Isolation and Antagonist Test of Arabica Coffee Endophyte Bacteria (*Coffea Arabica* L.) in Inhibiting Upas Fungus Growth (*Corticium Salmonicolor* B.Et. Br.)

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Abstract
One of the effective efforts in inhibiting the spread and growth of upas fungus on coffee plants is by using biological control agents utilizing endophytic bacteria. Research on the antagonistic test of endophytic bacteria in inhibiting the growth of upas fungus using a non-factorial Completely Randomized Design (CRD) using different endophytic bacteria in each treatment, endophytic bacteria were obtained from rubber plant leaf isolates. This study had 7 treatments with 2 replications. Endophytic bacteria were characterized based on colony morphology, namely colony shape, height, edge and color, and cell morphology. The selection test of endophytic bacteria obtained was able to inhibit *Corticium salmonicolor* fungus. The results of the inhibitory power test of endophytic bacterial filtrate against *Corticium salmonicolor fungus* in vitro showed the largest results shown to be able to inhibit the area of *Corticium salmonicolor colonies* of 13.20 mm at Sp 5 AR UII and the smallest inhibitor was at Sp2 AR UI 6.10 mm when compared to the control of 45.75 mm.

***Keywords*:** Endophytic Bacteria, Upas fungus, Microgranules

Introduction

Coffee is one of the plantation commodities in Indonesia that has a fairly high economic value among other plantation crops and plays an important role as a source of foreign exchange for the country. Several areas that are used as centers for coffee production and cultivation in Indonesia, one of which is Tanah Toraja. Coffee not only plays an important role as a source of foreign exchange but is also a source of income for no less than one and a half million coffee farmers in Indonesia [1]. In general, coffee is used as a processed product in the form of a drink that comes from the processing and extraction of coffee beans [2].

Coffee plants (*Coffea* spp.) are one of the plantation crops in Indonesia whose commodities are taken into account in strengthening the country's foreign exchange. Based on data from the Directorate General of Plantations, Arabica coffee production reaches >1,000 tons which is dominated by North Sumatra, Aceh, South Sulawesi, and several other regions [3]. The area of coffee plantations is dominated by smallholder plantations of 95.37% with robusta coffee covering 81.96% and Arabica coffee covering 18.04% [4].

The disease of the upas fungus (*Corticium salmonicolor*) on the trunk and branches of rubber plants is not as popular as leaf or root disease attacks, although the impact of losses caused by branch and stem damage is quite high, especially in areas with high rainfall and high humidity [5]. For this reason, it is necessary to find effective and efficient antagonistic microorganisms to control the disease of the upas fungus branches such as bacteria sp. By *Pseudomonas*, therefore, it is necessary to confirm the effectiveness of *Pseudomonas* sp. by conducting re-testing. Testing is carried out by inoculating *Pseudomonas* sp [6].

On branches attacked by upas fungus with various levels of attack. The treatment was carried out four times with an interval of one week. The research design used was a non-factorial Completely Randomized Design (CRD) with 7 treatments consisting of *Pseudomonas* sp (without storage, one month of storage, two months of storage, and three months of storage), and control (without application). Each treatment was repeated 2 times with 20 sample plants in each unit.

Method

This study used a non-factorial Completely Randomized Design (CRD) using different endophytic bacteria in each treatment, endophytic bacteria were obtained from rubber plant leaf isolates. This study had 7 treatments with 2 replications:

Isolation of Endophytic Bacteria

Endophytic bacteria were isolated from healthy rubber plant leaves. Rubber leaves were washed with water until clean, then cut into several parts and surface sterilized by soaking in 70% alcohol for 2 minutes, 1% sodium hypochlorite (NaOCl) solution for 2 minutes and rinsed using sterile distilled water [7].. The sample pieces were dried with sterile tissue paper. Then the surface-sterilized plant parts were macerated with a sterile mortar until smooth with the addition of 1:10 water. The suspension and suspension that had been diluted 10-1 were taken as much as 0.1 ml then spread evenly on Nutrient Agar media and then incubated at room temperature for 3 days .

Bacterial Purification

Purification was performed on all bacterial colonies that grew and were considered different based on morphological appearance including color and shape of the colony. Each bacteria was taken and separated into new sterile NA media using an ose needle. If the bacteria that grew were still mixed with other bacteria, they were purified again. This serves to obtain pure endophytic bacterial isolates [8]. A single colony of purified bacteria was used for antagonist testing.

Observation Parameters

Characterization of Endophytic Bacterial Isolates

Characterization includes colony shape, color, colony edge shape and gram type of endophytic bacteria.

