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Biology of Cellulosic Bacteria from Hindgut *Oryctes rhinoceros* as Antagonistic Agent of *Ganoderma boninense* In Vitro

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Abstract

Ganoderma boninense is a pathogenic fungus that causes the base rot of oil palm trunks. Antagonistic microorganisms can inhibit, suppress, or eliminate populations of plant pathogens. Antagonistic microorganisms can be utilized through symbiotic bacteria. The capability of symbiotic bacteria to degrade cellulose enables them to be more utilized as biological agents for controlling plant pathogens. *Oryctes rhinoceros* larvae are one of the insects that have symbiotic bacteria. This study aims to analyze the morphological and biochemical characteristics of symbiotic bacteria of *O. rhinoceros* larvae and see their effectiveness in inhibiting the growth of *G. boninense*. This research employed a non-factorial completely randomized design with six treatments: S0, *Ganoderma boninense* (control); S1, *G. boninense* + isolate of symbiotic bacteria (P2); and S2, *G. boninense* + isolate of symbiotic bacteria (P3). The results showed that treatment P2 that c, which contains *Bacillus subtilis*, was able to inhibit *G. boninense* by 35.21%, and the P3 bacterial isolate, which is *Bacillus* sp., was able to inhibit the growth of *G. boninense* by 23.66%. The identification showed that bacteria P2 is *B. subtilis*, and P3 is *Bacillus* sp.

Keywords: antagonist agents, *Ganoderma boninense*, *Oryctes rhinoceros*, *Symbiotic bacteria*

Introduction

Basal stem disease on oil palm, caused by *Ganoderma boninense*, is the most serious oil palm disease in Indonesia and worldwide. Socfindo (2022) reported that basal stem rot caused by Ganoderma is a deadly disease that can cause up to 80% mortality. Previously, basal stem rot disease was not considered economically important because it primarily affected older oil palms resulting from replanting. Effective control strategies for basal Stem rot in oil palms have so far proven to be difficult. Appropriate control techniques are necessary to minimize losses caused by Ganoderma, a biological control that is environmentally sustainable. *Ganoderma* can be controlled biologically using antagonistic microorganisms, which can serve as agents that inhibit or eliminate plant pathogen populations. Bacteria are one of the microorganisms that can be used as biological agents to control plant pathogens (Dabamona et al., 2019).

Bacteria and insects have various symbiotic relationships, including both mutualistic and commensal ones. According to Hadi et al. (2021) about 65% of insects have symbiotic bacteria. Symbiotic bacteria are primarily found in the gut of insects. The gastrointestinal tract serves as a link between the natural environment and the animal's physiology, thereby allowing for the permanent colonization of microbes from the environment. The symbiosis between bacteria and insects may be beneficial and pathogenic. Based on mutualism, symbiotic bacteria utilize the host as a habitat. On the other hand, symbiotic bacteria can increase the host insect's immunity to pathogen infection and environmental stress. *Oryctes rhinoceros* (Coleoptera; Scarabaeidae) larvae need biocatalysts, such as bacteria in their gut system, to metabolize food.

Symbiotic bacteria are types of bacteria that settle on a host and can produce the same secondary metabolite compounds as their host (Mokos et al., 2023). The type of bacteria symbiotic with the insect gut depends on the food source to be absorbed. Cellulosic bacteria are one of the symbiotic microorganisms found in the gut, and they can be identified in the gut of *O. rhinoceros* larvae. The presence of bacteria in the gut of *O. rhinoceros* larvae is because the food source of these larvae is the roots of softened oil palm trunks and empty oil palm bunches. Symbiotic bacteria also

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serve to improve host insect immunity to pathogen infection and environmental stress. Symbiotic bacteria are primarily found in the gut of insects. The gut is the link between the environment and the animal's physiology. Bacteria native to the gut have a mutualistic relationship with their host, utilizing the host as a habitat. Bacteria exhibit various biochemical activities, including the acquisition of nutrients from the environment (Marheni and Lubis, 2019).

