Growth and Reproduction of Trichoderma sp. in with presence Bacillus sp. or Fluorescent Pseudomonad

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Abstract. The application of biological agents can be done singly or in combination to increase its effectiveness. \textit{Trichoderma} sp. fungi, \textit{Bacillus} sp. and fluorescent pseudomonads bacteria has been widely used as a biological control agent for plant pathogens. The purpose of this study was to determine the growth and reproduction of \textit{Trichoderma} sp. in the presence of \textit{Bacillus} sp., or fluorescent pseudomonads. The research was carried out \textit{in vitro} by dual and double layer culture method. The variables observed were the inhibition zone, the total and, viability of conidia of \textit{Trichoderma} sp. The data obtained were compared using the two free sample T test and analysis of variance was continued with Duncan's multiple range test. Based on the results of this study concluded that the growth and reproduction of \textit{Trichoderma} sp inhibited by the presence of \textit{Bacillus} sp. or fluorescent pseudomonads, but does not reduce the viability of conidia. This shows that in \textit{Trichoderma} sp. with \textit{Bacillus} sp. or fluorescent pseudomonads can be applied simultaneously.

Keywords: \textit{Trichoderma} sp., \textit{Bacillus} sp., fluorescent pseudomonads, interaction, conidia viability production.

1. Introduction
Management of plant diseases by applying chemical inputs in a long time has been evident to have a significant negative impact on the environment, polluting, leaving dangerous residues and even developing resistance in certain pathogenic organisms. On the other hand plant associations with microbes such as Pseudomonas and Bacillus have been recognized for their important role in soil fertility and plant health [1]. Some bacteria and fungi as biological controller agents have been identified, such as the genera Pseudomonas, Bacillus, and \textit{Trichoderma} [2]. Besides being known as a biological controller, genus Pseudomonas, especially from the group of fluorescent pseudomonads, also plays a role in increasing plant growth by stimulating root growth as well as induces plant systemic resistance [3], likewise
Bacillus [4] also, Trichoderma in addition to being a biocontrol also promotes plant growth [5].

Fluorescent pseudomonads act as a natural agent for suppressing soil-borne diseases. This is thought to have occurred due to secondary metabolites being released into the root environment. The main secondary metabolites produced by fluorescent pseudomonad include phenazines, 2, 4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin, hydrogen cyanide (HCN). This metabolite is known to be antifungal, antibacterial, antiviral [6]. Other studies show that the majority of these bacteria secrete siderophore, which is a compound of chelating iron (affinity to iron) as a response to iron's limitations [7]. In addition to siderophores, pyoverdines (also called pseudobactins) have implications for the resistance of induction [8]. On the other hand, Bacillus produces secondary metabolites which include bacillomycin D and fengycin function to suppress pathogens directly and induce plant systemic resistance. Bacillomycin D is the most powerful anti-fungal than other antibiotics produced by Bacillus. Then bacillibactin secondary metabolites which are closely related to the production of siderophore, macrolactin, bacilaene, difficidin, bacilysin function to suppress pathogens directly [9]. In addition to siderophores and secondary metabolites classified as antibiotics Bacillus also produces other metabolites such as salicylic acid, lipopolysacharide, and hydrolytic enzymes to suppress pathogens directly or through enhancing the plant defense mechanisms [10]. Trichoderma is an effective biological control agent against pathogens fungal. The mechanism for controlling pathogens can act indirectly or directly. Indirectly through the ability to compete for nutrition and space, modify environmental conditions, or stimulate plant growth, increase plant defense mechanisms [11], and antibiosis by producing antibiotics or toxic. The antibiotics produced by Trichoderma can be distinguished from compounds with light and heavy molecular weights such as daucanes, pyrones, trichodermanides, viridins, azaphilones viridiofungins, peptaibols as anti-fungal compound or spore germination inhibitors [12; 13], or directly by mechanisms such as mycopathogenesis with the production of lytic enzymes such as chitinase, glucanase, and protease before entering the host mycelium. The existence of this enzyme plays a key role in the process of mycoparasitism [14].

