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Diversity of Endophytic Bacteria and their Potential as Biological Agents Against *Rigidoporus microporus* Causes White Root Disease in *Hevea brasiliensis*

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HIGHLIGHTS

- Endophytic bacteria have potential to suppress *Rigidoporus microporus* causes white root disease.
- Endophytic bacteria can solubilize phosphate, which plays a role in reducing the disease index.
- Endophytic bacteria have potential as biocontrol and biofungicide against white root disease.

Abstract: The big problems of rubber (*Hevea brasiliensis*) horticulture are the present of pathogenic fungus, *Rigidoporus microporus*, which causes white root disease. Endophytic bacteria have potential to inhibit the pathogenic fungus. This study aims to isolate, identify and evaluate the diversity of endophytic bacteria that can suppress the growth of *R. microporus* on rubber. Identification of endophytic bacteria based on morphology characters and molecular marker using 16S rRNA primer. In vitro assessments of biological agents were carried out using fermentation medium. In vivo tests were carried out in the nursery using rubber. The isolation results obtained 55 isolates of endophytic bacteria. Isolate B49 from bark showed the highest percentage of inhibition against the growth of *R. microporus* (82.47%), then followed by isolates D8 (81.44%) and D20 (72.16%) from leaves, and A27 (61.86%) from roots. The 16S rRNA gene sequence of the D8 isolate exhibited 98% similarity to *Bacillus paramycoides*, D20 isolate was shown 95% similarity to *Acinetobacter nosocomialis*, A27 isolates identified as *Enterobacter cloacae* (99%), and B49 identified as *Bacillus cereus* (99%). In addition to inhibiting the growth of *R. microporus*, several endophytic bacterial isolates used in this study were shown to be able to solubilize phosphate thus suppress white root disease. *Bacillus* D8, *Acinetobacter* D20, and *Bacillus* B49 can be used to control white root disease in rubber plantations. Selected bacteria isolated from rubber have the potential to be used as a biocontrol and as a biofungicide against white root disease caused by *R. microporus*.

Keywords: biological control; endophytic bacteria; *Rigidoporus microporus;* rubber; white root disease.

INTRODUCTION

Rubber (*Hevea brasiliensis* (Willd. Ex A. Juss) Mull. Arg.) is one of the important commodities for the Indonesian economy that can produce natural latex. Rubber plant is one of the leading commodities developed by Riau Province. Riau Province has a rubber plantation area of 494,106 (2019) and 517,317.00 Ha (2020) with a production of 373-726 tons [1]. The low rubber production is caused by several obstacles, namely the seedlings condition, planting season, and disease attacks. One of the diseases attack very detrimental to farmers is white root disease caused by *Rigidoporus microporus* [2,3].

The fungus lives parasitically over a long period of time by producing white mycelia attached to the the surface of the root bark [4]. The mechanism of *R. microporus* infection against rubber plants is that the fungus contacts the rubber roots and releases lignocellulase enzymes that can damage the roots of these plants and then colonize other parts of the roots [5]. This disease can cause plant death in high intensity, especially in plants aged 2 to 6 years [6]. The symptoms of white root disease attack are pale, yellow, and dull colored leaf crowns, which eventually dry and fall, leaving only the twigs visible. Sometimes diseased plants form young leaves or flowers and fruit at an earlier time [7]. When the roots are opened, it can be seen that the root surface is overgrown with white fungal mycelium or rhizomorphs that are firmly attached to the roots making it difficult to remove [8]. Infected roots eventually rot and turn brown [9]. So far, people have used chemicals to treat white root fungus.

The most commonly used fungicides with the active ingredient triadimefon, which are chemicals that have a low potential for cumulative toxic effects on plants. Synthetic fungicides have a fairly high toxic effect on humans [10], and cause accumulation of toxins and damage to the soil and other beneficial soil organisms [11]. Excessive use of pesticides and improper doses can cause the fungus to become more up resistant [12]. Another alternative is to use biocontrol agents capable of suppressing the growth of white root fungus, reducing the use of chemicals and environmental pollution [13]. Biological control can enhance the availability of certain nutrients phosphate and potassium solubilization [14] and nitrogen fixation [15], or synthesize the phytohormones such as gibberellins and indole-3 acetic acid) [16].

Several previous studies have identified fungi and bacteria that can act as biocontrols by suppressing the growth of white root fungi, such as *Trichoderma* sp. and *Penicillium*, in a study at the Rubber Research Institute of Nigeria (RRIN), which can inhibit *R. microporus* by 81.85% and 65.27% [17]. The research results by [18] using *Bacillus* sp. and *Bacillus apiaries* had inhibition zones of *R. microporus* of 5.57 mm and 6.12 mm using the dual culture method. In addition, *Streptomyces* sp. TM32 with biomass measurement can suppress the growth of *R. microporus* is close to 100%. The survival and health test results on rubber plants, suppression of white root disease by *Streptomyces* sp. TM32 was shown to suppress *R. microporus* 20% greater than with metalaxyl chemicals in rubber plants. Modification of *Streptomyces* sp. TM32 with organic matter carrying sorghum seeds at week 14 of the experiment showed an average increase in the most optimal cumulative height of rubber trees of 35 cm [13]. *Streptomyces* application in vivo can inhibit and control *R. microporus* in long-term rubber plantations [19].

Studies on the use of rubber endophytic bacteria that can inhibit *R. microporus* have not been widely reported. The use of local isolates of rubber endophytic bacteria isolated directly from the rubber plantation of Siabu Village, which was attacked by the fungus *R. microporus* was expected to inhibit *R. microporus* more effectively than other biocontrol agents. Thus, the presence of indigenous endophytic bacteria is expected to suppress and fight the growth of *R. microporus* on rubber seedlings. This study aims to isolate, identify and evaluate the diversity of endophytic bacteria that can suppress the growth of *R. microporus* on rubber plants.

MATERIAL AND METHODS

Isolation of endophytic bacteria from a rubber

The specimen of rubber plants *(Hevea brasiliensis*) was collected from Siabu Village, Salo, Riau Province with coordinates 01^{o}99’2.06”N, 101^{o}05’99.14”E. Bacteria were isolated using a modified technique described previously in [20]. The root, bark, and leaf were cut to a size of 2 cm x 2 cm and then thoroughly cleaned under running tap water. The surface of each sample was sterilized by immersing it in 70% ethanol for two min, soaking it in NaOCl (3%) for two min, rinsing it three times in distilled water, and drying it with sterile filter paper. Aliquots of the completed rinse's sterile distilled water were splattered onto the surface of Nutrient Agar (NA) plates for 48 hours to ensure that the surface had been sterilized successfully. After surface sterilization, sample were used to isolate endophytic bacteria using two methods: First, each leaf was placed

onto NA plates and incubated at room temperature until the bacteria started to grow; second, each small piece of leaves was crushed with the addition of 1 ml of sterile 0.85% sodium chloride, and then spread over the surface NA plates followed by incubation at room temperature until the bacteria started to grow [21].

Characteristics endophytic bacteria from a rubber

The morphology of endophytic bacteria was observed, including the color, shape, elevation, and margin. Cell shape and Gram staining were used to observe the morphology of bacteria cells [22].

Screening inhibition of endophytes bacteria against *Rigidoporus microporus*

Isolates of *R. microporus* at five days aged at Potato Dextrosa Agar (PDA) medium were chopped with a diameter of 0.5 cm. Fungus disk pieces then inoculated into the middle petri dishes containing NA medium. Endophytic bacteria disk isolates aged 24 hours then inoculate at four sides of the edge of petri dishes with 2.5 cm at length from fungi. Isolates of *R. microporus* were inoculate at NA medium without treatment were consider as control [23]. The petri dishes were incubated for 5 days at room temperature the inhibition was observed by the inhibition of mycelia of *R. microporus* [24].