Symbiotic bacteria settle on a host and can produce the same secondary metabolite compounds as their host. Bacteria exhibit various biochemical activities using raw materials, or nutrients, obtained from their surrounding environment (Marheni and Lubis, 2019).

Oryctes rhinoceros larvae are one of the insects that have a symbiotic bacterium. The type of bacteria symbiotic with an insect's digestive tract depends on the food source to be digested. Cellulolytic bacteria are one type of symbiotic microorganism found in the digestive tract. Cellulolytic bacteria can be found in the digestive tract of *O. rhinoceros* larvae because the larvae's food source is oil palm stems and empty fruit bunches. The utilization of cellulolytic microbes can be a direct competitor of pathogens, such as *G. boninense*, in terms of competition for nutrients in the form of cellulose, thereby preventing fungi from utilizing cellulose in the roots and stems of oil palms. The identification results of Marheni et al. (2021) revealed six bacterial isolates from the hindgut of *O. rhinoceros* larvae, including *B. stratosphericus, B. siamensis, B. cereus, Haemophilus parainfluenzae, Achromobacter xylosoxidans,* and *Alcaligenes faecalis.*

The capability of symbiotic bacteria to decompose cellulose may enable them to be utilized as biological agents to control plant pathogens. Cellulose is a constituent component of the cell wall of pathogenic fungi. Symbiotic bacteria are expected to be capable of breaking down cellulose in the pathogen cell wall, thereby inhibiting pathogen growth. The research related to the use of cellulosic bacteria includes *Steronophomonas rhizophila*, which inhibits the growth of *G. boninense* by 63% and produces cellulase enzymes with a cellulase index of 0.46 (Rupaedah et al., 2018). There has been limited research on symbiotic bacteria from the digestive tracts of insects. Based on the topic, researchers aim to investigate the symbiotic bacteria found in the gut of *O. rhinoceros* larvae in relation to the fungus *G. boninense*.

Materials and Methods

Research Location and Specimen Preparation

The experiment was conducted at the Plant Disease Laboratory of the Agrotechnology Study Department, Faculty of Agriculture, University of Sumatra Utara, from January to June 2024. *Oryctes rhinoceros* 3rd instar larva was collected from empty palm oil bunches in smallholder plantations in Langkat Regency, North Sumatra, and pure isolates of the fungus of *G. boninense* from the collection of Marihat Oil Palm Research Center, Simalungun Regency. This study employed a completely randomized design with six replications. The treatments are
S0 = *G. boninense* (Control)
S1 = *G. boninense* + symbiotic bacterial isolate (P2)
S2 = *G. boninense* + symbiotic bacterial isolate (P3)

Preparation for Symbiotic Bacteria Isolate

Oryctes rhinoceros larvae from empty bunches were collected from the field and brought to the laboratory. Bacteria from the gut of *O. rhinoceros* larvae were isolated by piercing the larvae's abdomen and opening it using sterile scissors. The digestive tract was crushed and put into an Erlenmeyer flask containing 100 ml of sterile water. The Erlenmeyer was shaken at 100 rpm for 1 hour to obtain a suspension that was expected to include potential bacterial isolates. The suspension was diluted with serial dilutions (10^{-1} to 10^{-8}) by taking 1 mL of suspension and putting it into a test tube which contained 9 mL of sterile water and taking the suspension at a dilution of 10^{-8} and then spreading it with the spreader method on Nutrient Agar (NA) media. The isolate was then incubated for 24 hours at 35°C in an incubator; after 48 hours, bacterial isolation was subjected to several steps of the quadrant method to obtain a pure isolate.