Based on this description, it appears that one of the mechanisms in controlling or suppressing the growth of pathogens or their surrounding microbes, fluorescent pseudomonad, Bacillus, or Trichoderma is by producing antibiotics or secondary metabolite compounds. Furthermore, what if these biological control agents grow at the same time and place, is there an antagonism that occurs with one another? The purpose of this study was to determine how interaction or the growth, production, and viability of spores produced by Trichoderma with in the presence of bacillus or fluorescent pseudomonads.

2. Materials and Methods

*Trichoderma* sp. fungus was obtained from the collection of institute seedling and plantation plant protection (BBPPTP) Surabaya. Jalan Raya Mojoagung No. 52 Gambiran, Mojoagung Kab. Jombang. The isolate of *Bacillus* sp. and fluorescent pseudomonad were obtained from the Laboratorium collection of the plant ecology of the Trunojoyo Madura University which has been known can suppress the growth of the fungus Colletotrichum, Sclerotium, Fusarium.

2.1. Antagonistic

Antagonistic is determined by measuring the inhibition zone that is a) Measuring the difference between the diameter of the *Trichoderma* sp. colony on control and treatment on
the double-layer culture method, measuring the difference from the center to the edge of the colony away and towards the streaking of Bacillus sp. or fluorescent pseudomonad on dual culture method. 1) Control, in petri dish with a diameter of 9 cm, on the PDA media cultured colony disc mycelium, Trichoderma sp. which has been three days old with a diameter of the disc is 1 cm. 2) Double-layer culture method, 1 ml of colony suspension of fluorescent pseudomonads or Bacillus sp. from agar slant that has been incubated 24 hours was cultured by pour plate method in 6 ml of PDA media. After solidifying was cultured disc of mycelium colony Trichoderma sp. which has been three days old with a diameter of one cm. 3) Dual culture methode, in petri dish with a diameter of 9 cm, disc colony mycelium Trichoderma sp. which has been three days old with a diameter of colony one 1 cm was cultured on the PDA agar, then colony fluorescents pseudomonads or Bacillus on culture agar slant that has been incubated 24 hours was streaked at a distance of three cm from the disc of colony mycelium the Trichoderma sp. Differences between inhibition zone of the Trichoderma sp. by fluorescents pseudomonads and Bacillus sp. were analyzed by T-test α: 0.05

2.2. Conidia total
The calculation of conidia total was carried out on the control and treatment, by the made suspension of the conidia from 1cm the disc of Trichoderma sp. colony which had been incubated for 5 days, in 10 ml of distilled water. The total of conidia is the conidia found in 1 ml of conidia suspension. The calculation is done on a hemocytometer.

2.3. Viability of Conidia
Conidia viability is the percentage of conidia Trichoderma sp. germinating in either control or treatment, ie counting conidia germinating from 1 ml of conidia suspension on the drop of PDA 0.5 cm in diameter above object-glass, after incubating for 16 hours. To determine the differences between treatments to total and viability of conidia on the data obtained were carried out variance analysis was followed by Duncan’s multiple range tests.

3. Results and Discussion
Based on the analysis, it appears that the colony growth inhibition zone of Trichoderma sp. due to the presence of fluorescent pseudomonad is greater than Bacillus, as shown in tables 1 and 2, however, in the double-layer method there is no significant inhibition zone difference
between fluorescent pseudomonad and *Bacillus* sp. In the dual culture method starting from 2 days after incubation, the zone of growth inhibition of the colony *Trichoderma* sp. with in the presence of *Bacillus* sp. is always smaller than the fluorescent pseudomonad, even in some experimental units the colony of *Trichoderma* sp. can grow beyond or pass through the streaked of Bacillus colony (Figure 1). This shows that there are differences in the types of secondary metabolites produced by Bacillus sp. and fluorescent pseudomonad, in which the anti-fungal levels of these metabolites may differ. As explained that 2,4-diacetylphloroglucinol is the metabolite of the most powerful antifungal produced by fluorescent pseudomonads [6].

| Dual culture          | Days to |
|-----------------------|---------|
|                       | 1      | 2      | 3      | 4      |
| Fluorescent pseudomonads | 0,2 a  | 3,4 b  | 11,5 b | 15,5 b |
| *Bacillus* sp.         | 0,1 a  | 2,2 a  | 9,6 a  | 13,6 a |

Note: Numbers followed by the same notation in the same column indicate not significantly different based on the T-test (α: 0,05).

