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Characterization and Potential of *Trichoderma* spp. from Leaves Waste of Protective Plants

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A B S T R A C T

*Trichoderma*sp. is a microorganism that has a fairly wide habitat distribution in various geographical regions and climate zones. *Trichoderma*sp. could be isolated on a variety of organic materials that contain lignocellulosic materials such as leaf waste of protective plants. The aim of this study was to determine the character and potential of each *Trichoderma*sp was isolated from various leaves of protective plants. The results of this study found 5 different isolates based on their morphological shape both macroscopically and microscopically. *In vitro* potential test results showed that T.KTP isolates (*Trichoderma*sp.from *Terminaliacatappa* leaves) had higher cellulolytic index and lignolytic index values. It shows that T.KTP isolates have more potential in degrading organic material containing lignocellulose.

Introduction

*Trichoderma*sp is a microorganism that has a fairly wide habitat distribution in various geographical regions and various climate zones. It can be isolated on a variety of organic materials containing lignocellulose. Materials that contain lignocellulose are produced by many protective plants that are usually found along urban roads.

Lignocellulose is a complex structure of plant cell walls with three main components, namely cellulose, lignin and hemicellulose (Saha, 2004).

*Trichoderma*sp. known to be able to produce several extracellular enzymes including cellulase enzymes. Cellulase enzyme is an enzyme that is able to degrade cellulose with its main products namely glucose, cellobiose and cellooligosaccharides which degrade cellulose with its main products namely glucose, cellobiose and cellooligosaccharides. Cellulase has an enzyme system consisting of endo-1,4-β-glucanase, ekso-1,4-β-glucanase and β-D-glucosidase. These three enzymes work synergistically to degrade cellulose and release reducing sugars as the final product. Endo-1,4-β-glucanase cuts the chain bonds in cellulose to produce shorter cellulose molecules, ekso-1,4-β-glucanase cuts the ends

of the cellulose chain to produce cellobiose molecules, whereas β-D-glucosidase cuts the cellobiose molecules into two molecules glucose (Kim, 2001).

This fungus is also a micoparasite that produces a large number of secondary metabolites including phytohormone (Shoresh *et al.,* 2010). Phythohormone produced by *Trichoderma*sp. which can be used to accelerate plant growth. Currently, *Trichoderma* is widely used as a biopesticide, biological fertilizer and decomposers of organic matter (Woo *et al.,* 2014).An interesting aspect of *Trichoderma* as biocontrol is its ability to colonize roots and induce systemic resistance to fungi, bacteria, and even insects that attack (Salas *et al.,* 2011). mechanism that occurs in the soil by the activity of *Trichoderma*sp. namely competitors both space and nutrition, and as mycoparasites so as to suppress soil borne pathogenic activity (Sudantha *et al.,* 2011).

Materials and Methods

This study was conducted by survey method to determine the sampling location. Furthermore, there are several steps carried out, namely isolation of *Trichoderma*spp. from leaves waste of protective plant, macroscopic and microscopic characterization and *in vitro* potential testing.

Isolation of *Trichoderma*spp.

Isolation of *Trichoderma*spp. derived from the leaves waste of protective plant and carried out using a multilevel method, then observed the growth of fungus colonies and aseptically separated into petridish using Potato Dextrosa Agar (PDA) medium.

Characterization of *Trichoderma* spp.

Characterization of *Trichoderma*spp isolates was carried out through macroscopic and microscopic observations. Macroscopic observation is based on the color of the colony and the surface of the colony, while microscopic observation is based on the phialid, spore and conidiophoric forms (Samson *et al.,* 1988).

Potential isolate test *in vitro*

In vitro isolate potential test was carried out by using a specific medium of Carboxy Methyl Cellulose Agar (CMC) and Tanat Agar (PDA + tannic acid 0.1%). The *in vitro* potential of fungal isolates was observed by calculating cellulolytic index and lignolytic index. The index calculation was done by finding the ratio between the diameter of the clear zone and the diameter of the fungal colony (Jamilah *et al.,* 2009).

