Antagonistic evaluation of *Trichodermaviride* and *Aspergillusflavus* against wood-decay fungus *Pleurotusostreatus*

Erwin, E Iskandar, N Husien

Faculty of Forestry, Mulawarman University
Kampus Gunugn Kelua Jl. Penajam,Samarinda (75119), Kalimantan Timur, Indonesia

E-mail: erwin@fahutan.unmul.ac.id

Abstract. *Pleurotusostreatus* cultivation is often limited by the presence of antagonistic microorganisms that can also grow together on a variety of substrates that are used as growth media of *P. ostreatus*. As a preliminary study, this research aims to evaluate the antagonism of*Trichodermaviride* and *Aspergillusflavus* in interacting with the *P.ostreatus* fungus by in-vitro. An antagonism test using a dual culture method of these fungi with 1 month incubation time on Poteto Dextrose Agar (PDA) media was performed. Evaluation of the antagonistic properties of these fungi is conducted through macroscopic and microscopic observations. Macroscopically, the growth of *T. viride* and *A. flavus* mycelium showed that both colonies were more dominant than *P. ostreatus* colonies. Microscopically, hyphae of *T. viride* and *A. flavus* indicate the presence of very strong micoparasitic properties that characterized by attachment and convolution of both hyphae to *P. ostreatus* hyphae. It was concluded thatthe growth of *T. viride* and *A. flavus* fungi was more dominant and had potential as an inhibiting agent of *P. ostreatus* fungal growth, respectively, in dual culture on PDA media.

1. Introduction

Pleurotusostreatus, has long been recognized as one of the most widely used wood fungi (mushroom) for food because of its high levels of protein, minerals and vitamins [1]. Naturally, this fungus is not only grows on dead wood but also easily cultivated on various agricultural industry and residual material substrates, and other agriculture-cellulosic materials [2] because they produce oxidative enzymes [laccase and manganese peroxidase (MnP)] and hydrolytic (cellulase, xylanase and tannase) [3].The use of different substrates is able to effect on biological and economic yield of *P. ostreatus* (oyster mushroom) cultivation [4].

However, it is common to find problems in the cultivation of the *P. ostreatus* mushroom, one of which is the existence of contamination on the growth substrate media which regularly found, so that variations of heat treatment are required to eliminate the growth of other fungi such as *Trichoderma* sp., *Altemaria* sp., *Aspergillus* sp., *Fusarium*sp ., *Monilia* sp., *Mucor* sp., *Rhizopus* sp. etc.[5]. The growth of these other fungi as a competitor often leads to impaired growth of *P. ostreatus* so that it can reduce the production of its cultivation.

In the same habitat, it is well known that mushrooms have mutually supportive relationships and inhibit the growth of one another in obtaining nutritional sources for its survival. Schmidt[6] notes that

the inhibiting growth relationship or better known as the antagonism (competitive reciprocal effect) is based on production of toxic metabolites,on mycoparasitism, and on nutrient competition.

In the effort of handling *P. ostreatus* growth disorders, the initial information required on how the characteristics of interdependent or antagonistic relationship between *P. ostreatus* with competitors in a substrate. Therefore, antagonistic studies of fungi were performed using two species of antagonistic fungi, *Trichodermaviride* and *Aspergillusflavus* to find out the interaction pattern of each of these fungi in inhibiting the growth of *P. ostreatus* fungus by in-vitro.

2. Materials and methods

2.1. Fungal isolates and culture media

The fungal isolates used were *Trichodermaviride*, *Aspergillusflavus* and *Pleurotusostreatus* stored in the collecting of the Forest Protection Laboratory of Faculty of Forestry, Mulawarman University, Samarinda.

Potato Dextrose Agar (PDA) material is made aseptically in laboratory following the procedure ofHiMedia[7]. PDA is used as a culture media for fungal growth of *P. ostreatus*, and antagonistic test as well.

2.2. Antagonistic test

An antagonistic test using a dual culture method of Bruce and Highley[8] on PDA media was performed on *T. viride* and *A. Flavus* against *P. ostreatus*, respectively, with an incubation period of 1 month.

Materials and equipment used in the antagonistic test were conducted aseptically in Laboratory of Wood Biology, Faculty of Forestry, Mulawarman University, Samarinda.

*2.3. Observation*s

Interaction of two fungal colonies in vitrodual culture on PDA media wasexaminedon macro-and microscopic observations.

*2.3.1. Macroscopic observation.*In macroscopic observation,NIKON SMZ645 Stereoscopic Microscope at 10-30x magnification was used to evaluate mycelium growth and the contact limit between the two fungal colonies growing on the PDA media plates.