In vitro antagonistic activity test

The ability of endophytic bacteria isolates to suppress the growth of *R. microporus* in fermentation media was tested by adding 1 mL of endophytic bacteria (10^8 cfu/ml) and 1 mL of *R. microporus* (10^8 cfu/ml) into 100 mL of Potato Dextrose Broth (PDB) aseptically. Each treatment was made with three replications and incubated for five days at a temperature of ± 28ºC on a shaker incubator with a speed of 150 rpm. At the end of incubation, the mass of the fungus *R. microporus* was separated from the PDB medium by filtration using Whatman paper No. 42 (dried and weighed the initial weight (W1)). The fungus mass was then dried for 16 hours at 80ºC in an incubator. Furthermore, the dry weight of fungal biomass (W2) was weighed. WT = W2 – W1, where WT = total dry weight of the fungus after being treated with endophytic bacteria (g), W2 = dry weight of the fungus after being treated with endophytic bacteria (g), W1 = dry weight of Whatman paper (g) [25]. The percent inhibition = control biomass endophytic – bacteria treatment biomass x 100%. At the end of the incubation time the fungal hyphae of *R. microporus* that grew were observed under a microscope to know physiology respond. In vitro antagonistic activity test using a completely randomized design with 3 replicates.

Screening phosphate solubilizing

Each isolate of endophytic bacteria was inoculated in petri dish contain Pikovkaya media. Pikovkaya media consisting of bacto agar 20 g, glucose 10 g, $Ca_3(PO_4)_2$ 5.0 g, $(NH_4)_2SO_4$ 0.5 g, KCl 0.2 g, $MgSO_4$ $7H_2$ 0.1 g, yeast extract 0.5 g, $MnSO_4$ $7H_2$ 0.002 g, $FeSO_4$ $7H_2O$ 0.002 g, 1000 mL of distilled water [26]. Petri dishes were incubated for 3 days at room temperature. Then the clear zone formed was observed and measured every day using a caliper [27]. The Phosphate Solubilization Index (PSI) value can be calculated using the following formula [28]:

$$\text{PSI} = \frac{\text{colony diameter + clear zone diameter}}{\text{colony diameter}} \text{x}100$$

Coldentification of endophytic bacteria based on 16S rRNA.

Each isolates DNA template was extracted using the Presto™ Mini gDNA Bacteria kit. The 16S rRNA genes have been amplified using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') [22] and performed in 50 µL reaction volumes of 25 µL 2X Taq Master Mix, 1.0 µL of each primer, 1 µL DNA template and 22 µL of deionized water. The reaction was carried out under conditions of amplification of 94°C at 2 min for pre-denaturation followed by 35 cycles of denaturation (94°C final extension was at 72°C; 1 min), annealing (46.4°C; 1 min) and extension (72°C; 1 min). For the PCR products, gel electrophoresis was used 2% agarose with TBE buffer at 100 V for 40 min. The PCR product was then sequenced at 1st BASE Laboratory.

In vivo antagonistic as biological control white root disease in rubber seedling

The biocontrol ability of endophytic bacteria based on disease suppression in rubber colonized by the *R. microporus* strain. Research with a completely randomized design, each with five replicates referring to [10]

with modifications. The test rubber seedlings used were seedlings from rubber plantations. Two-month-old seedlings were cut off the taproot until 5 cm remained from the root neck. Then the rubber seedlings were transferred and regrown in polybags measuring 30 x 25 cm containing 3 kg of sterile soil media. Plants were maintained for two months. Pathogen inoculum was prepared by inoculating 5 x 1 x 1 cm of rubber wood with *R. microporus* culture and incubated for 7 days. Pathogen inoculation was done by planting two pieces of inoculum tangent to the taproot of the test rubber seedlings.

The endophytic bacterial isolates selected were the four isolates that were highest in suppressing the growth of *R. microporus* fungal colonies. Potential isolates were cultured on Nutrient Broth (NB) medium for 24 hours and prepared as cell suspension at the final concentration 1 ml of 10^8 CFU/ml in pot containing sterilized mixed soil (cornmeal: sawdust; 1:1), then incubated for 7 days [29].

Endophytic bacteria were applied together with the pathogen inoculum. After the rubber seedlings were two months old, the pathogen inoculum was placed around the rubber taproot. Next, 50 grams of endophytic bacterial isolates were inoculated around the rubber taproot by immersion. Two months after inoculation, observations were made on the pathogen *R. microporus* and endophytic bacteria. The parameters observed were increased stem height, number of leaves, disease intensity, number of soil microbes, and an overview of rubber wood infection by *R. microporus* [10,30]. The Disease Index (DI) is the percentage of yellowing of rubber leaves with percentage, healthy: 0% yellow leaves, light: 1-25%, medium: 26-50%, heavy: 51-75%, and extra heavy: 76-100% [30].

Data Analysis

Isolation result of endophytic bacteria from rubber tree and screening of inhibition were analyzed descriptively. The homology of the 16s rRNA gene sequence was analyzed using the Mega BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). One-way analysis of variance (ANOVA) and if there is a significant difference, it is continued with Duncan's Multiple Range Test at the 5% level (DMRT). Data processing was carried out using the SPSS program version 17.0.

RESULTS

Isolation and characterization endophytic bacteria from rubber

From this study, 55 isolates of endophytic bacteria were obtained, including 17 isolates from roots, 17 isolates from bark and 21 isolates from leaves. Different organs show different endophytic bacteria. Isolation of endophytic bacteria from rubber by direct and grinding methods. The direct isolation technique causes the possibility of endophytic bacteria to grow to be limited. The use of this technique causes not all endophytic bacteria to be isolated or cultured. In addition to the direct isolation and grinding method can be used to isolated bacteria endophytic.

Isolates of endophyte bacteria were tested towards *R. microporus.* Screening test were carried out first before test of antifungal activity. Screening test were conducted to select isolates of endophytic bacteria that have antifungal activity against *R. microporus.* Based on this study, 11 out of a total of 55 endophytic bacterial isolates were found to have inhibitory power characterized by slow growth of *R. microporus*.

Partial characterization results generally isolate obtained have a white color, flat elevation, and entire edges with coccus cell shape. Morphology colony and Gram staining of endophytic bacterial it is obtained that 7 isolates were Gram negative and 4 isolates were Gram positive (Table 1; Figure 1).

Table 1. Morphology and cultural characterization of endophytic bacterial.

