Antagonism of Lentinus Cladopus Lc4 Extract, Trichoderma sp. Jpa Extract on Bacillus sp., Xanthomonas sp. and E. Coli

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Antagonism of Lentinus Cladopus Lc4 Extract, Trichoderma sp. Jpa Extract on Bacillus sp., Xanthomonas sp. and E. Coli

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Abstract. Microbes that have antimicrobial abilities, which produce antimicrobial compounds are bacteria, actinomycetes, and fungi. Antimicrobial compounds produced by microbes which are generally secondary metabolites that are not used for the growth process. The purpose of this study was the antagonism test of *Lentinus cladopus* LC4 and *Trichoderma* sp. JPA against *Bacillus* sp., *Xanthomonas* sp. and *E.coli*. This research method is the descriptive method, filtrate culture extraction, and activity test of *Trichoderma* sp. JPA antimicrobial compounds and *Lentinus cladopus* LC4 against pathogenic bacteria *Xanthomonas oryzae, Escherichia coli* and *Bacillus subtilis* by disc method. Isolate extract of *Trichoderma* sp. JPA has the antagonistic potential for *E.coli*, *X. oryzae*, and *B. subtilis*. Whereas *Lentinus cladopus* LC4 has the antagonistic potential for *B. subtilis.*

1. Introduction

One effort to fight microbes is to use other microbes that have antagonistic abilities. Antimicrobials act as an inhibitor of the metabolism of other microbes. Antagonistic microbes that have antimicrobial ability can produce antimicrobial compounds [1]. Antimicrobial compounds produced by microbes are generally secondary metabolites that are not used for the growth process, but for self-defense and competition with other microbes in obtaining nutrients, habitat, oxygen, light, and others [2]. These antimicrobial compounds can be classified as antibacterial or antifungal [3]. Some antimicrobial compounds are phenol, formaldehyde, antibiotics, acids, and toxins [4]. Microbes that have the antimicrobial ability and produce antimicrobial compounds are bacteria, actinomycetes, and fungi. The fungus that has antimicrobial activity is the genus *Aspergillus, Penicillium, Paecilomyces, Trichoderma* [5].

Trichoderma sp. produces antimicrobial compounds, namely enzyme 1.3 glucanases, and chitinase which can destroy the walls of hyphae from several fungi and isocyanide-3 - (- isocyanocyclopent-2-enylidene) propionic acid which can inhibit the growth of *E. coli* bacteria [4]. Besides *Trichoderma* sp., *Lentinus* also has the potential to produce various types of metabolites that can be used for health and

industrial purposes. Several types of *Lentinus* that have been studied are *L. squarrosulus* originating from tropical Africa and other species from subtropical regions namely *L. trabeum, L. lepideus, L. adhaerens,* and *L. degener*. The potential of *Lentinus* as an antagonist was reported by [6]; [7] who reported that *L. squarrosulus* produced two antibiotic compounds isolated from the culture filtrate. One of these compounds is Ls2 compounds which can inhibit the growth of *Bacillus subtilis*, *Mucor ramannianus,* yeast, and *Rigidoporus lignosus*. Based on the previous description, the purpose of this study was the antagonism test of *Lentinus cladopus* LC4 extract, *Trichoderma* sp. JPA extract against *Bacillus* sp., *Xanthomonas* sp. and *E.coli*.

2. Methods

2.1 Culture filtrate extraction

Extract of *Trichoderma* sp. JPA Culture filtrate and *Lentinus cladopus* LC4 were obtained by separating the filtrate first from the mycelium with filter paper, then the filtrate was extracted twice with n-butanol (1:1 v/v). Butanol extract was dried with the rotary evaporator, under vacuum, at a temperature of 40 °C, then dissolved with methanol until it dissolved completely. Extract of *Trichoderma* sp. JPA Culture filtrate and *Lentinus cladopus* LC4 were obtained by filtrate directly evaporated to dry with the rotary evaporator, under vacuum, at a temperature of 30 ^{0}C, then dissolved with methanol until dissolved. The mycelium from each of the above cultures is separated from the culture filtrate with filter paper.

Then the mycelium is destroyed with the help of mortar and extracted twice with 50 ml of methanol and shaker for 24 hours for each extraction. The extract in methanol was separated from the mycelium fragment with number 3 fritted glass filter with the help of a vacuum pump. Then the methanol extract was dried with the rotary evaporator, under vacuum, at a water temperature of 30 ^{0}C. The dried extract is then dissolved again with methanol until it dissolves. Activity test of antimicrobial compounds of *Trichoderma* sp. JPA and *Lentinus cladopus* LC4 against pathogenic bacteria *Xanthomonas oryzae*, *Escherichia coli* and *Bacillus subtilis* with discs method

Activity test of *Trichoderma* sp. JPA and *Lentinus cladopus* LC4 are carried out using the disc method. Sterile PDA media poured into Petri dishes aseptically. Liquid cultures of *Xanthomonas oryzae* isolates, *Escherichia coli*, and *Bacillus subtilis* were distributed evenly on PDA media. Disc paper containing *Trichoderma* sp.JPA extract and *Lentinus cladopus*LC4 extract are placed in the middle of a petri dish. Antimicrobial activity is characterized by a clear zone. Observation of antimicrobial activity was carried out after 24 hours incubation.

