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The Potentiality of Forest Litter Fungi as IAA (Indole-3-Acetic Acid) Producer

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Abstract

Many industrial forest in Indonesia are replanted under intensive silviculture program. This program includes utilizing of soil microorganism for promoting better plant growth. Preliminary study was done on the exploration of forest litter fungi that are able to produ 2 IAA. Six thermotolerant fungi (*Aspergillus* sp B.10, *Aspergillus* sp 20.2, *Penicillium* sp 4.2, *Aspergillus* sp 9.2, *Penicillium* sp 1.2 and *Trichoderma* sp B.2) have been isolated from forest litter. All isolates produced IAA with various concentration, ranges from 15.56 ppm to 76.81 ppm. Two isolates (*Penicillium* sp 1.2 and *Trichoderma* sp B.2) which produced the high IAA contents were further examined. Bioassay of 10^{-2} ppm, 10^{-4} ppm and 10^{-6} ppm of crude extract IAA using mung bean (*Vigna radiata*) seedling indicated that the extract only significantly effected on the number of lateral root. Filtrate from those isolates induced lateral root formation more than those of IAA standard on the same concentration.

Keywords: *IAA, fungi, plant growth*

BACKGROUND

Intensive silviculture program implemented to achieve Indonesia's forests healthy, sustainable and prospective. This program was developed in the Industrial Plantation Forest (HTI). Procurement of quality seeds are given priority in this program. One of the seeds that interest to developed is meranti (*Shorea*).

Intensive silviculture component that are being developed is the utilization of forest plants and litter microbe to support the growth of plant seedlings. This could be supported by examining the potential of microbes to enhance growth of seed plants. Hindersah and Tualar (2004) states that the microbes capable as agents that influence on the plant growth increase (plant growth promoting agents). These microbes produce a variety of growth hormone (GH), vitamins and organic acids.

Some fungi 9 pable to produce GH. Several species of *Fusarium* (Hasan 2002) and *Sclerotium* (Sarma et al. 2002) has been known capable to produce auksin (IAA). IAA produced by fungi as s 5 condary metabolites (Ünyanyar 2000). IAA is a common product of the metabolism of L-tryptophan in several microbes (Ahmad et al. 2004).

Most fungi from the litter exist as decomposers that require capability to growth on high temperature conditions (thermotolerant), grow at thermophylic temperatures 60 ^{0}C or more (Maheswari et al. 2000), at decomposition process (Sylvia et al. 2005).

Fungi play a role in the decomposition of litter organic matter (Kurzatkowski et al. 2004).

Litter from planting area in some plantation in East Kalimantan and Central Kalimantan is often used in Dipterocarpaceae breeding process. Plants that were given litter from the planting area grow better than plants that were not given (Tjitrosoemitro S in December 2009, personal communication). The nutrients from leaf litter can improve seedling growth of Dipterocarpaceae (Brearly et al. 2003).

The study of the fungi that have potentiality to produce IAA not much done. Therefore, research of the fungi from plant litter in producing IAA need to be conducted.

METHOD

Fungal Isolation

0.1 grams litter add into sterile aquades of 99 ml in 250 ml Erlenmeyer. Heat treated of the suspension was done in the water bath at 60 ^{0}C for 15 minutes. 1 ml sample

*enicillium*sp 9.2, *gillus**Asper*sp 4.2, *Penicillium*sp 20.2, *Aspergillus*sp B.10, *Aspergillus* fungi hermotolerance Six types of t

and incubated at 60 ^{0}C. Colonies that grew then purified, and identified to genus according to Burnet and Hunter(1972).

IAA Assay

Crude extract IAA production

Stock cultures of fungi (7-10 days) on PDA medium used as working cultures. Three pieces of culture work inoculated into 100 mL of Czapek Dox liquid medium with 1% pepton. Cultures incubated at shaker machine for 7 days. Filtrate separated from the biomass fungi by centrifugation at 4500 rpm for 30 minutes. The filtrate used as a source of ZPT (IAA crude extract).

IAA content was detected by adding 4 ml Salkowsky reagent (400 ml H_2SO_4, 20 ml 0.1 M $FeCl_3$, sterile aquadest 580 ml) into 2 ml of the filtrate (Hasan 2002). The change of the color into pink indicates the IAA. OD read using spectrophotometer with 500 nm wavelength. IAA concentration (ppm) obtained through the conversion of IAA standard curve (Ahmad et al. 2005). Two isolates that produce the highest concentrations of IAA to be further investigated its influence on the growth of mung bean sprouts (*Vigna radiata*) as the plant test.