No	Isolates Code	Colony morphology			Cell morphology		
		Upper surface color	Lower surface color	Elevation	Edges	Shape	Gram staining
1	D7	White	White	flat	undulate	Coccus	Negatif
2	D8	White	White	flat	undulate	Bacill	Positif
3	B14	White	White	flat	undulate	Coccus	Positif
4	D20	Milky white	Milky white	convex	entire	Coccus	Negatif
5	A27	White	White	flat	entire	Bacill	Negatif
6	A28	White	White	flat	entire	Coccus	Negatif
7	D36	White	White	flat	undulate	Coccus	Negatif
8	A44	Milky white	Milky white	convex	entire	Bacill	Negatif
9	B46	Milky white	Milky white	flat	entire	Coccus	Negatif
10	B49	Milky white	Milky white	flat	undulate	Bacill	Positif
11	D54	Milky white	Milky white	flat	entire	Bacill	Positif

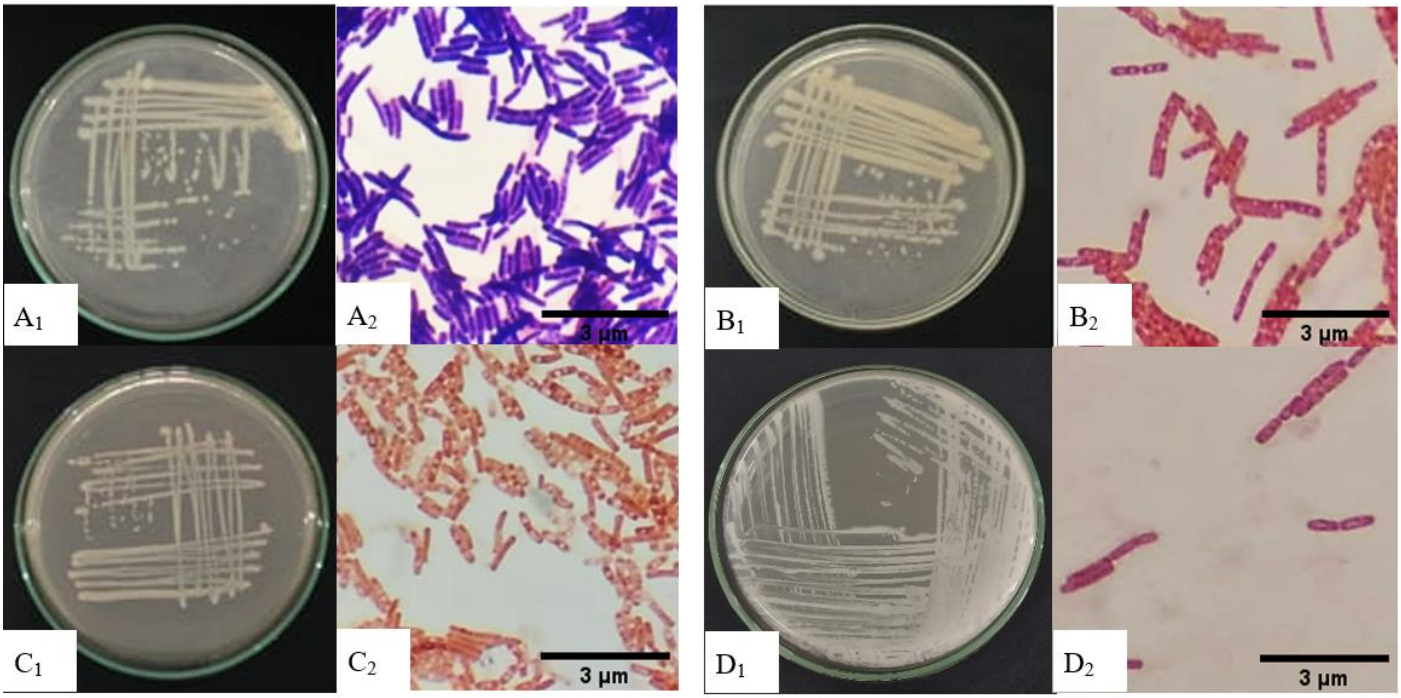

Figure 1. Morphology of four isolates with suppressing the growth of white root disease. 1. colony morphology; 2. gram staining. A. D8, B. A27, C.D20, D.B49.

Gram positive bacteria were characterized with purple color while Gram negative bacteria characterized with pink color. Based on this partial characterization, in line with the research conducted by [20] who have isolated endophytic bacteria from rubber in South Sumatra, the results showed that most bacteria are Gram-negative bacteria. The same research on different plants conducted by [31] reported that 78% of the total isolates of endophytic bacteria were Gram-negative bacteria.

In vitro antagonistic activity

Endophytic bacteria from rubber live and colonize in the rubber plant tissue, roots, bark, and leaves. The results of the study in Table 2. show that the biomass of *R. microporus* with the administration of each endophytic bacterial isolate had an effect on suppressing the growth of white root disease (*R. microporus*) with different compressive abilities compared to the control. Isolate B49 from bark showed the highest percentage of inhibition against the growth of *R. microporus* (82.47%). This is indicated by the least biomass of *R. microporus*, which is 113.33a ± 11.55 mg compared to the control (646.67 mg), then followed by isolates D8 and D20 from leaves, and A27 from roots.

Table 2. Biomass of *R. microporus* inoculated with rubber endhophytic bacteria in fermentation media for 5 days incubation and the phosphatase index.

No	Isolate	Average Biomass (mg)	% Inhibition
1	D7	540.00^{cd} ± 62.45	16.49%
2	D8	120.00^{a} ± 26.46	81.44%
3	B14	590.00^{de} ± 36.06	8.76%
4	D20	180.00^{ab} ± 65.57	72.16%
5	A27	246.67^{b} ± 92.92	61.86%
6	A28	520.00^{cd} ± 52.92	19.59%
7	D36	560.00^{de} ± 30.00	13.40%
8	A44	453.33^{c} ± 61.10	29.90%
9	B46	526.67^{cd} ± 87.37	18.56%
10	B49	113.33^{a} ± 11.55	82.47%
11	D54	573.33^{de} ± 70.95	11.34%
12	Control	646.67^{e} ± 11.55	-

Numbers followed by the same notation in the same column group show no significant difference at the 5% level according to DMRT.

The different inhibition results was because each endophytic bacterium has different antifungal abilities. The four isolates could inhibit fungal growth with suppression of mycelial biomass. In addition, the inhibitory effect of potential endophytic bacterial isolates on *R. microporus* was investigated using light microscopy. Morphology of *R. microsporus* hypha growth that endophytic bacteria have suppressed in comparison with hypha without treatment. Fungal hyphae from the antagonistic test against *R. microporus* compared to the control showed that the hyphae of *R. microporus* treated with endophytic bacteria wilted (Figure 2).

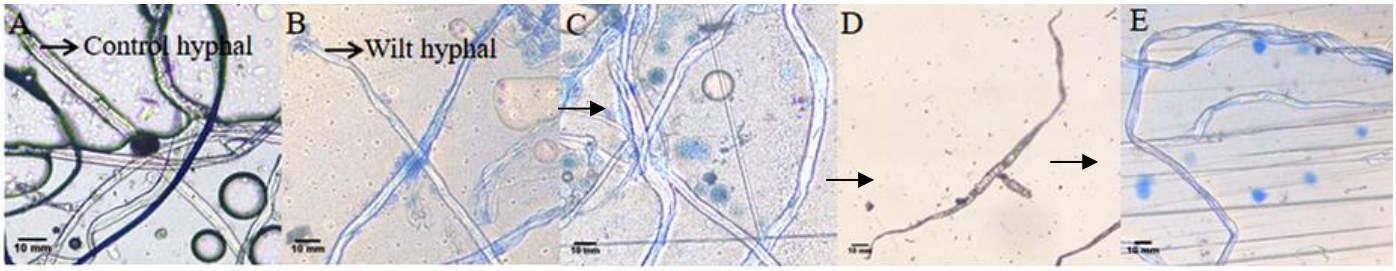

Figure 2. Aerial hypha under light microscopy at 400x magnification. A. Control, B. Isolate D8, C. Isolate D20, D. Isolate A27, and E. Isolate B49.

