Characteristics of rhizobacteria on healthy and white rot-infected rubber trees

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Abstract. White root disease is an important disease in rubber plants caused by the fungus Rigidoporus microporus. The severity of white root disease in smallholder rubber plantations is up to 15%. Various controlling technique has not been effectively eliminate this disease. To develop an integrated white root disease management, a comprehensive study including the ecology and rhizobial characteristic of rubber tree is needed. This study was aimed to describe the characteristics of rhizobacteria on healthy and infected rubber trees. The results showed that the population of antagonistic and non-fluorescence bacteria was higher in the rhizosphere of healthy rubber plants, vice versa. The observation of bacterial morphology showed that the bacterial community was more diverse in the rhizosphere of healthy rubber than the rhizosphere infected by R. microporus.

1. Introduction
White root disease is one of important diseases in rubber plants. The disease severity in smallholder rubber plantations is up to 5% [1]. White root disease is caused by the fungus Rigidoporus microporus Swartz (syn. Fomes lignosus Klotzsch). Dissemination of this disease is by surface contact between infected and healthy rubber roots. Soil conditions are also affecting the development of R. microporus. White root disease causes considerably high economic losses, not only about the yield loss but also the expensive controlling cost. White root disease may cause plant death which directly reduces the productivity [1]. White root disease control was carried out in various ways including stump demolition, planting legumes cover crop, and sowing of sulphur and treatment to infected plants. Biotic factors that inhibit the development of white root disease antagonistic activity of several beneficial soil microbes i.e. bacteria, actinomycetes, and fungi; while the abiotic factors involved chemical and physical properties of the soil, such as low soil acidity, low moisture/saturated water, and solid/clay soil structure [2].

Although various controlling method has been carried out, this disease have not been controlled effectively. This difficulty is strongly related to the absence of specific study about the ecology of white root disease. Therefore, this study was carried out to describe the characteristics of healthy rubber rhizobacteria and those of infected with white root disease in order to design a comprehensive disease management.
2. Methods
Research was carried out at Protection Laboratory, Sembawa Research Centre, Indonesian Rubber Research Institute from Nov 2017 to Feb 2018. This research was a descriptive study by describing the bacterial community based on antagonistic activity in vitro on R. microporus, bacterial biomass, fluorescence, Gram reaction, and the number of colonies types with detailed methods as follows:

2.1. Sampling of rhizospheric soil
A sample of rubber rhizosphere was taken from Experimental Field of Sembawa Research Centre from 4 yr-old healthy and infected rubber plants in each 3 locations. The healthy lateral roots (diameter 1 to 2 mm) and soil from each samples were collected. Samples of roots and soil were immediately put into a plastic bag and in a cooling box for testing in the laboratory.

2.2. Isolation, enumeration, and characterization of bacteria from rubber rhizosphere
Bacterial isolation was carried out from samples of the rhizosphere of healthy and infected rubber plants. As many as 1 g of sample was put into a test tube containing 10 mL of MgSO4 0.01 M then shaken with a vortex for 20 sec. The bacterial suspension was diluted to 10^6 then 0.1 mL of the suspension from each dilution series was spreaded on nutrient agar (NA) medium then incubated at 28 °C for 24 hr. Several observations involved in this examination were: (1) bacterial population, bacteria grown after isolation were observed by the number of bacteria in colony forming units (cfu); (2) observation of colony morphology, observation of bacterial colonies morphology included colony color, surface texture (smooth/rough) and size, slime production; (3) fluorescence test, fluorescence observation on rhizobacteria from isolation was carried out by culturing bacteria on the King's B media. Observations were carried out under ultraviolet light by looking at the ability of bacteria to fluorescent; (4) Gram reaction, observation of Gram reactions was carried out on rhizobacterial isolates using KOH 0.53 M solution. Observation was carried out by dripping KOH on the object glass, then 1 use of bacterial was mixed with KOH solution by stirring using the needle. Slimmy bacteria are indication of Gram negative, and the non slimmy bacteria are indication of Gram positive.

2.3. Antagonism test of rhizobacteria against R. microporus
In vitro antagonistic test was carried out by culturing the rhizobacteria against R. microporus on yeast agar media. Observation of antagonistic activity of rhizobacterial colonies against R. microporus was carried out by observing the colony interactions. R. microporus colonies growth may form an inhibition zone when it have direct interaction with rhizobacteria. Meanwhile, the R. microporus colonies that can penetrate rhizobacterial colonies indicates a non-antagonistic interaction.

3. Results and discussion
Symptoms of rubber plants infected by R. microporus were characterized by yellowing and fallen out leaves. In addition, the death of the twigs also occurred in mature trees. Occasionally, infected trees produced early flowers and fruit, then the roots become rotten and causing tree falls. White mycelium (rhizomorph) colonized the root surface of infected plant. White root disease may form a fruiting body on the plant root and on the stump. Naturally, there are many root exudates produced and released to the soil [3]. The root exudate produces amino acids that could support the growth of antagonistic microorganisms and these exudates are used by many soil microbes in survival and reproduction [4].

3.1. Population and characteristics of bacteria
Based on the observations of rhizobacterial population, population and diversity of microbes was higher in healthy rubber rhizosphere than infected rubber rhizosphere (Table 1). The bacterial colonies forming are varies for each species [5]. The high diversity of microbes in healthy rubber plants showed that healthy plants delivered a favorable conditions for the microbes to live. Microorganism plays role in enhance plant nutrition either through symbiosis or trough mineral nutrient observation
ability [6]. In addition, the carbon and nitrogen cycle plays a role in producing hormones and stimulating plant growth, as well as plant protection.