Results and Discussion

Morphological Characteristics of *Trichoderma*spp. (Macroscopic and microscopic)

Based on the isolation results, there were five isolates *Trichoderma* namely; T.MH (derived from *Swietenia mahagoni* leaves), T.KTP (derived from *Terminalia catappa* leaves), T.ASN (derived from the leaves of *Pterocarpus indicus*), T. KP (derived from the leaves of *Filicium decipiens*), T. TPG (derived from *Wodyetia bifurcata* leaves). Morphological characters of the five isolates of *Trichoderma* spp. observed macroscopically and microscopically could be seen in the following image:

Five *Trichoderma* isolates which were characterized based on their morphology, the color development of the colonies differed from day 1 to day 7. The development of the colony's color begins with clear white, then becomes pure white, and changes to a light green color, then becomes dark green after 7 days of age. This is supported by the

statement of Stamet (2000) which states that most saprophytic fungi at first had the mycelium is white and then the color can change when the mycelium is mature.The development of the colonies' color that occurred was almost the same for every *Trichoderma*spp. This is thought to be influenced by pigmentation and the amount of conia. Watanabe (2002) also stated that the *Trichoderma*spcolony on PDA media was initially white and then turned to a greenish color as the age of the isolates increased.

The colonies formed from all isolates were rounded in a circle with a flat colony surface and looked rough like fibrous, green and the edges were smoother. The same traits were stated by Soesanto *et al.,* (2011) who found 4 *Trichoderma* isolates with a circular colony shape and a dark green colony color on the circle. *Trichoderma* isolates spp. have different spore distribution patterns, T.KP, T.ASN, T.KTP and T.MH isolates (Figure 1) show concentric lines or look circular like a ring, whereas T.PNG has radial spore spread patterns.

The average of *Trichoderma* spp. observed to grow to full reach of the periphery of the petri dish within 4 days, faster than the study conducted by Taribuka *et al.,* (2016) The average colony of *Trichoderma* sp. which they observed grew fully to the periphery of the Petri dish within 5 days. This indicates that, the isolates obtained in this study have a high adaptability to the growth media use,d so that mycelium growth was relatively faster. Growth speed of *Trichoderma*sp. it was also influenced by the carbon source in the media where *Trichoderma* grows. One source of carbon that is very supportive for the growth of *Trichoderma* sp. include glucose (Kucuk and Kinavic: 2003).

Microscopically, *Trichoderma*spp. isolates observed under a microscope have almost the same characteristics, namely having branched conidiophores, round conidia, and fialids in T.KTP isolates with short-shaped edges and tapered edges compared to the middle, while the other four T.KP isolates, TMH, T.ASN and T.PNG have a longer fatial tube with a pointed tip (Figure 1).

Each isolate has 2-3 series of fialids. It has explained based on the identification book from Watanabe (2002) which states that *Trichoderma* sp. has a pyramid-like branched conidiophore that is at the bottom of the repetitive lateral branches, while getting to the end of the branching becomes shorter. Fialid looks slim and long, especially in the aspect of branches, conidia are semi-round to oval. *Trichoderma* spp. isolates observed microscopically there were have many similarities so it was difficult to determine the species in each isolate.

In vitro potential of cellulolytic and lignolytic

Cellulolytic and lignolytic activity of *Trichoderma*sp. can be seen in the following image:

Cellulolytic fungal screening was carried out by using Carboxy Methyl Cellulose Agar (CMC) medium. CMC is a cellulose derivative so that it can be used as a medium to test cellulase activity. Carboxy Methyl Cellulose Agar (CMC) media has a substrate that provides a carbon source for the growth of *Trichoderma*. The resulting clear zone shows the hydrolysis of organic matter in the substrate due to the presence of cellulase enzymes from its fungi.The clear zone formed around the *Trichoderma* sp colony is the result of the degradation of CMC media by cellulase enzymes as shown in figure 2 (B). *Trichoderma* sp colonies that form clear zones indicate that the *Trichoderma* produces cellulase enzymes.