Phosphate Solubilizing

The formation of a clear zone on Pikovskaya media characterizes the ability of phosphate-solubilizing microbe, indicating that the microbes can dissolve tricalcium phosphate ($Ca_3[PO_4]_2$) [32]. The phosphate solubilizing ability of bacteria can be seen from the value of its phosphatase index. Four of the 11 isolates used in this study had the most significant inhibition percentage against *R. microporus*. And of the four isolates, two are stable positive dissolving phosphate in Pikovkaya selective medium, namely isolates D20 and D49 (Table 3).

Table 3. Potential of bacteria to solubilize phosphate in Pikovkaya agar medium.

No	Isolate	Phosphatase index
1	D8	-
2	D20	1.74± 0.04
3	A27	-
4	B49	1.88± 0.05

Molecular identification of selected endophytic bacteria

The partial 16S rRNA gene sequences of the isolates were analyzed for homology using the BLAST algorithm in GenBank. The 16S rRNA gene sequence of the D8 isolate exhibited 98% similarity to *Bacillus paramycoides*, while D20 isolate was shown 95% similarity to *Acinetobacter nosocomialis*. Isolate of A27 identified as *Enterobacter cloacae* (99%) *and* B49 identified as *Bacillus cereus* (99%). Phylogenetic tree obtained using the neighbour-joining method showed a close relationship among strains (Figure 3).

In vivo evaluation of endophytic bacterial antagonists on *R. microporus*

Endophytic bacterial isolates selected on the basis of their potential to inhibit the growth of the *R. microporus* were then evaluation tested of fungal antagonists in vivo on rubber. In vivo experiments are essential to verify the effectiveness of potential antagonists. The parameters observed were the number of leaves and plant height increase in two months of inoculation (Table 4 and 5).

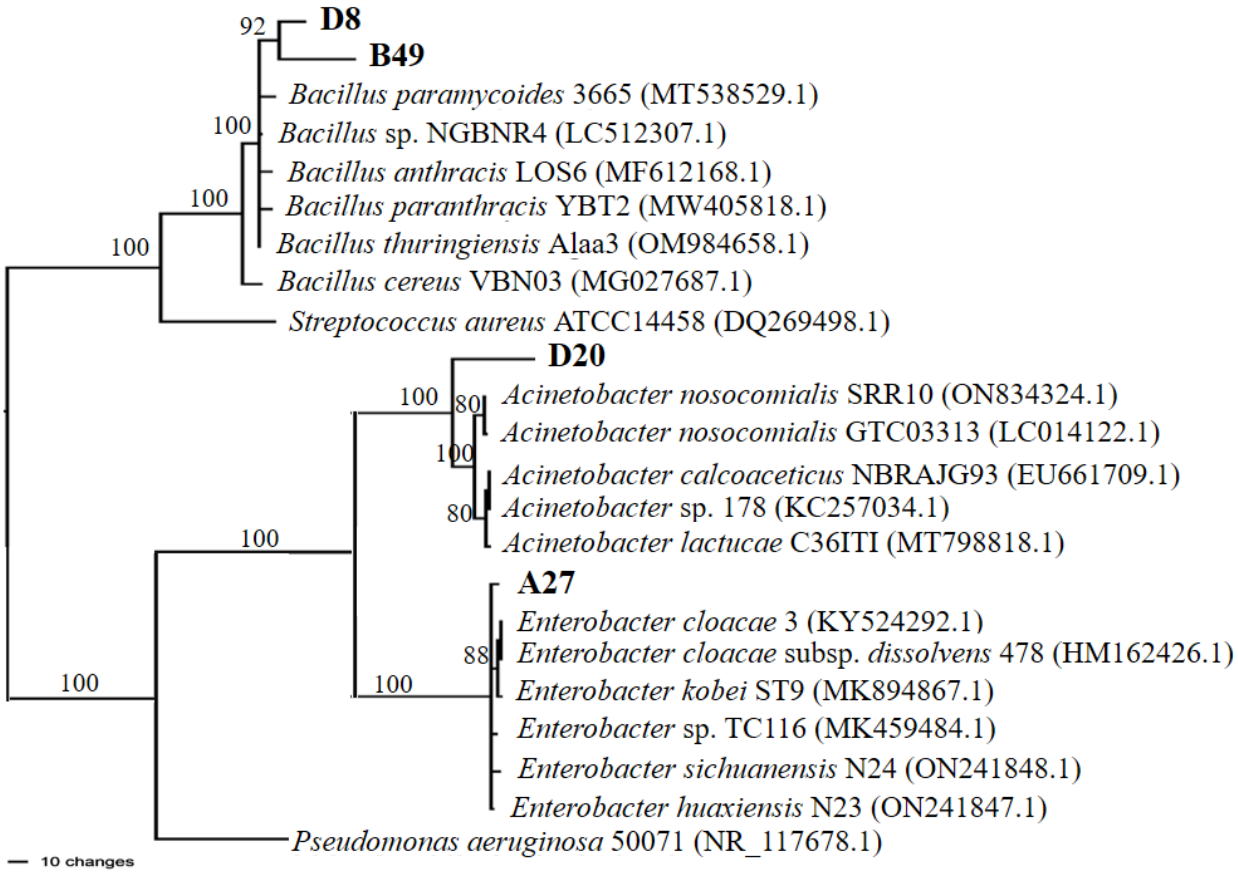

Figure 3. Neighbour-joining trees based on 16S rRNA gene sequences showing the positions of D8, D20, A27, and B49 isolates and related species. Bootstrap values greater than 50% of 1000 replications are shown as percentages at branching points of the tree.

Table 4. Effect of endophytic bacteria on the number of leaves of rubber seedlings inoculated with *R. microporus* during the two-month application period.

		Leaf number	
Treatments	**Pre-application**	**1st month**	**2nd month**
Control with *R. microporus*	9.330	10.33 a	10.33 a
Control without *R. microporus*	11.67	12.67 a	10.67 a
D8 + *R. microporus*	10.00	8.67 a	10.33 a
D20 + *R. microporus*	10.67	10.33 a	11.00 a
A27 + *R. microporus*	13.67	12.33 a	13.00 a
B49 + *R. microporus*	12.67	12.67 a	12.67 a

Numbers followed by the same notation in the same column group indicate not significantly different at the 5% level according to DMRT.

Table 5. Effect of endophytic bacteria on rubber plant height gain (cm) inoculated with *R. microporuss* over a two-month application period.

		Height increment (cm)	
Treatments	**Pre-application**	**1st month**	**2nd month**
Control with *R. microporus*	44.57	2.77[a]	7.79[ab]
Control without *R. microporus*	39.70	1.47[a]	5.31[a]
D8 + *R. microporus*	43.60	3.27[a]	13.65[bc]
D20 + *R. microporus*	49.80	8.97[b]	17.96[c]
A27 + *R. microporus*	51.13	1.37[a]	3.94[a]
B49 + *R. microporus*	43.80	3.20[a]	9.67[ab]

Numbers followed by the same notation in the same column group indicate not significantly different at the 5% level according to DMRT.

The results obtained on the number of leaves parameter did not show a significant effect. This may be due to the fact that the sawdust planting medium contains cellulose as the main ingredient for the growth of *R. microporus*. In addition, the age of the rubber used was younger at 4 months, with an incubation period of only 2 months. In general, other researchers use 15 months old or more rubber to have more lignin content and a longer incubation period of up to 3 months to 12 months [33]. While the increase in the height of rubber plants treated with endophytic bacteria isolate D8, D20, B49, experienced a greater increase in the height of 3.27, 8.97, and 3.20 compared to the control treatment after one month of application and 2 months of application, but the isolate A27 did not experience significant height gain.