Bioassay IAA

Sterilized seeds of mung bean with chlorox 3% for 3 minutes and rinsed with sterile water. Mung bean seed was grown until the first leaves appear (approximately 7 days) and treated with crude extract IAA. Seeds grown in liquid culture in a sterile jar containing the crude extract IAA with different concentrations (10^{-2} ppm, 10^{-4} and 10^{-6} ppm). Growth of seedlings in aquadest used as negative control.

RESULTS

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Those six isolates able to produce IAA (from 15.56 ppm to 76.81 ppm, table 1) when grown in Czapex Dox medium with 1% pepton.

Table 1. IAA content of crude extract of some isolates

Isolate	IAA Crude extract (ppm)
Aspergillus sp. B.10	15.56
Aspergillus sp. 20.2	19.21
Penicillium sp. 4.2	19.52
Aspergillus sp. 9.2	24.10
Penicillium sp. 1.2	32.23
Trichoderma sp. B.2	76.81

Isolates that produce filtrate with the highest IAA contents (*Trichoderma* sp B.2 (Fig. 1a) and *Penicillium* sp 1.2 (Fig. 1b)) are

used for bioassay. Crude extracts from the fungus only affects in the number of lateral roots (Table 2). Standard IAA was also only affects the number of lateral roots. Crude extracts IAA from the filtrate in general gives a better effect on the number of lateral roots compared with standard IAA. The number of lateral roots varied from 29.5 to 73.17.

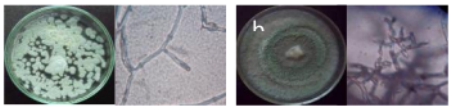

Figure 1 colony isolates on PDA medium and microscopic cross-section of *Penicillium* sp 1.2 (a) and *Trichoderma* sp B.2 (b) on 1000x magnification.

Table 2. The influence of IAA on lateral root number of mung bean seedling 3 weeks after planting

Isolate (source)	IAA (ppm)	Average of lateral root number
Penicillium sp 1.2	10^{-2}	60 f
	10^{-4}	73.17 g
	10^{-6}	53.67def
Trichoderma sp B.2	10^{-2}	57.17ef
	10^{-4}	60.83 f
	10^{-6}	49.33de
standar IAA	10^{-2}	32.14ab
	10^{-4}	40.00bc
	10^{-6}	46.00c
Sterile aquadest	0	29.50a

The numbers which followed the same letter is not significantly different according to Duncan's test results on P <0.05.

The influence of the IAA content on fungal filtrate (crude extracts IAA) on lateral root number varies by fungal tipe. Crude extracts IAA from *Penicillium* sp 1.2 stimulate lateral root growth better than crude extracts IAA from *Trichoderma* sp B.2. 10^{-4} ppm of crude extract IAA of *Penicillium* sp 1.2 is the optimum concentration that encouraging lateral roots growth. Generaly, the number of lateral roots produced by treatment with crude extract of the fungus filtrate IAA better when compared with standard IAA.

DISCUSSION

Isolation of forest litter fungi were performed at temperature of 40 ^{0}C and 60 ^{0}C to obtain the fungus that survive at high temperatures. The six fungi survive when grown at high temperatures, these fungi are thermotolerant (Maheswari et al., 2000). The ability of this fungus lives in the high temperature will facilitate adaptation to the thermophilic phase in composting process.

IAA Production Capabilities

IAA concentrations of crude extracts from six thermotolerant fungi varied between 15.56 ppm to 76.81 ppm. The isolates potential to be developed as IAA producer. 10^{-4} ppm crude extract IAA and 10^{-6} ppm IAA standard provide optimum effect in the number of lateral roots, at each treatment. Auxin encourage the growth of lateral roots and root hair development (Casimiro et al., 2001) and increase the number of lateral roots (Woodward and Bartel, 2005).

Treatment with crude extracts of all IAA concentrations from *Penicillium* sp 1.2 and *Trichoderma* sp B.2 generally give better results when compared with standard IAA at the same concentration. Possibility this is occur because the filtrate contain amount of IAA and also other organic compounds that affect the growth of lateral roots, whereas IAA standard consisted of pure IAA components.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSION

All fungal isolates from forest litter

(*Aspergillus* sp B.10, *Aspergillus* sp 20.2, *Penicillium* sp 4.2, *Aspergillus* sp 9.2, *Penicillium* sp 1.2 and *Trichoderma* sp B.2) is thermotolerant and capable to producing IAA in crude extract of various concentrations (15.56 ppm -76.81 ppm). *Penicillium* sp 1.2 and *Trichoderma* sp B.2 filtrate produce the highest concentration of IAA . 10^{-4} ppm crude extract IAA from *Penicillium* sp 1.2 significantly affected to increase the number of lateral roots.

Further research on the extraction and utilization of fungal isolates for plant growth needs to be done

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