Identification of Cellulosic Bacteria by Morphology, Physiology, and Biochemistry

Cellulosic bacteria are identified based on their morphology, including shape, margin, surface texture, size, elevation, and colony color. Physiological characteristics include bacterial Gram staining and biochemical tests, such as citrate utilization, catalase production, starch hydrolysis, gelatin liquefaction, triple sugar iron agar (TSIA), and protease activity.
Bacterial Cellulosic Activity Test

The bacteria to be tested were inoculated on carboxy methyl cellulose (CMC) media. The presence of cellulolytic activity from symbiotic bacteria was indicated by the formation of a clear zone on CMC media after being treated with Congo red dye. The

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clear zone formed is caused by the process of cellulose degradation by cellulolytic bacteria. The cellulolytic activity of each isolate can be observed and measured with a measuring rod. The cellulotic index is measured using the following formula (Hidayatullah et al., 2022).

$$\text{Cellulotic index} = \frac{\text{Diameter of Clear Zone (mm)} - \text{Diameter Of Colony (mm)}}{\text{Diameter Of Colony (mm)}}$$

According to Choi et al. (2005), the cellulosic index value is categorized as low if CI ≤ 1, medium if CI is 1-2, and high if CI≥ 2.

Inhibition Test of Cellulosic Bacteria against Ganoderma boninense In Vitro

Ganoderma boninense inoculum was isolated using a 10 mm cork borer and inoculated on PDA media. The fungi's inoculum was placed 3 cm from the edge of the Petri dishes. A total of 1 mg of the cellulolytic bacterial isolate was streaked 3 cm opposite to 3 cm from the pathogenic fungi and incubated at room temperature (Figure 1B). In the control treatment (Figure 1 A), the PDA media contained only *G. boninense* inoculum on one side.

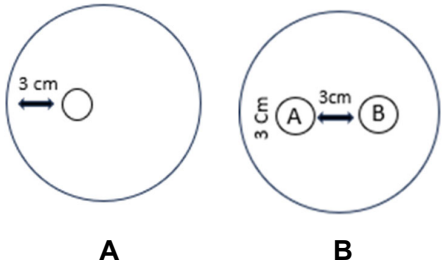

Figure 1. Placements of fungi and symbiotic bacteria inocula. A = *G. boninense* inoculum on PDA media; B = the cellulolytic bacterial isolate was streaked opposite to the pathogenic fungi.

Measurement of inhibition was determined by using the Farhan et al. (2023) formula:

$$\text{Inhibition} = \frac{R1 - R2}{R1} \times 100\%$$

Where R1 is the diameter of fungal colonies on the control treatment (mm), and R2 is the diameter of fungal colonies on the tested treatments (mm)

The inhibition percentage is based on the growth inhibition category (GIC), with a scale of 0-4 (Živković et al., 2010).

According to Prastya et al. (2014), the determination of the antagonistic capability is categorized into four groups based on the percentage of inhibition zone (Table 1). Table 2 presents the categories based on the percentage of the inhibition zone and their corresponding symbols, as described by Prastya et al. (2014).

Table 1. Inhibition percentage of pathogen growth scales based on Živković et al. (2010)

Scale	Inhibition of pathogen growth (%)
0	No inhibition
1	1-25
2	26-50
3	51-75
4	76-100

Table 2. Categories of the inhibition zones and their corresponding symbols (Prastya et al., 2014)

Categorized by percentage	Symbol
Strong (>40%)	+++
Medium (40%-30%)	++
Weak	-

16S rDNA Bacterial Identification

Bacterial 16S rDNA identification was analyzed at Genetics Science Indonesia. 16S rDNA sequencing was determined based on the Gene Bank database using the BLAST program at the National Biotechnology Information Center https://www.ncbi.nlm.nih.gov/. The sequences obtained from the sequencing results were combined and analyzed with the BLAST program.

Results and Discussion

Bacterial Morphology, Physiology, and Biochemistry

The results of the bacterial isolation that was carried out showed that two bacteria were selected for testing in the next step. Table 1 presents the bacterial morphological characterization of both symbiotic bacterial isolates from the gut of *O. rhinoceros* larvae collected from empty bunches. Differences in morphological characteristics include shape, color, margin, and elevation of colonies (Table 3).