2.3.2. Microscopic observation. By using NIKON Eclipse E400 Light Microscope at 40-400x magnification, microscopic observation was made on the growth and interaction of the two fungal hyphae growing on the PDA media plates.

3. Results and discussion

3.1. Growth inhibition of Pleurotusostreatus by Trichodermaviride

Macroscopically, in the dual culture method, the growth of both fungi in one Petri dish at 1-2 weeks incubation showed *Tricodermaviride* colony with mycelium and greenish spores more dominant than *Pleurotusostreatus* colony with whitish mycelium (Figure 1a).

Since the beginning contact, *T. viride* colony dominates almost thoroughly the surface area of PDA media. On the contrary, the growth of *P. ostreatus* mycelium with very limited growth area,cover the surface of PDA media, even tend to grow in the opposite direction from the contact zone. In the contact zone, there is no clear boundary separating between the two fungal colonies (Figure 1b).

The dominance of *T. viride* colonies is not only from the fungal growth fully covering the surface of the PDA media but also the fungus grows on top of the *P. ostreatus* mycelium (Figure 1). This condition shows *T. viride*plays a role micoparasite[9] by utilizing the mycelium of *P. ostreatus* as a

source of nutrients other than obtained from the media. It is certain that with the dominant colonization, *T. viride* is antagonistic fungus to *P. ostreatus.*

The interaction stage of both colonies can also be shown by microscopic observations (Figure 2) in which appears to be attachment and convolution of *T. viride* hyphae on the *P. ostreatus* hyphae. The attachment is an early stage of theconvolution process, which, according toKlein Dand Eveleigh[10], is a frequent response between the *Trichoderma* spp. with other fungi in case of contact.

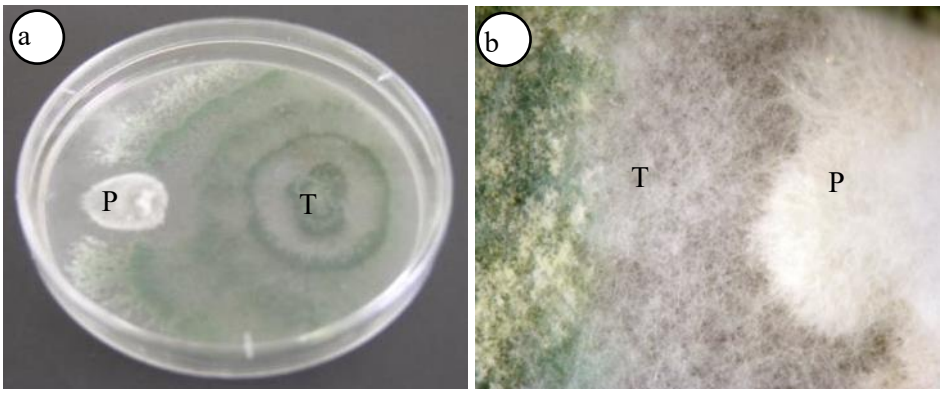

Figure 1. The dominant growth of the *Tricodermaviride* colony(T) against *Pleurotusostreatus* (P) on PDA dual culture media: (a) The overall surface of the media in Petri dishes; (b) At the contact boundary of the colonies' encounter [28 x magnifications].

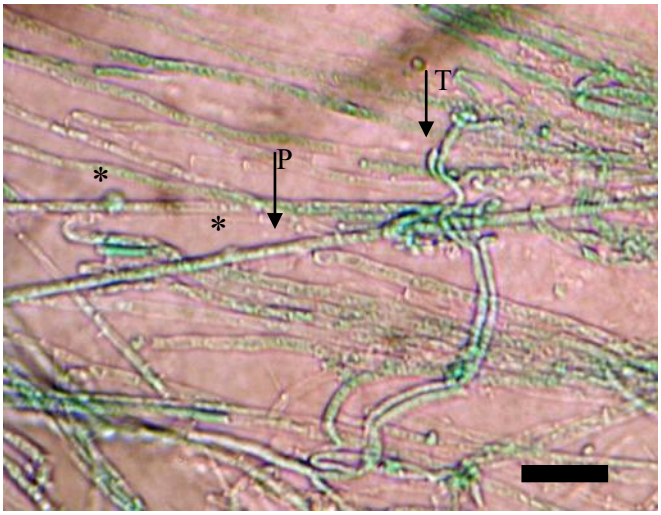

Figure 2. Attachment and convolution of*Tricodermaviride*hyphae (T) on*Pleurotusostreatus* hyphae (P) on dual culture of PDA media. Sign * denotes clamp connection of *P. ostreatus*hyphae, which is known as main characteristic of basidiomycetefungi [Bar = 20 µm].