Table 1. Characteristics of the rhizobacterial community from the rhizosphere of healthy rubber plants and infected by white root disease.

| Rhizosphere     | Bacterial population (cfu/g) | Number of isolates (%) |
|-----------------|-----------------------------|------------------------|
|                 |                             |                        | Antagonistic | Fluorescens | Gram |
|                 |                             |                         | (+)         | (-)         | (+)  | (-)  |
| Healthy plants  | 1.41 x 10^{11}              | 60.00                   | 40.00       | 37.20       | 62.8 | 66.52 | 33.48 |
| Infected plants | 1.10 x 10^{11}              | 56.00                   | 44.00       | 57.00       | 43.0 | 46.40 | 53.60 |

Based on fluorescence observations of the rhizobacteria, healthy rhizosphere of rubber plants has higher number of non-fluorescence bacteria than fluorescence ones. Whereas, in infected rubber plants rhizosphere, the number of fluorescence bacteria was higher than the number of non-fluorescence ones (Table 1).

Antagonistic fluorescence bacteria were more dominant in healthy rubber plants. It indicated the bacteria might suppress pathogen infection and trigger plant growth. Based on another research, *Pseudomonas fluorescens* strain isolated from the roots of lettuce plants has a broad spectrum of antibiotics against pathogens of fungi and bacteria and also can trigger plant growth [7]. In cultures, this bacteria produces hydrogen cyanide (HCN) 24 diacetylfloroglucinol (phl) and does not produce pyrolnitrine, pioluteorin, or carboxyl-1 fenazine acid (PCA).

The other results regarding antibiotic-producing fluorescence bacteria reported that the population of *P. putidae* was well known dominating the total population of *P. fluorescens* bacteria on suppressive soils of *Rhizoctonia solani* Kuhn. on apple rhizosphere. The decline in the fluorescent bacterial population will increase the pathogenic population of *Phytophthora*, *Pythium*, and *Rhizoctonia* on favorable soil condition.

![Figure 1](image1.png)

**Figure 1.** Fluorescence bacteria from rubber tree rhizosphere on King’s B medium.

Based on the Gram reactions, it was found that rhizobacterial community of healthy rubber plants were dominated by Gram positive bacteria (66.52%) compared to Gram negative bacteria(33.48%). On the contrary, rhizobacterial community of infected rubber plants were dominated by Gram negative bacteria (53.6%) compared to Gram positive bacteria (46.4%). Gram-positive bacteria such as *B. subtilis* can produce antibiotic named Iturin A. Iturin A is an amino acid, which produces 7 remaining amino acids α and 1 remaining amino acid β. The working power of the antibiotic iturin A is so strong that it can suppress the pathogen *R. solani* RB 14 in tomato plants [9, 11].
The rhizosphere of healthy rubber plants has higher number of bacterial population, diversity of morphological types, presence of fluorescent and Gram-positive bacteria, and antagonistic bacteria against *R. Microporus* than the infected rubber plants.

3.2. Antagonistic test of rhizobacteria against *R. microporus*

Antagonistic test of rhizobacteria against pathogenic fungi *R. microporus* on 7 d after inoculation had shown that there was a difference in the ability of the bacteria to inhibit the growth of *R. microporus* colonies. The colony interactions were performed in 3 types. The 1st type was the cessation of *R. microporus* growth when in contact with bacterial colonies and without inhibition zone. The 2nd type was the formation of an inhibition zone on the side of the *R. microporus* colonies that faces bacterial colonies. Both types was antagonistic interactions. Whereas in the 3rd type, the fungus colonies of *R. microporus* grew through bacterial colonies resulting in non-antagonistic interactions (Fig. 2).

![Figure 2](image_url)

**Figure 2.** Interaction of rhizobacterial colonies and *R. microporus*; (A) *R. microporus* colonies across bacteria; (B) rhizobacterial colonies induced inhibition zone (antibiosis); (C) colonies *R. microporus* was in contact with bacterial colonies.

Antagonistic interactions are a form of inhibition by beneficial microorganisms by creating unfavorable condition for the pathogenic microorganisms [10]. This situation can be maintained to control biological plant pathogens. There are several antagonistic mechanisms, i.e. (1) competition, (2) acidic substance diffusion, (3) antibiosis, (4) hyperparasitism, and (5) predation [12].

From this research, as many as 30 antagonistic rhizobacteria against *R. microporus* were successfully isolated. We measured the width of inhibition zone to categorize the bacterial isolates. We found that the number of bacteria which has less than 5 mm inhibition zone was same either in the healthy or infected rubber plants. However, the number of bacterial which has more than 5 mm inhibition zone in healthy rubber plants was higher than in infected rubber plants. The inhibition zone indicates the amount of metabolites produces by antagonistic bacteria in inhibiting the growth of *R. microporus*. In percentage wise, antagonistic bacteria either in healthy or in infected rubber plants are relatively similar at 60%. Moreover, mutualistic and antagonistic interaction are naturally occurred not only in healthy but also in infected plant ecosystem.

4. Conclusion

Based on the observations of rhizobacteria in infected and healthy rubber plants, it can be concluded that the diversity of morphological types, the population of antagonistic bacteria, and the number of Gram positive were higher in the healthy rhizosphere of rubber plants. Moreover, the antagonistic ability of bacteria with a inhibition zone width of more than 5 mm was dominated by the bacterial isolates from healthy rubber plants.
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