Based on the two isolates of symbiotic bacteria, there are differences in colony morphology, color, margin, and elevation of the colonies.

Isolate P2 has a circular shape (round), white color, entire margins (flat), and convex elevation, (Figure 2 A

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and B), whereas P3 has an irregular shape (irregular), yellowish-white color, undulated edges (wavy), and umbonate elevations (convex with prominent center) (Figure 2 C and D).

Gram staining is a procedure used to classify bacteria into two large groups: gram-positive and gram-negative. Gram staining of both bacteria showed purplish-blue results. This indicates that the symbiotic bacteria P2 and P3 are gram-positive and have a *Bacillus* cell shape (Figure 2). In Gram staining, cells that cannot release the color and remain colored, such as those stained with crystal violet, which is purplish-blue, are called Gram-positive bacteria. In contrast, cells that can release crystal violet and bind saffranin appear pink and are classified as Gram-negative bacteria. According to Amin et al. (2023). The difference in response to the Gram-staining mechanism in bacteria is based on the structure and composition of the bacterial cell wall. Gram-positive bacteria contain protein, whereas gram-negative bacteria contain a higher percentage of lipids. The structure of the cell wall also affects the color of Gram-negative bacteria. The walls of Gram-negative bacteria have a higher lipid content compared to those of Gram-positive bacteria. Gram-negative bacteria have three layers of cell walls. The outer layer of lipopolysaccharide (lipid) is likely to be washed away by alcohol, and when stained with saffranin, it will appear red. The presence of Lugol iodine causes the bonding of crystal violet with iodine, which increases the affinity of the dye for bacteria.

P2 and P3 have a *Bacillus* form. According to Dwimartina et al. (2021), commonly, bacilli are rod-shaped or cylindrical cells and one of the most common forms of bacterial morphology found in prokaryotic cells (bacilli, cocci, and spirals). Bacteria with *the Bacillus form are divided into three forms, i.e., single rod-shaped bacilli (monobacilli), rod-shaped bacteria arranged in pairs (diplobacilli), and bacilli-shaped* bacteria arranged like a chain (*Streptobacillus*).

Based on the observation of symbiotic bacteria colonies isolated from the gut of *O. rhinoceros* larvae using biochemical tests, which include starch hydrolysis test, gelatin test, TSIA test, citrate test, catalase test, and protease test. The results of citrate testing showed that symbiotic bacteria isolate P2 and P3 could not use citrate as a carbon source. This is indicated by the color of the medium not changed. Fallo et al. (2022) suggest that the change in medium color indicates that microorganisms use citrate as the only source of carbon and energy. A change in the color of the medium from green to blue will indicate positive results. Changes in medium color from green to blue due to increased pH in the media. The use of citrate by bacteria causes the acid to disappear from the culture, resulting in an increase in pH and changing the color of the media from green to blue.

The catalase test results showed that symbiotic bacteria with code P2 produced catalase enzyme. This is characterized by the formation of bubbles around the colony with the addition of 3% H_2O_2. Catalase test results of symbiotic bacteria with code P3 showed negative results because they did not produce bubbles in the tested preparations. According to Purba et al. (2024), the catalase enzyme works by breaking down hydrogen peroxide (H_2O_2) into dihydrogen-oxide (H_2O) and oxygen (O_2). Therefore, bacteria that break H_2O_2 with the enzyme catalase

Table 3. Characteristics of symbiotic bacteria isolated from the gut of *Oryctes rhinoceros* larvae

Isolate codes	Morphology of bacterial colonies			
	Shape	Color	Margin	Elevation
P2	Circular	White	Entire	Convex
P3	Irregular	White yellowish	Undulate	Umbonate