Indication of the micoparasite process of *T. viride* as an antagonist fungus, according to Ranasingh *et al*[9], itis usually followed by releasing process of various enzymes such as chitinase, glucanase and pectinase.

Basically, the antagonistic properties are due to the ability of *T. viride* to produce extracellular enzymes capable of metabolizeon polycyclic aromatic hydrocarbons (PAHs) such as naphthalene, phenanthrene, chrysene, pyrene, and benzo[a]pyrene [11], which is indicate his dominating ability and

even the destructive structure of the *P. ostreatus* mycelium. Such event was also observed in dominating of other Tricoderma species, i.e. *T. citrinoviride,T. harzianum and T. Atroviride* associated with the *Camptomyia* pestagainst the mycelial growth of ashiitake mushroom, *Lentinulaedodes* [12].

3.2. Growth Inhibition of Pleurotusostreatus *by* Aspergillusflavus

The *Aspergillusflavus* mycelial colony also showed growth dominance fulfilling the PDA media against *Pleurotusostreatus* (Figure 3a). The condition also shows that the *P. ostreatus* mycelium could not develop as it could grow in mono-culture media.

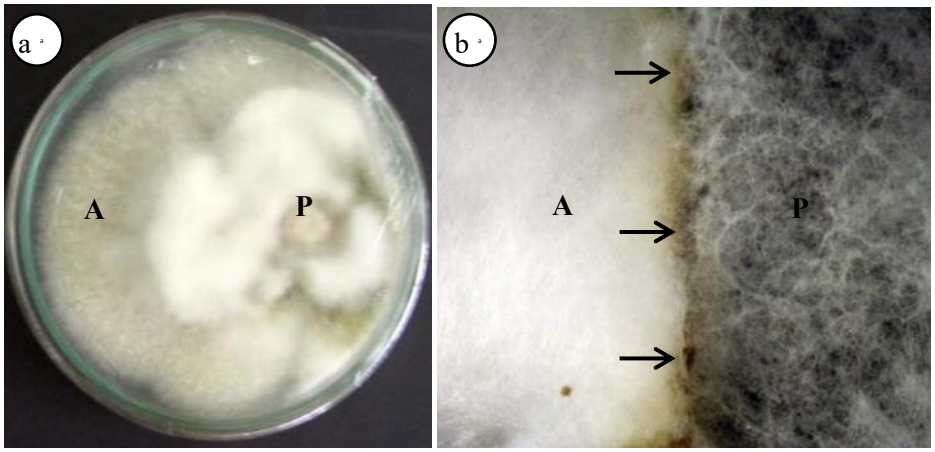

Figure 3.*Aspergillusflavus* colony (A) Dominates the surface of the media and covers the mycelium of *Pleurotusostreatus* (P) on PDA dual culture media method: (a) The overall surface of the media in Petri dishes; (b) At the contact boundary of the colonies (arrows) [28 x magnifications].

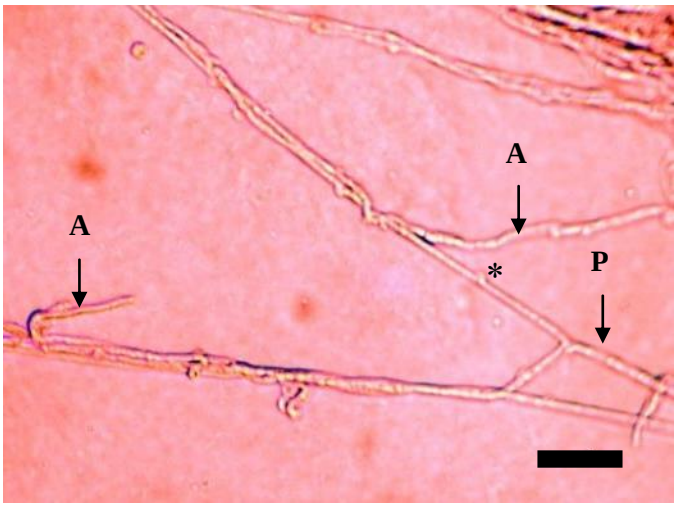

Figure 4. Attachment and convolution of *Aspergillusflavus* hyphae (A) to the *Pleurotusostreatus* hyphae (P) on dual culture of PDA media Sign * denotes clamp connection of *P. ostreatus* hyphae, which is known as main characteristic of basidiomycete fungi [Bar = 20 μm].

Covering of PDA media by *A. flavus* mycelium was also characterized by presence of a limiting zone that forms like a long line span on the second encounter of mycelium (Figure 3b). The properties

of *A. flavus*micoparasite on dual-culture media were demonstrated by exploiting *P. ostreatus*mycelium as a source of nutrients beside those that obtained from PDA. This could be indicative that for the fact that *A. flavus* has in common with *T. viride*, which acts as an antagonist fungus against *P. ostreatus*.