Table.1 Cellulolytic and lignolytic index of five isolates of *Trichoderma*spp

Isolate Code	Lignolytic Index	Cellulolytic index
T.PNG	1,25	1,21
T.MH	1,13	1,06
T.KTP	1,53	1,35
T.KP	1,22	1,17
T.ASN	1,18	1,14

Description: T.PNG = *Trichoderma*sp.from*Wodyetia bifurcata* leaf, T.MH: *Trichoderma*sp.from*Swietenia mahagoni* leaf, T.KTP: *Trichoderma*sp.from*Terminalia catappa leaf*, T.KP: *Trichoderma*sp.from*Filicium decipien* leaf, T.ASN: *Trichoderma*sp.from*Pterocarpus indicus* leaf

Fig.1 *Trichoderma*spp. isolates (A1-A5) Macroscopic, (B1-B5) Microscopic (1000x magnification). T.PNG: *Trichoderma* sp. from*Wodyetia bifurcata*leaf, T.ASN: *Trichoderma*sp.from *Pterocarpus indicus*leaf, T.KP: *Trichoderma*sp. From *Filicium decipiens* leaf, T.MH: *Trichoderma*sp.from of *Swietenia mahagoni*leaf, T.KTP: *Trichoderma*sp. from of the *Terminalia catappa* leaf

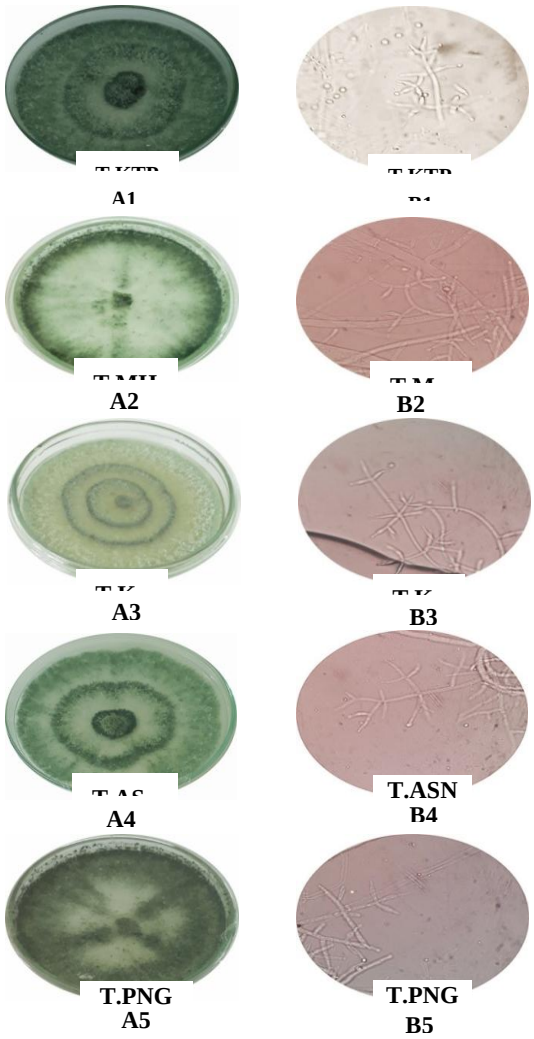
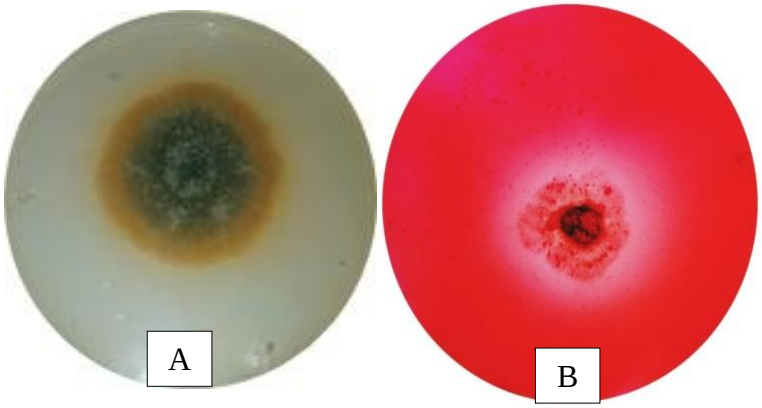