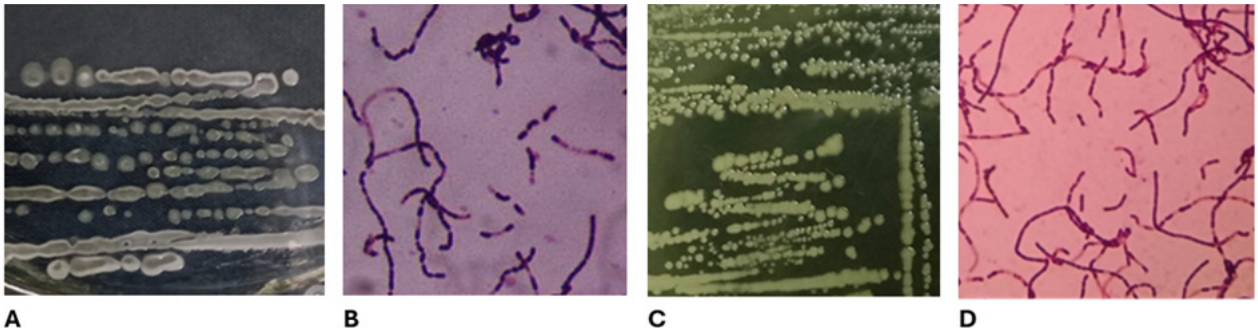

Figure 2. Morphological characteristics of symbiotic bacteria: *G. boninense* + symbiotic bacterial isolate P2 (A and B), *G. boninense* + symbiotic bacterial isolate P3 (C and D).

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will immediately form a defense system from toxic H_2O_2 that produces itself.

The results of the starch hydrolysis test showed that both isolates of symbiotic bacteria derived from the gut of *O. rhinoceros* larvae could hydrolyze starch by producing an amylase enzyme. The observation of the starch hydrolysis test results was characterized by the formation of a clear zone after iodine was dripped on the symbiotic bacterial isolates. The positive starch hydrolysis test was characterized by a clear zone after dropping iodine on bacterial isolates. This happens because starch molecules are water-soluble molecules that give a blue color when mixed with iodine. They will form a clear zone when hydrolyzing starch. According to Nuryanti et al. (2021), the starch hydrolysis test is carried out to determine the ability of bacteria to utilize starch that produces the enzyme amylase.

The gelatin test aims to test whether bacteria can hydrolyze gelatin into simpler compounds, including amino acids. The gelatin test results showed that both symbiotic bacteria isolates can hydrolyze gelatin. The symbiotic bacteria gelatin test results indicate a positive result because the gelatin medium remains liquid after being put into the refrigerator. The statement of Raharja et al. (2023) bacterial isolates in gelatin test observations that react positively are characterized by melting of the media, while if negative, the test media will freeze. The thawing is caused by several microorganisms that can produce proteolytic extracellular enzymes called gelatinase. The enzyme works by hydrolyzing proteins into amino acids.

The results of the TSIA test of P2 show the yellow slant and red butt. The P3 isolate showed yellow slant and butt results. TSIA test is used to determine the ability of bacterial isolates to ferment glucose, sucrose, and lactose. According to Raharja et al. (2023), the red color in the TSIA test indicates an alkaline reaction, the yellow color indicates an acidic reaction, and the black precipitate is due to the formation of H2S gas. The TSIA test results are characterized by the yellow top and yellow bottom, which indicates glucose, sucrose, and lactose can be fermented. If the top is red and the bottom is yellow, only glucose can be fermented; if the top and the bottom are red, the bacteria cannot ferment carbohydrates. If there is black sediment, it indicates that the bacteria can produce gas from H_2S. Isolate of P2 indicates that bacteria only ferment glucose, while the isolates of P3 indicate that bacteria can ferment glucose, lactose, and sucrose.

Bacterial isolates P2 and P3 showed positive protease test results. This is because there is a clear zone around the bacterial colonies on Skim Milk Agar (SMA) media. This indicates that symbiotic bacteria from the gut of *O. rhinoceros* can produce protease enzymes and are included in proteolytic bacteria. According to Simamora and Sukmawati (2020), the clear zone that appears indicates the breakdown of protein molecules contained in bacteria on SMA media. Based on the literature of Nurikhsanti et al. (2024) bacteria that can produce protease enzymes can be used, and protease enzymes play a role in hydrolyzing the cell walls of plant pathogens.