Endophytic Bacterial Antagonist Test Against Fungi In

This test aims to obtain a consortium of endophytic bacteria that have the potential as a biological control agent against Pestalotiopsis sp. The test was carried out by growing Pestalotiopsis fungi together with endophytic bacteria on PDA media. Endophytic bacteria were grown in the middle of the petri dish, then Pestalotiopsis sp. fungi were grown on ¼ of the petri dish and three replications were carried out. Measurement of the growth diameter of Pestalotiopsis fungi began on the second day to the seventh day. According to [9]. the

percentage of inhibitory power of antagonistic bacteria can be determined through colony growth calculated using the formula:

$$\boldsymbol{DE = \frac{d1 - d2}{d1} \times 100\%}$$

Information:

DE: Efficacy (inhibition) power (%)

d1: Area of fungal growth in control (cm)

d2: Area of fungal growth on endophytic bacterial isolates (cm)

Result and Discussion

Isolation and Characteristics of Endophytic Bacteria from Coffee Plant Roots and Stems

From the isolation of endophytic bacteria from the roots and stems of coffee (*Coffea arabica* L), four isolates of endophytic bacteria were obtained, namely 2 isolates from the stems and 5 isolates from the roots. The 8 isolates have varying characteristics both in terms of morphology and color [10]. Isolation and characteristics of endophytic bacteria are shown in Table 1

Table 1. Characteristic of Colony Morpholgy Endophyte Bateria from Coffee Plant.

Species	Colony Color	Elevation	Edge (Margin)	Form (Whole)
Sp. 1 Ar	White	Flat	Filamentous	Filamentous
Sp. 2 Ar	White	Flat	Rhizoid	Rhizoid
Sp. 3 Ar	White	Flat	Irregular	Irregular
Sp. 4 Ar	White	Flat	Lobate	Irregular
Sp. 5 Ar	White	Flat	Filamentous	Rhizoid
SP. 1 Br	White	Flat	Irregular	Filamentous
SP. 2 Br	White	Flat	Irregular	Rhizoid

Observation Results of Antagonistic Test

The results of the antagonistic test of endophytic bacteria on the growth of upas mushrooms showed the presence of an inhibition zone indicating that endophytic bacteria were able to inhibit the growth of upas mushroom mycelium. The results of quantitative observations can be seen in the following table.

Tabel 2. Diameter of inhibition zone of antagonistic test of endophytic bacteria on growth of upas fungus.

Species	Mushroom Diameter (mm)
Control	45.75 mm
Sp. 1 Ar UI	12.30 mm
Sp. 2 Ar UI	6.10mm
Sp. 2 Ar UII	12.20mm
Sp. 3 Ar UI	10.50 mm
Sp. 3 Ar UII	7.20mm
Sp. 5 Ar UI	12.20mm
Sp. 5 Ar UII	13.20 mm

SP. 1 Br UI	9.15 mm
SP.1 Br UII	12.80 mm
SP. 2 Br UI	17.10 mm
SP. 2 Br UII	12.10mm

Figure `1. Growth of antagonist test against upas fungus *(Corticium salmonicolor*)

Discussion

Characteristics of Endophytic Bacteria from Coffee Plant Roots and Stems

AA hormone-producing bacteria are characterized based on colony morphology, namely colony shape, height, edge and color, and cell morphology through bacterial staining, namely cell shape and grammatical characteristics of bacteria. Based on the results of bacterial isolation and endophytic properties in coffee plants, four different isolates were obtained, then all isolates showed gram (+) bacteria types from IAA-producing endophytic bacterial isolates. From previous studies, six different endophytic bacterial isolates were obtained, namely Bacillus sp., Pseudomonas sp., Klebsiella sp., Xanthomonas sp. [11]. These results were confirmed by [12]. who obtained 7 endophytic bacterial isolates from coffee plants. From these results, different characteristics were obtained which were observed in terms of colony shape, bacterial morphology, and bacterial physiology. These results are in accordance with previous research [13]. which stated that the growth of microorganisms on solid media is characterized by different colony shapes such as round, irregular and so on.

Antagonistic Test

The data shows that bacteria have the smallest average diameter of fungal colonies, which is 6.10 mm, followed by the largest endophyte at 17.10 mm. The suppression of endophytic bacteria on the growth of *Corticium salmonicolor* is characterized by abnormal fungal growth and the presence of a clear zone between endophytic bacteria and fungi. The clear zone is thought to occur due to the production of antifungal compounds produced by endophytic bacteria. The mechanism of inhibition against pathogens is by producing antibiotics, toxins, competition for space and nutrients [14].

Conclusion

The endophytic bacterial selection test was found to be able to inhibit the *Corticium salmonicolor fungus*. The results of the endophytic bacterial filtrate inhibition test against the *Corticium salmonicolor fungus* in vitro showed the largest results shown to be able to inhibit the area of the *Corticium salmonicolor colony* of 13.20 mm at Sp 5 AR UII and the smallest inhibitor was at Sp2 AR UI 6.10 mm when compared to the control of 45.75 mm.

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