Fig.2 *In vitro* potential of *Trichoderma*sp. on specific medium (A) PDA + Tanic acid (B) CMC



The size of the clear zone was also an early indication of the size of the activity of the cellulase enzyme produced, the greater the area of the clear zone produced was the possibility of the activity of the cellulase enzyme produced being even greater (Subowo, 2012). The same thing was explained by Razic (2011) who stated that if the value of the cellulolytic index is greater then the ability to degrade CMC substrates was also greater.

Based on Figure 2. It could also be seen that *Trichoderma*sp. was able to grow in a specific PDA medium that is modified with the addition of 0.1% tannic acid, tanic acid in this study was used because it has a molecular structure that is almost the same as the molecular structure of lignin (Amrullah, 2013), so that it could be used as a medium to indicate the presence of enzymes ligninase and from the morphological form of the colony there is a brown zone. Brown zone formed indicates that the *Trichoderma*sp. colony produces ligninase enzyme, it has known that ligninase enzyme has the potential to degrade lignin compounds.

Table 1. Shows lignolytic and cellulolytic index values for each of the five isolates obtained (T.PNG, T.MH, T.KTP, T.KP, and T.ASN) and it was known that each isolate has a lignolytic index value and cellulolytic is different. Lignolytic index values were higher than cellulolytic index values. Lignolytic index values range between 1.13 - 1.53, while cellulolytic index ranges from 1.06 - 1.35.

Lignolytic index which has the highest value is T.KTP isolates (*Trichoderma* from *Terminalia catappa* leaves) while the lignolytic index that has the smallest value is T. MH isolate (*Trichoderma* from *Swietenia mahagoni* leaves). It indicates that T.KTP isolates have more potential in degrading lignin than with four other isolates (T.PNG, T.MH, T.KP and T.ASN). The difference in the index values obtained from these study was thought to be because the substrate used at the beginning of isolation is different.The substrate is a carbon source for fungal growth. It also explained by Meryandini *et al.*, (2009) each microorganism produces a complex of cellulase enzymes that differ depending on the genes they have and the carbon source used.

The process that occurs in lignin degradation is the breakdown of complex lignin molecules into simpler monomer constituents to be used

as a source of nutrition or carbon source for the growth of *Trichoderma* isolates. The formation of brown deposits or zones in the media used shows that the mold has the ability to oxidize phenols, which were compounds found in lignin (Siregar *et al.,* 2012). The brown zone formed also the result of the secretion of lignolytic enzymes due to the ability of *Trichoderma* isolates to use tannic acid as a carbon source, and it was assumed to be the result of polyphenol activity into a quinon that produces dark colored polymers (Asadi *et al.,* 2007). Saili *et al.,* (2014) explained that the development of dark brown color was confirmed through the polyphenol oxidation activity by fungal isolation.

For cellulase activity of five isolates, it was known that the largest cellulolytic index is T.KTP while the smallest cellulolytic index is T.MH. This difference in cellulolytic index values shows that five *Trichoderma* isolates produced had differences in their enzyme activity. It also indicates that T.KTP isolates have greater potential to degrade cellulose compared to other isolates because they have the highest cellulolytic index values. Bajya *et al.,* (2015) stated that, the highest and lowest cellulolytic activity seen from the clear zone diameter which has the ability to degrade cellulose on CMC media.

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