These studies indicated that treatment effectively eliminated the pathogen by the tested isolates. Observations on the rubber bark showed the effectiveness of endophytic bacteria, which was better than the control attack by white root disease. Rubber plants treated with endophytic bacterial isolates have a good and clean bark texture without any fungal hyphae (Figure 4).

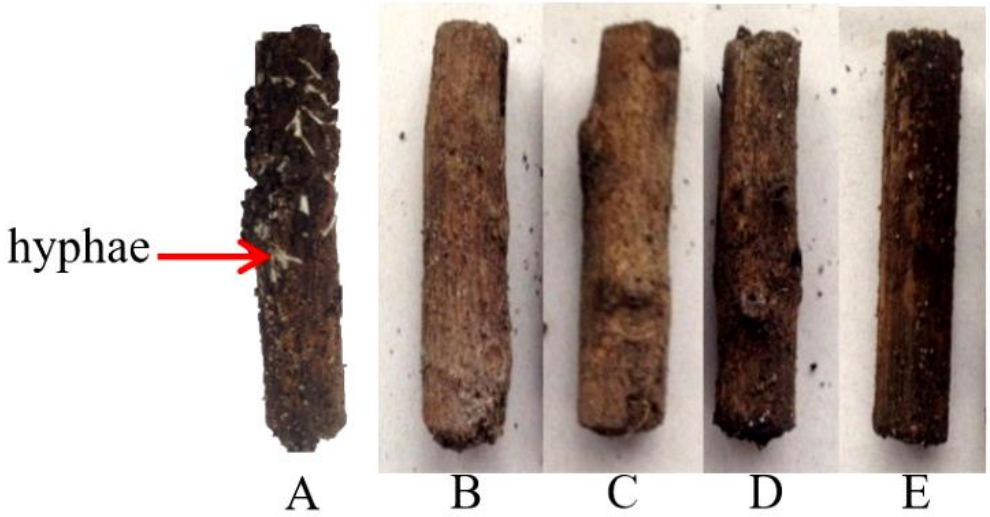

Figure 4. Rubber wood pieces treated with *R. microporus* fungus after two months of application (A) Control, (B) isolate D8, (C) isolate D20, (D) isolate A27, and (E) isolate B49.

The observation results were also seen in the condition of the leaves. Disease index (DI) was calculated according to the score by [30] by calculating the percentage of yellowing leaves during two months of application of rubber endophytic bacteria and *R. microporus* (Table 6). Isolate D20 has a light disease index and isolates of D8, A27, and B49 have medium disease. Phosphate dissolving ability is thought to cause suppress of disease. Isolates that can dissolve phosphate have light disease, namely isolates D20 and D49. Leaves are yellow and will fall on rubber attacked by white root disease, while those treated with endophytic bacteria have normal green and healthy leaves (Figure 5).

Table 6. Effect of endophytic bacteria on disease Index in rubber seedlings after 2 months of application period.

Treatments	**Disease Index (%)**	**Intensity**
Control with *R. microporus*	65,61	Heavy
Control without *R. microporus*	16,88	Light
D8+ *R. microporus*	35,83	Medium
D20+ *R. microporus*	23,43	Light
A27+ *R. microporus*	30,03	Medium
B49+ *R. microporus*	28,71	Medium

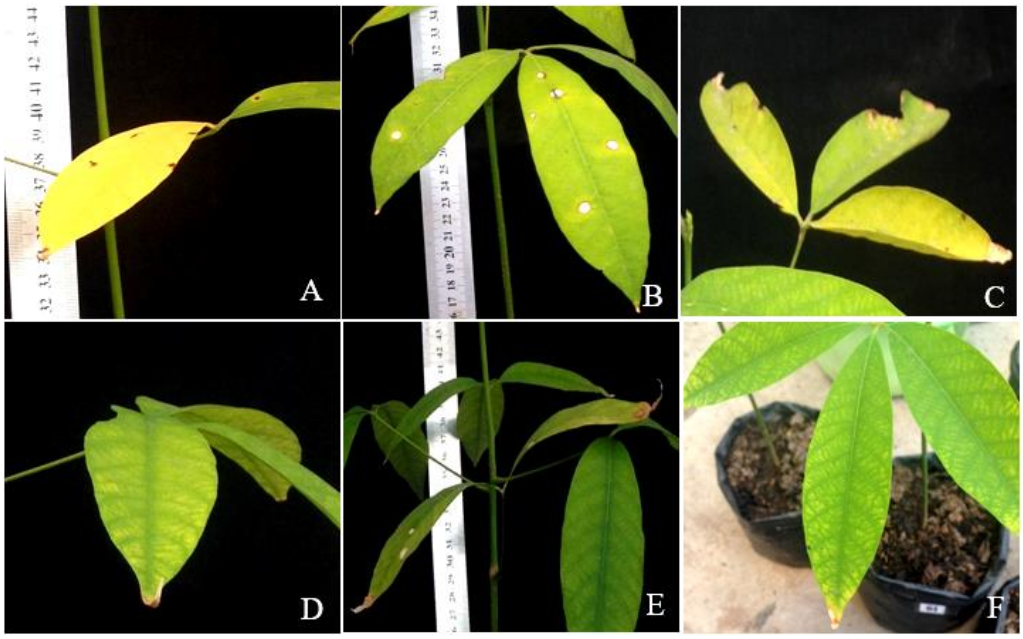

Figure 5. Rubber seedling leaves after two months of application (A) Control with *Rigidoporus microporus*, (B) Control without *R. microporus*, (C) isolates D8, (D) isolate D20 (E) isolate A27, and (F) isolate B49.

DISCUSSION

Endophytic bacterial populations derived from epiphytic populations on root surfaces and leaf surfaces that are known to be able to enter plant tissues (endophytes) can be invested into seedlings and planting materials [34,35,36]). When in plant tissue, endophytic bacteria will occupy certain parts of the plant tissue or systemically colonize (37,38]. Microorganisms can live inside the cell, in the space between cells between cells, or in the transport tissue system of the plant (34,39,40]

Previous research has reported that of the five endophytic bacterial isolates, there is one isolate that can inhibit the growth of *R. microporus* by 0.3 cm [41]. Meanwhile, the study [42] obtained endophytic bacterial isolates from rubber roots that were able to inhibit *Phytophthora meadii*. This condition shows that endophytic isolates isolated from rubber plants have different inhibitory activities on different pathogen tests. *Klebsiella variicola* from rubber plant leaves have the potential to biocontrol against pathogenic fungi and are indicated to have chitinolytic activity known as chitinase chitinolytic Index (IC) of 1.66- 2.08 [22].

According to the study, endophytic bacteria from rubber plants (root, bark, and leaves) were identified using partial 16S rRNA sequencing, *Bacillus* D8*, Acinetobacter* D20, *Enterobacter* A27*,* and *Bacillus* B49 were identified. The impact of the application of biological agents is not immediately visible in a short time but takes a long time to provide environmental stability to suppress the development of pathogen infection and reduce disease intensity.

From this study, there were two *Bacillus* spp isolates had a high percentage of inhibition of more than 80%, namely isolates D8 from leaves and B49 from bark. *Bacillus* spp. are endophytic bacterial species that have proven to be widely used for the biocontrol of phytopathogenic fungi [43,44,45]. This phenomenon can be attributed to their distinct secondary metabolites and rapid growth that allow them to be integrated into biocontrol studies [46]. *Bacillus* spp. can suppress white root disease with an intensity of 80.95%-82.91%. The percentage of inhibition from the results of research using *Bacillus* strains isolated from rubber rhizosphere can inhibit *R. microporus* by 72.69-90.94% [47]. *Bacillus* sp. isolates can produce various antibiotics, such as zwittermicin (aminoglycoside) antibiotics in *Bacillus*, which can control damping-off disease in alfalfa plants [48].

