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 °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 °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 cladopusLC4 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 cladopusLC4  |         |           | 2.4         |
| Lentinus cladopusLC4  |         | 1.9       |             |
| Lentinus cladopusLC4  |         |           | 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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