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