Some *Bacillus* species, such as *B. cereus* from rubber roots, can inhibit the growth of *R. microporus* fungal colonies in vitro [40]. *B. cereus* AR156 from tomato root exudates was involved in biocontrol against the fungus *Acalstonia solanacearum* [49]. *B. cereus* IB311 which is antagonistic to plant pathogens *Pseudomonas syringae* and *Agrobacterium tumefaciens* [50]. *B. velezensis* NH-1 was used for biocontrol of Fusarium wilt caused by *F. oxysporum* on cucumber [51], *B. paramyco*ides for biocontrol of *Fusarium* wilt on fava beans [52]. *Bacillus proteolyticus* from rubber plants were shown to be able to produce α-amylase inhibitors by 29.44%, which has the potential as a candidate for antidiabetic agents [53].

This study is the first report to use native *Acinetobacter* and *Enterobacter* from rubber to control white root disease caused by *R. microporus* in vitro. There are studies that have proven in vitro that Acinetobacter

and Enterobacter strains can be used as potential biocontrol agents against *Ralstonia* wilt disease in tomato [54]. Several species used as biocontrol, such as *Acinetobacter lwoffii* PTA-113 and PTA-152 from the rhizosphere, can efficiently protect grape leaves from gray mold disease caused by *Botrytis cinerea* [55]. *Acinetobacter calcoaceticus* SJ19, Algerian rhizobacteria protecting tomato plants against tomato grey mould [56]. *Acinetobacter nosocomialis* is known for antimicrobial resistance, surface motility, and biofilm formation [57].

Some Enterobacter species such as *E. cloacae* can suppress the plant pathogen *Pythium ultimum* on cucumber [58]. *E. cloacae* PS14, as an endophytic bacterium, is effective in controlling *R. solanacearum,* which causes wilt disease in potatoes with mode of action by producing siderophores, indole-3-acetic acid, hydrogen cyanide, and salicylic acid [59]. *Enterobacter cloacae* isolated from banana roots and leaves have been studied to potentially suppress the black Sigatoka fungus and support plant growth in soils lacking organic matter [60].

The percentage of inhibition can be influenced by the type of isolate, inoculum concentration and incubation time. According to [61], the ability of endophytic bacteria to suppress pathogens involves one or several inhibitory mechanisms including rapid bacterial growth and the production of secondary metabolites such as chitinase, proteinase, glucanase and lipase. The ability of a biological agent, especially endophytic bacteria, to suppress pathogens usually involves one or several inhibitory mechanisms, such as rapid bacterial growth. It can produce cell wall degrading antibiotic compounds such as chitinase, proteinase, glucanase, and lipase enzymes to inhibit the pathogen *R. microporus* [62]. Chitinase activity is known to destroy fungal cell walls consisting of chitin [20]. Research conducted by [34], showed that *Bacillus cereus* A10 isolates isolated from the roots of healthy rubber plants in white root fungus endemic areas could inhibit white root fungi with produces proteinase and gelatinase enzymes.

The different biomass of *R. microporus* at the end of incubation with the addition of the tested bacteria was suspected to produce secondary metabolites with different contents and concentrations. According to [17], isolates of endophytic bacteria produced secondary metabolites such as chitinase enzymes and siderophores. In addition, differences in the biomass of *R. microporus* produced could be influenced by the growth rate and concentration of metabolites of each tested bacterium.

The treatment of endophytic bacteria explored around rubber plants has a percentage of height gain that is significantly different from the control treatment. Endophytic bacteria are known to provide many beneficial effects on their host plants such as stimulating plant growth, fixating nitroogen and inducing plant resistance to pathogens [62,63,64]. From the results of this study, it can be seen that endophytic bacteria are able to stimulate plant height growth.

Although it has not shown a significant effect from observing plant height parameters and the number of leaves, the intensity of disease severity can prove a significant effect. The four endophytic bacterial isolates applied to rubber seedlings showed low and moderate severity compared to the control with *R. microporus*. This indicates an interaction endophytic bacteria thus suppressing the population of *R. microporus.* The disease saverity is related to the phosphate solubilization index. Light disease saverity showed in endophytic bacteria that can solubilize phosphate. So it can be proven that, in addition to inhibiting the growth of *R. microporus*, these endophytic bacteria can also solubilize phosphate, so the disease saverity is light. Phosphate solubilizing bacteria can dissolve phosphate from unavailable to available [65]. Phosphate solubilizing bacteria are said to increase plants disease intensity by spurring root cells to produce compounds inhibiting pathogen growth [66].

From this study, *Bacillus* D8 and *Acinetobacter* D20 isolated from rubber leaves and B49 isolated from rubber bark can be used to control white root disease in nurseries in rubber plantations. While *Enterobacter* A27 isolated from rubber root in vivo has not shown maximum results even though in vitro it has. From this study, selected bacteria isolated from rubber have the potential to be used as a biocontrol and as a biofungicide against white root disease caused by *R. microporus*.

CONCLUSION

The conclusions of this research are endophytic bacteria that successfully isolated consisted of 55 isolates. 11 of total endophytic bacterial isolates were found to have inhibitory power characterized by slow growth of *R. microporus*. Isolate B49 from bark showed the highest percentage of inhibition against the growth of *R. microporus* (82.47%), then followed by isolates D8 and D20 from leaves, and A27 from roots. The 16S rRNA gene sequence of the D8 isolate exhibited 98% similarity to *Bacillus paramycoides*, while D20 isolate was shown 95% similarity to *Acinetobacter nosocomialis*. Isolate of A27 identified as *Enterobacter cloacae* (99%) *and* B49 identified as *Bacillus cereus* (99%). The results of the effectiveness test of isolates on the growth response of rubber showed a significant difference between rubber treated with endophytic

bacterial isolates and plants without endophytic bacterial treatment. In addition to inhibiting the growth of *R. microporus*, several endophytic bacterial isolates used in this study were shown to be able to solubilize phosphate, thus suppress white root disease. *Bacillus* D8, *Acinetobacter* D20, and *Bacillus* B49 can be used to control white root disease in rubber plantations. From this study, selected bacteria isolated from rubber have the potential to be used as a biocontrol and as a biofungicide against white root disease caused by *R. microporus*.