Bacterial Cellulotic Activity Test

The utilization of cellulosic-producing microbes can be a direct competitor of pathogens. Based on the literature of Kurniawan et al. (2021) the utilization of cellulolytic microbes can be a direct competitor of pathogens, one of them is *G. boninense* in terms of competition for nutrients in the form of cellulose, to prevent *G. boninense* from being able to utilize cellulose in the roots and base of the oil palm trunk. Analysis of cellulosic bacterial activity can be done by measuring the clear zone formed around the colony. According to Talantan et al. (2018), cellulosic activity is measured based on the clear zone formed around the colony using a vernier and calculated using the bacterial cellulase activity index formula.

Table 5. Biochemical test results of symbiotic bacteria.

Biochemical Test	Code of bacterial isolates	
	P2	P3
Citrate Test	+	-
Catalase Test	+	-
Starch Hydrolysis Test	+	+
Gelatin test	+	+
TSIA Test	Y/R	Y/Y
Protease Test	+	+

Notes: + = Positive, - = Negative, */* = Slant/Butt, R = Red, Y = Yellow.

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Based on the observation results (Table 3), the symbiotic bacteria with isolate code P2 were classified in the medium category, with a cellulosic index of 1.26 mm. Meanwhile, symbiotic bacteria with isolate code P2 belongs to the low category with an isolate code of 0.57 mm. The magnitude of the bacterial cellulosic index is evident from the inhibitory ability produced by these bacteria. In Table 3, bacteria with the highest cellulosic index have great inhibitory power against the fungus *G. boninense*. According to Alpandari et al. (2022), cellulosic bacteria possess a greater ability to decompose materials rich in cellulose and lignin. Additionally, cellulosic bacteria can inhibit the growth of pathogenic microorganisms, producing lactic acid and acetic acid, and the resulting compost does not harm plants. According to Sembiring (2019), the differences in clear zones produced by each cellulosic bacterium vary because each bacterium produces a distinct cellulase enzyme complex, depending on the genetic makeup and the carbon source used. The research results by Nisa et al. (2020) showed that cellulosic bacteria can inhibit the growth of the fungus *G. boninense.*

The cellulosic index value does not affect a bacteria's inhibition amount. High cellulase enzyme activity does not correlate positively with the ability to inhibit plant pathogens. The research results by Nisa et al. (2020) found that isolate DBS1 has the highest antagonistic power in inhibiting the growth of the pathogenic fungus *G. boninense*, which is 40.17% and has a low cellulosic index. In contrast, LBS1 and DBS6 have a high cellulosic index, namely 4.38 and 2.81. The results have a weak inhibition percentage (27.92% and 1.16%). Other mechanisms or activities are involved in bacterial antagonism, such as the production of compounds that can function as inhibitors of an enzyme.

Inhibition test of Symbiotic Bacteria Against G. boninense

Measurement of inhibition based on the results of the growth of *G. boninense* fungal colonies. The measurement of the percentage of inhibition aims to determine the number of symbiotic bacteria inhibiting the growth of *G. boninense* fungus after 10 days.

The results of inhibition testing using symbiotic bacteria at 10 days against the growth of *G. boninense* showed that all treatments applying symbiotic bacteria can inhibit its growth with varying inhibition (23.66 - 35.21%). Treatment P2 has the highest percentage of inhibition, at 35.21%, while treatment P3 has the lowest percentage of inhibition, at about 23.66%.

Based on the inhibitory power research results, both symbiotic bacteria can antagonistic agents against the pathogenic fungus *G. boninense*. According to Flori et al. (2020) bacteria are antagonistic agents for pathogenic fungi because they can produce several growth-inhibitory compounds. The inhibitory compounds produced by antagonistic bacteria function in degrading the fungus cell wall, affecting cell membrane permeability, inhibiting fungal enzymes, and inhibiting protein synthesis.