The Bacillomycin D is metabolites the most powerful antifungal of produced by Bacillus [9], of which 2,4-diacetylphloroglucinol and Bacillomycin D have the not same shape and molecular weight. On the other hand, it is explained that the Bacillus sp. can inhibit the grown of mycelia of Trichoderma, however, the level of Bacillus antagonism towards Trichoderma is determined by the species of both Bacillus and Trichoderma [15]. Likewise, it has been explained that interaction in vitro of fluorescent pseudomonad with Trichoderma in the dual culture method inhibits the growth of mycelial Trichoderma [16]. On the double-layer method, there is no difference the zone of inhibition of growth of colony Trichoderma sp.

| Double layer-culture | Days to |
|----------------------|---------|
|                      | 1      | 2      | 3      | 4      |
| Fluorescent pseudomonads | 11,2 a | 19,4 a | 23,4 a | 59,5 a |
| *Bacillus*            | 12,4 a | 21,5 a | 25,2 a | 62,2 a |

Note: Numbers followed by the same notation in the same column indicate not significantly different based on the T-test (α: 0,05).
with the presence of fluorescent pseudomonads and Bacillus sp., but the color of colony Trichoderma growing on the fluorescent pseudomonad colony layers appears more clearly than those that grow in Bacillus colony layers (Figure 1), this shows that is the colony has denser conidia. This condition explains that secondary metabolites produced by fluorescent pseudomonad might inhibit mycelial growth but not inhibit the production of conidia Trichoderma sp. Inhibitory levels by the metabolite compound produced by Bacillus sp. are lower than by fluorescent pseudomonad but reduce the level of conidia production. This is evident in the results of the analysis of the conidia total (Table 3).

Tabel 3. Total and viability of conidia Trichoderma in each treatment 5 days after cultivation

| Treatment                              | Total of conidia (conidia/drop) | Viability conidia (%) |
|----------------------------------------|----------------------------------|-----------------------|
| Control                                | 4.3 x 10^7 a                     | 63.7 a                |
| Dual culture                           |                                  |                       |
| Fluorescent pseudomonads               | 9.2 x 10^6 b                     | 70.5 a                |
| Bacillus                               | 8.8 x 10^6 b                     | 73.2 a                |
| Double-layer culture                   |                                  |                       |
| Fluorescent pseudomonads               | 9.3 x 10^6 b                     | 65.0 a                |
| Bacillus                               | 5.4 x 10^6 c                     | 64.3 a                |

Note: Numbers followed by the same notation in the same column indicate not significant different based on Duncan’s multiple range test (α: 0.05).

Based on the analysis of variance to conidia viability (Table 3), there appears to be no significant difference in conidial viability. This condition indicates that the antibiosis shown by fluorescent pseudomonad and Bacillus on the growth of Trichoderma colonies does not reduce the viability of conidia. Lower conidia viability was likely to be present in controls than with other treatments. This shows that secondary metabolites produced by fluorescent pseudomonad and Bacillus sp. on the one hand inhibit the growth of colonies, but the possibility on the other hand otherwise increases the viability of conidia. It is not surprising,
as has been found [16], that in vivo interactions between fluorescent pseudomonad with Trichoderma sp. initially the population of Trichoderma sp. will decrease until 7 days after incubation, however, 14 days after incubation the population increases again.

This shows that at the beginning of the interaction will result in depressed Trichoderma growth, but as time passes Trichoderma can adapt and increase its growth through increased viability of conidia, as well as the possibility of Bacillus sp. Based on the results of this study it can be stated that although at the beginning of the interaction there is an effect that suppresses the development of Trichoderma sp. in its development Trichoderma sp. can improve its growth, this shows that the application of Trichoderma sp. can be done simultaneously with fluorescent pseudomonad or Bacillus. It has been shown [2] that inoculation of Trichoderma sp and fluorescent pseudomonad by single can prevent Ralstonia solanacearum infection, but if combined can increase prevention effectiveness up to 97%.

4. Conclusio

The growth and reproduction of Trichoderma sp inhibited by the presence of Bacillus sp. or fluorescent pseudomonads, but does not reduce the viability of conidia. This shows that in Trichoderma sp. with Bacillus sp. or fluorescent pseudomonads can be applied simultaneously.

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