3. Result and Discussion

Test results in antimicrobial activity of *Trichoderma* sp. JPA and *Lentinus cladopus* LC4 are active against bacterial assays as indicated by the formation of inhibitory zones around disc paper on agar medium overgrown with *Escherichia coli, Xanthomonas oryzae, Bacillus subtilis.* This shows that the type of extract used has antimicrobial compounds that have activity in inhibiting the growth of *Escherichia coli, Xanthomonas oryzae, Bacillus subtilis* (Figure 2 and Table 1).

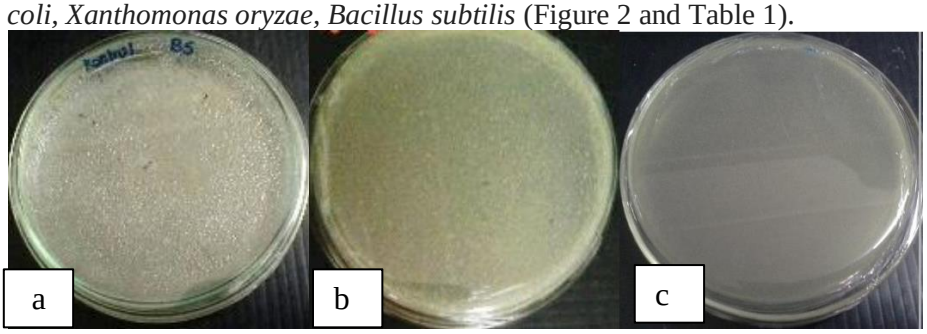

Figure 1. Control without *Trichoderma* sp. JPA and *Lentinus cladopus* LC4; (a) *Escherichia coli* (b) *Xanthomonas oryzae*, (c) *Bacillus subtilis*

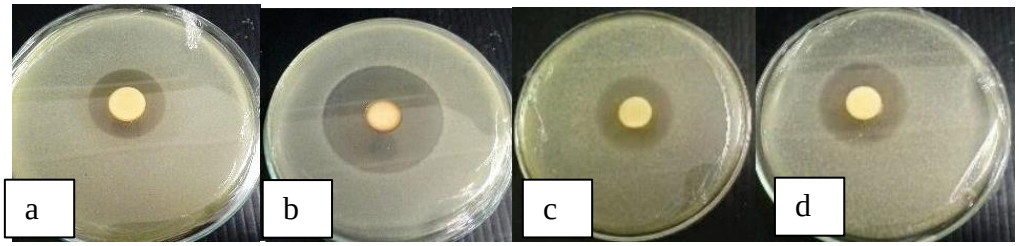

Figure 2 (a) Antagonists of E. coli by *Trichoderma* sp. JPA; (b) Antagonists against *Xanthomonas oryzae* by *Trichoderma* sp. JPA; (c) Antagonists against *B. Subtilis* by *Trichoderma* sp. JPA; (d) Antagonists against *B. Subtilis* by *Lentinus cladopus* LC4

Table 1 Results of the antibacterial activity test

Type of extract	The diameter of the Inhibitory Zone(mm)		
	E. coli	*X. oryzae*	*B. subtilis*
Trichoderma sp. JPA	2.6	2.6	2.4
Trichoderma sp. JPA	2.6	2.0	1.5
Trichoderma sp. JPA	3.1	2.3	2.6
*Lentinus cladopus*LC4			2.4
*Lentinus cladopus*LC4			1.9
*Lentinus cladopus*LC4			2.3

The results showed that *Trichoderma* sp. JPA Each can inhibit the growth of *E. coli, X. oryzae, B. subtilis.* While *Lentinus cladopus* LC4 can only inhibit *B. subtilis* which is characterized by the formation of clear zones around the paper disc. *Trichoderma* sp. JPA and *Lentinus cladopus* LC4 contain compounds that are antimicrobial to *E.coli, X. oryzae, B. subtilis. Lentinus cladopus* LC4 has antagonism ability for Ganoderma boninense which causes root rot of palm oil [7–9].

Trichoderma sp. produce antimicrobial isocyanide (3- isocyanocyclopent-2-enylidene) propionic acid [4, 10–13] have also suggested that various types of tropical *Lentinus* were effective against *B. subtilis*, each producing at least more than one antimicrobial compound isolated from both culture and mycelium filtrate. Even from one source, such as from mycelium alone can be obtained more than one antimicrobial compound. *Trichoderma* is known to have antagonistic ability against pathogenic fungi. This fungus is a beneficial microorganism, avirulent to host plants, and can parasitize other fungi [14]–[17]. *Trichoderma* is also known to reduce *P. nicotianae* attacks on tobacco plants [16, 18–20]. *Trichoderma* spp. Able to control *Fusarium* sp. causes of wilting in strawberry plants [21, 22]. *Trichoderma* is a fungus that can be used as a pathogen control agent, the antagonistic mechanism by *Trichoderma* in inhibiting the growth of pathogens, including competition, parasitism, antibiosis, and lysis [4].

4. Conclusion

Isolate extract of Trichoderma sp. JPA has the antagonistic potential for *E.coli, X. oryzae,* and *B. subtilis.* Whereas *Lentinus cladopus* LC4 has antagonistic potential *B. subtilis*. Each is characterized by the formation of clear zones.

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