On the control treatment, the growth of the mycelium of *G. boninense* after 10 (the days after inoculation) did not fill the entire Petri dish because the mycelium growth of *G. boninense* tended to be slow. In about 10-12 days, the new mycelium can grow to a diameter of approximately 9 cm in a whole petri dish. According to Alviodinasyari et al. (2015), the growth of *G. boninense* takes 15-40 days. The growth of *G. boninense* mycelium is influenced by several factors, such as culture media, pH, temperature, and nutrition. Based on laboratory tests, *G. boninense* can grow at

Table 3. Bacterial cellulolytic activity

Codes of bacterial isolate	Diameter		Cellulosic index (mm)	Category
	Clear zone (mm)	Colony (mm)		
P2	42.55	18.80	1.26	Medium
P3	10.80	6.84	0.57	Low

Table 4. Inhibition test of symbiotic bacteria against *G. boninense*

Treatments	Inhibition (%)	Activity value	Scale
Control	0	-	0
P2	35.21	++ (medium)	2
P3	23.66	+ (low)	1

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pH 3.0 - 8.5 with an optimal temperature of 30°C. The mycelium growth was disrupted at temperatures of 15°C and 35°C and could not grow at 40°C. Mycelial growth of *G. boninense* tends to be inhibited on PDA. Media containing symbiotic bacterial isolates P2 and P3. This suggests that the symbiotic bacterial isolates P2 and P3 exhibit antagonistic properties and possess antibiotic capabilities. Sihombing et al. (2019) stated that bacteria with antibiotic abilities can typically interfere with the morphological and physiological growth of fungi.

On antagonistic tests, it was shown that there was activity from each symbiotic bacterium against *G. boninense* fungi. The macroscopic inhibition mechanisms of symbiotic bacterial isolates are competition and antibiosis. In the P1 treatment, there was competition between bacteria and *G. boninense*; both utilized the PDA media as a growth space and source of nutrients. P2 has rapid growth and the ability to spread. According to Marilna and Hakim (2023), the competition mechanism occurs when the pathogen lacks space for development, rendering it unable to thrive. The inhibition is caused by the antibiotic compounds damaging the cell wall of the pathogen, thereby disrupting its metabolic activities.

The P2 isolate showed the mechanism of antibiosis. Antibiosis is a condition where an organism produces one or more metabolites that negatively affect other organisms. Metabolite compounds that can inhibit or destroy another organism are called antibiotics. In their research, Jatnika et al. (2013) stated that bacteria produce antibiotic compounds, such as chitinase enzymes, which can hydrolyze fungal cell walls, siderophores, and other antibiotics, thereby inhibiting the development of pathogens. The mechanism of bacterial antibiosis against pathogenic fungus is related to the ability of bacterial isolates to produce degradation enzymes such as chitinase, protease, cellulase, and other related compounds.

Macroscopic and Microscopic Observations of G. boninense After Treatments with Symbiotic Bacteria

Macroscopic observations of *G. boninense* fungi in all treatments do not vary too much, i.e., the margin of the fungi is entire (control P0 and P1) and undulate (P2). The texture of the colony is cottony; the elevation has a central protrusion (umbonate), the growth of mycelium up (aerial), there is exudate (droplets on the surface), and has a slow growth type. This is following the literature of Susanto et al. (2013), which states that the fungi *G. boninense* has a velvety white mycelium with slow mycelial growth.