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REFERENCES

1. Central Bureau of Statistics. Riau in Number [Internet]. Pekanbaru: Central Bureau of Statistics of Riau Province. 2020. Available from: https://riau.bps.go.id/indicator/54/220/1/produksi-perkebunan.html.
2. Longsaward R, Pengnoo A, Kongsawadworakul P. Viboonjun U. A novel rubber tree PR-10 protein involved in host-defense response against the white root rot fungus *Rigidoporus microporus*. BMC Plant Biol. 2023; 23(157):1-21.
3. Shabbir I, Abd Samada MY, Othman R, Wong MY, Sulaiman Z, Jaafar NM, et al. White root rot disease suppression in rubber plant with microbial co-inoculants and silicon addition. Rhizosphere. 2020;15(5-6):1-8.
4. Damiri N, Rofiqi R, Mulawarnam, Rahim SE, Febyanti TP. Effect of three composts with active ingredients of *Pseudomonas fluorescens* on the development of white root disease and production of rubber plants. Biodiversitas. 2021;22:3237-42.
5. Oghenekaro AO, Kovalchuk A, Raffaello T, Camarero S, Gressler M. Genome sequencing of *Rigidoporus microporus* provides insights on genes important for wood decay, latex tolerance and interspecific fungal interactions. Sci Rep. 2020;10:5250.
6. Go WZ, Kit LC, Paik SH, Mui YW, Chuah AL, Arthy S, et al. Virulence of *Rigidoporus microporus* isolates causing white root rot disease on rubber trees (*Hevea brasiliensis*) in Malaysia. Plants. 2021; 10(10):2123.
7. Parlindo F, Tondok ET, Wiyono S. Etiology of white root disease of cashew (*Anacardium occidentale* L.). IOP Conf Ser: Earth Environ Sci. 2022;974:012007.
8. Wiyono S, Widodo, Natsuaki KT. Detection of white root fungus (*Rigidoporus microporus* (fr.) overeem) in soil using cassava root baits J. Int. Soc. Southeast Asian Agric Sci. 2020;26:93–9
9. CABI. *Rigidoporus microporus (*white root disease of rubber), CABI Compendium: CABI International; 2022.
10. Manurung L, Lubis L, Marheni, Dalimunthe CI. Testing of various types of active ingredients against white root disease (WRD)*(Rigidoporus microporus (Swartz: Fr))* without tillage area (WTA*).* J Agroekotek. 2015;3(1):168-78.
11. Evueh AG, Okhuoya JA, Osemwegie OO, Attitalla IH, Ogbebor ON. Valuation of phylloplane fungi as biocontrol agent of *Corynespora* leaf fall disease of rubber (*Hevea brasiliensis* Muell. Arg.). World J Fungal Plant Bio. 2011; 2:1-5.
12. Murtado A, Mubarik NR, Tjhajolesono A. Isolation and characterization endophytic bacteria as biological control of fungus *Colletotrichum* sp. on onion plants (*Allium cepa* L.). IOP Conf. Series: Earth Environ Sci. 2020; 457(2020):1-15.
13. Nakaew N, Rangjaroen C, Sungthong R. Utilization of rhizospheric *Streptomyces* for biological control of *Rigidoporus* sp. causing white root disease in rubber tree European. J Plant Pathology. 2015;142:93-105.
14. Jaffar NS, Jawan R, Chong KP. The potential of lactic acid bacteria in mediating the control of plant diseases and plant growth stimulation in crop production - A mini review. Front Plant Sci. 2023 Jan;13:1047945.
15. El-Saadony MT, Saad AM, Soliman SM, Salem HM, Ahmed AI, Mahmood M, et al. Plant growth-promoting microorganisms as biocontrol agents of plant diseases: Mechanisms, challenges and future perspectives. Front. Plant Sci. 2022;13:923880.
16. Gouda S, Kerry RG, Das G, Paramithiotis S, Shin HS, Patra JK. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiol. Res. 2018;206,131–40.
17. Omorusi VI, Omo-Ikerodah EE, Mokwunye MUB. Evaluation of effect of antagonistic fungi and *Arbuscular mycorrhizal* fungi (AMF) on incidences of some disease of *Hevea brasiliensis* (Muell. Arg). Nat Sci. 2011;9:151-4.
18. Muharni, Widjajanti H. Screening of chitinolytic bacteria antagonistic to the growth of white root fungus (*Rigidoporus lignosus*) from the rhizosphere of rubber plants. J Sains. 2011;14:51-6.
19. Chaiharn M, Sujada N, Pathom-aree W, Lumyong S. Biological Control of *Rigidosporus microporus* the cause of white root disease in rubber using PGPRs in vivo. Chiang Mai J Sci. 2019;46(5):850-66.
20. Hidayati U, Chaniago IA, Munif A, Siswanto, Santosa DA. Potency of plant growth promoting endophytic bacteria from rubber plants (*Hevea brasiliensis* Mull. Arg.). J Agronomy. 2014;13(3):147-52.
21. Linda TM, Berlyansah A, Fibriarti BL, Sofiyanti N, Devi S. Isolation and analysis of bioactive compounds endophytic bacteria of sea fern (*Acrtostichum aureum* L.) from Bengkalis Island, Riau. J Bio Trop. 2022;22(1):46-54.
22. Linda TM, Siregar S, Fitri WD, Martina A, Lestari W, Roslim DI, et al. Isolation and screening of culturable endophytic bacteria from leaf of rubber plant that produces of chitinase. J Phys: Conf Ser. 2018:1116-052038.