Microscopically, the antagonistic featuresare seen that *A. flavus* hyphae also attached and convoluted *P. ostreatus* hyphae (Figure 4). The antagonistic ability of *A. flavus*hyphae is related to the extracellular xylanase enzymes that released [13], which may play a role as a metabolic toxin [5] and also has the greatest cellulolytic ability [14] against *P. ostreatus* hyphaeso that its growth in PDA media becomes inhibited. Meanwhile, aflatoxinB_1 (AFB_1)as a secondary metabolite that produced by several members of Aspergillus fungi could be degraded by ligninolytic enzymes of *P. ostreatus*strains [15,16].

4. Conclusions

Based on results of this study, we conclude that*Trichodermaviride* and *Apergillusflavus* mycelial colonies dominate the *Pleurotusostreatus* mycelia colony in utilizing nutrient in dual culture of PDA media. Both of the antagonistic fungi are very powerful microparasite against *P. ostreatus* hyphae, thereforethe preventionand inhibition oftheirgrowth in media of *P. ostreatus* cultivation must be undertaken early.

Acknowledgements

The authors are grateful to Dr. Djumali Mardji of the Forest Protection Laboratory, Faculty of Forestry, Mulawarman University for valuable suggestions in microscopic analysis of fungi.

References

[1] Wahid M, Sattar A and Khan S 1988 Composition of wild and cultivated mushrooms of Pakistan *Mushroom in Trop.***8** 47
[2] Sa'nchez C 2010 Cultivation of *Pleurotusostreatus* and other edible mushrooms *Appl. Microbiol. Biotechnol.***85** 1321
[3] Luz J M R, Nunes M D, Paes S A, Torres D P, Silva M C S and Kasuy M C M 2012 Lignocellulolytic enzyme production of *Pleurotusostreatus* growth in agroindustrial wastes *Braz.J.Microbiol.*1508
[4] Girmay Z, Gorems W, Birhanu Gand Zewdie S 2016 Growth and yield performance of *Pleurotusostreatus*(Jacq. Fr.) Kumm (oyster mushroom) on different substrates *AMB Expr.* **6** 87
[5] Yildiz S, ü. Cafer, Derya-Gezer E and Temiz A 2002 Some lignocellulosic wastes used as raw material in cultivation of the *Pleurotus ostreatus*culture mushroom *Process Biochem.***38** 301
[6] Schmidt O 2006 *Wood and Tree Fungi:Biology, Damage, Protection, and Use*(Berlin: Springer-Verlag) p 334
[7] HiMedia 2016 *Potato Dextrose Agar M096* (Mumbai: HiMedia Laboratories Pvt. Ltd) p 2
[8] Bruce A and Highley T L 1991 Control of growth of wood decay basidiomycetes by *Trichoderma* spp. and other potentially antagonistic fungi *Forest. Prod. J***41** 63
[9] Ranasingh,Subhat A and NeduchezhiyanM2006 Use of trichodermain disease management. *Orissa Rev.* 68
[10] Klein Dand EveleighD E1998Ecology of trichoderma*Trichoderma and Gliocladium* (*Basic Biology, Taxonomy and Genetics v*ol 1) ed CP Kubicek and GE Harman (London: Taylor & Francis Ltd) chapter 3 pp 57-74
[11] Zafra G and Espinosa DVC 2015 Biodegradation of polycyclic aromatic hydrocarbons by Trichoderma species: a mini review *Environ. Sci. Pollut. Res.* **22** 1942
[12] Kim J Y, Kwon H W, Yun Y H and Kim S H 2016 Identification and characterization of Trichoderma species damaging shiitake mushroom bed-logs infested by Camptomyia pest*J. Microbiol. Biotechnol.***26** 909
[13] Bhushan B, Pal A, Kumar S and Jain V 2015 Biochemical characterization and kinetic

comparison of encapsulated haze removing acidophilic xylanase with partially purified free xylanase isolated from*Aspergillus flavus*MTCC 9390 *J.Food.Sci. Technol.***52** 191

[14] Handayani T and Purwantisari S 2015 Isolation and identification of mold contaminants on mushroom growing medium (bag log) and their cellulolytic performance test *J. Sains dan Matematika***23** 55

[15] Das A, Sourav B, Muthusamy P and Jayaraman A 2015Aflatoxin B1 degradation during co-cultivation of *Aspergillusflavus* and *Pleurotusostreatus* strains on rice straw*3 Biotech.***5**279

[16] Jackson L W and Pryor B M 2017 Degradation of aflatoxin B_1 from naturally contaminated maize using the edible fungus *Pleurotus ostreatus AMB Expr* **7** 110