23. Zhang M, Li J, Shen A, Tan S, Yan Z, Yu Y, et al. Isolation and identification of *Bacillus amyloliquefaciens*IBFCBF-1 with potential for biological control of *Phytophtora* blight and growth promotion of pepper. J Phytopath. 2016:1012-21.
24. Saputra R, Puspita F, Hamzah A, Irfandri, Suryani E. Morphological characterization of *Trihoderma* spp. isolated from the oil palm rhizosphere in peat soils and its potential as a biological control for *Ganoderma* sp. in vitro. J Ilmiah Pertanian. 2022;19(2):56-68.
25. Li J, Gu S, Zhao Z, Chen B, Liu Q, Sun T, et al. Dissecting cellobiose metabolic pathway and its application in biorefinery through consolidated bioprocessing in *Myceliophthora thermophila*. Fungal Biol Biotechnol. 2019; 13(6):21.
26. Sonia AV, Setiawati TC. Activity of phosphate-solubilizing bacteria towards increasing phosphate availability in acid soils. J Agroekoteknologi. 2022;15(1):44-53.
27. Sudarmini DP, Sudana IM, Sudiarta IP, Suastika G. Utilization of phosphate solubilizing bacteria inducing the hormone IAA (Indole Acetic Acid) for the enhancement of soybean (*Glycine Max*) growth. J Agric Sci Biotechnol. 2018;7(1):1-12.
28. Sugianto SK, Shovitri M, Hidayat A. Potential of rhizobacteria as phosphate solvents. J Sains Seni ITS. 2018;7(2):71-74.
29. Suwandi. Evaluation of a combination of mycoparasitic *Trichoderma* isolates in controlling white root disease in rubber seedlings. J HT Tropika. 2008;8(1):55-62.
30. Kaewchai S, Soytong K. Application of biofungicides against *Rigidoporus microporus* causing white root disease of rubber trees. J Agric Tech. 2010;6:349-63.
31. Ramli NR, Mohamed MS, Seman IA, Zairun MA, Mohamad N. The Potential of endophytic bacteria as a biological control agent for *Ganoderma* disease in oil palm. Sains Malaysiana. 2016;45(3):401-9.
32. Mei C, Chretien RL, Amaradasa BS, He Y, Turner A, Lowman S. Characterization of phosphate solubilizing bacterial endophytes and plant growth promotion in vitro and in greenhouse. Microorganisms. 2021;9:1935.
33. Yulia, Suciatmih, Supriyati D, Rahmansyah M. Biodiversity of endophytic bacteria and their antagonistic activity to *Rizoctonia solani*a and *Fusarium oxysporum.* Global J Bio. 2013;2(4):111-8.
34. Frank AC, Saldierna Guzmán JP, Shay JE. Transmission of Bacterial Endophytes. Microorganisms. 2017; 5(4):70
35. Mbai FN, Magiri EN, Matiru VN, Ng'ang'a J, Nyambati VCS. Isolation and characterization of bacterial root endophytes with potential to enhance plant growth from Kenya bramasti rice. American Int J Contemporary Res. 2013;3(4):25-40.
36. Compant S, Cambon MC, Vacher C, Mitter B, Samad A, Sessitsch A. The plant endospherr world-bacterial life within plants. Environ Microbiol. 2020;23(4):812-1829.
37. Sekhar AC, Thomas P. Isolation and identification of shoot-tip associated endophyitic bacteria from banana cv. grand naine and testing for antagonistic activity against *Fusariumoxysporum* f. sp. *cubense*. American J Plant Sci. 2015; 6:934-54.
38. Suryanto D, Yeldi N, Munir E. Antifungal activity of endophytes bacterial isolates from torch Ginger (*Etlingera elicitor* (Jack.) RM Smith)) root to some pathogenic fungal isolates. Int J Pharm Tech Res. 2016;9(8):340-7.
39. Kandel SL, Joubert PM, Doty SL. Bacterial endophyte colonization and distribution within plants. Microorganisms. 2017;5(4):77-89.
40. Noran AS, Maiden NA, Fauzi MAFA. Rhizospheric microorganism from *Havea brasiliensis* and their antagonism on *Rigidoporus microporus*. Int J Agric For. 2015;1:59-65.
41. Setyawan B, Berlian I, Prasetyo NE. Exploration of endhophytic bacteria and their potention in inhibited white root disease (*Rigidoporus microporus).* J Penelitian Karet. 2016;34(2):175-88.
42. Abraham A, Philip S, Jacob CK, Jayachandran K. Novel bacterial endophytes from *Hevea brasiliensis* as biocontrol agent against *Phytiphthora* leaf fall disease. Springer. 2013;58:675-84.
43. Xue C, Penton CR, Shen Z, Zhang R, Huang Q, Li R. Manipulating the banana rhizosphere microbiome for biological control of Panama disease. Sci Rep. 2015;5(1):11124.
44. Lee T, Park D, Kim K, Lim SM, Yu NH, Kim S. Characterization of *Bacillus amyloliquefaciens* DA12 showing potent antifungal activity against mycotoxigenic *Fusarium* species. Plant Pathol J. 2017;33(5):499-507.
45. Khalil MM, Fierro-Coronado RA, Peñuelas-Rubio O, Villa-Lerma AG, Plascencia-Jatomea R, Félix-Gastélum R. Rhizospheric bacteria as potential biocontrol agents against *Fusarium* wilt and crown and root rot diseases in tomato. Saudi J Biol Sci. 2021;28(12):7460-71.
46. Fira D, Dimkić I, Berić T, Lozo J, Stanković S. Biological control of plant pathogens by *Bacillus* species. J Biotechnol. 2018;11:285:44-55.
47. Nasrun, Nurmansyah. The potency of rizhobacteria and natural fungicide for control white root disease in rubber plant. TIDP. 2015;2:61-8.
48. Beneduzi A, Ambrosini A, Passaglia LM. Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Genet Mol Biol. 2012;35(4):1044-51.
49. Wang N, Wang L, Zhu K, Hou S, Chen L, Mi D, et al. Plant root exudates are involved in *Bacillus cereus* AR156 mediated biocontrol against *Ralstonia solanacearum*. Front Microbiol. 2019;10:98.
50. Banerjee G, Gorthi S, Chattopadhyay P. Beneficial effects of bio-controlling agent *Bacillus cereus* IB311 on the agricultural crop production and its biomass optimization through response surface methodology. Anais da Academia Brasileira de Ciências. 2018;90(2):2149-59.

51. Luo W, Liu L, Qi G, Yang F, Shi X, Zhao X. Embedding *Bacillus velezensis* NH-1 in Microcapsules for biocontrol of cucumber *Fusarium* wilt. Appl Environ Microbiol. 2019;85(9):1-13.
52. El-Sersawy MM, Hassan SE, El-Ghamry AA, El-Gwad AMA, Fouda A. Implication of plant growth-promoting rhizobacteria of *Bacillus* spp. as biocontrol agents against wilt disease caused by *Fusarium oxysporum* Schlecht. in *Vicia faba* L. Biomol Concepts. 2021 Dec;12(1):197-214.
53. Elvani VS, Rahmani SS, Dwiningsih S, Linda TM, Fitri WD. Molecular identification of endophytic bacteria from the rubber plants (*Hevea Brasiliensis*) and activities in producing inhibitor α-amylase. Metamorfosa. 2023;10(1):13-22.
54. Xue QY, Chen Y, Li SM, Chen LF, Ding GC, Guo DW, et al. Evaluation of the strains of *Acinetobacter* and *Enterobacter* as potential biocontrol agents against *Ralstonia wilt* of tomato. Biological Control. 2009;48(3):252-8.
55. Aziz PT, Couderchet M, Biagianti S, Aziz A. Characterization of new bacterial biocontrol agents *Acinetobacter*, *Bacillus*, *Pantoea* and *Pseudomonas* spp. mediating grapevine resistance against *Botrytis cinerea.* Environ Experiment Bot. 2008;64(1):21-32.
56. Foughalia A, Bouaoud Y, Chandeysson C, Djedidi, Tahirine M, Aissat K, et al. A*cinetobacter calcoaceticus* SJ19 and *Bacillus safensis* SJ4, two Algerian rhizobacteria protecting tomato plants against *Botrytis cinerea* and promoting their growth. Egypt J Bio Pest Control. 2022;32:12.
57. Knight DB, Rudin SD, Bonomo RA, Rather PN. *Acinetobacter nosocomialis*: defining the role of efflux pump n resistance to antimicrobial therapy, surface motility, and biofilm. Frontiers microbiol. 2018;9:1902.
58. Robberts DP, Short Jr NM, Maloney AP, Nelson EB, Schaff DA. Role of colonization in biocontrol: studies with *Enterobacter cloacae*. Plant Sci. 1994;101(1):83-9.
59. Mohamed BFF, Sallam NMA, Alamri SAM. Approving the biocontrol method of potato wilt caused by *Ralstonia solanacearum* (Smith) using *Enterobacter cloacae* PS14 and *Trichoderma asperellum* T34. Egypt J Biol Pest Control. 2020*;*30:61.
60. Macedo-Raygoza GM, Valdez-Salas B, Prado FM, Prieto KR, Yamaguchi LF, Kato MJ, et al. *Enterobacter cloacae*, an endophyte that establishes a nutrient-transfer symbiosis with banana plants and protects against the black sigatoka pathogen. Front Microbiol. 2019;10:804.
61. Li FX, Ma HQ, Liu J, Zhang C. Antagonistic effects of *Bacillus cereus* strain B-02 on morphology, ultrastructure and cytophysiology of *Botrytis cinerea*. Polish J Microbiol. 2012;61:119-28
62. Gayathri. Isolation of endophytic bacteria from mangoes, bananas and sugarcane for their biological activities. Asian J Bio Pharmaceu Sci. 2013;1(1):19-27.
63. Abla E, Souad E, Abdelhadi H, Bazdi O, Khadija O. Antagonistic activity of endophytic bacteria isolated from *Menth arotundifolia* L. Int J Sci Tech Res. 2015;12(4):36-9.
64. Afzal I, Shinwari ZK, Sikandar S, Shahzad S. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. Microbiol Res. 2019; 221:36-49.
65. Tariq MR, Shaheen F, Mustafa S, Ali S, Fatima A, Shafiq M, et al. Phosphate solubilizing microorganisms isolated from medicinal plants improve growth of mint. Peer J. 2022;17(10):1-30.
66. Timofeeva A, Galyamova M, Sedykh S. Prospects for using phosphate-solubilizing microorganisms as natural fertilizers in agriculture. Plants. 2022;11(16):1-23.