Microscopic observation of *G. boninense* in each treatment, using 1000x magnification, is shown in Figure 4 to illustrate the growth of hyphae after applying symbiotic bacteria. The development of fungal hyphae in isolates P1 and P2 is abnormal, such as hyphae that curl, twist, frizzle, branch, and swell (Figure 4 C, D, E). Abnormal hyphal growth can be indicative of plasma membrane damage in *G.*

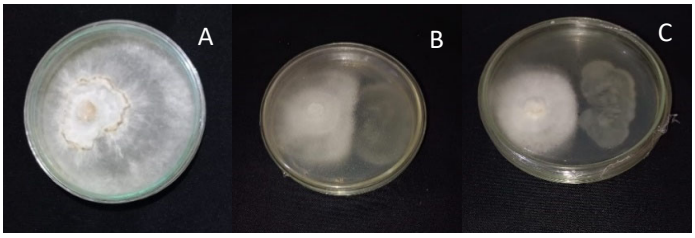

Figure 3. Macroscopic fungus of *G. boninense* after being treated with symbiotic bacteria. A) Control (A); *G. boninense* + symbiotic bacterial isolate P2 (B); *G. boninense* + symbiotic bacterial isolate P3 (C). Magnification: 1000x

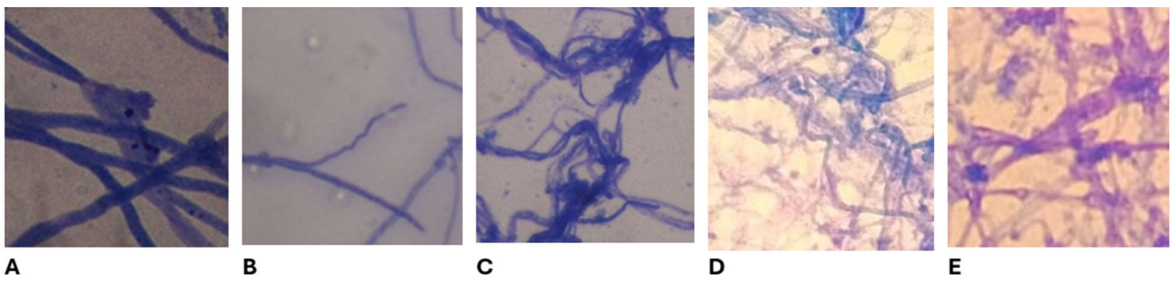

Figure 4. Microscopic fungi of *G. boninense* in the control (A), P2 (B and C), and P3 (D and E): normal hyphae (A), branched hyphae (B), twisted and curled hyphae (C), frizzy hyphae (D), and swollen hyphae (E). Magnification: 1000x.

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boninense. Eliza et al. (2007) stated that antifungal compounds produced by bacteria generally result in the abnormal development of hyphae, characterized by malformation, which is indicated by swelling and shortening of the hyphae, leading to abnormal growth.

Based on the literature of Maulidia et al. (2021), antagonistic bacteria produce cellulase, protease, and chitinase enzymes that can degrade the fungal cell wall by penetrating and absorbing the contents of the fungal cell. Antibiotic compounds produced by symbiotic bacteria also cause abnormal hyphal growth. This agrees with the statement by Masyitah et al. (2023) that hyphal growth becomes abnormal, such as curling, lysis, and malformation, due to the presence of antibiotic compounds that can degrade cell walls and affect fungal growth.

Results of Bacterial Identification Based on 16S rRNA

The results of bacterial identification based on 16S rRNA showed that P2 bacteria are *B. subtilis* and P3 bacteria are *Bacillus sp*. The *Bacillus* genus is recognized as one of the genera that controls various types of phytopathogens in plants, and B. subtilis is a type of antagonistic bacterium primarily used to control soil-borne pathogens. Bacteria, as a biological agent, can produce metabolite compounds that have antifungal effects. *B. subtilis* can suppress the growth of its pathogen by releasing antibiosis, which inhibits the growth of other fungi, and by releasing toxic enzymes that can destroy fungi.

Conclusions

The two isolates of symbiotic bacteria from the digestive tract of *O. rhinoceros* showed morphological and biochemical differences. Symbiotic bacteria can inhibit the growth of *G. boninense* with a variable percentage of inhibition (23.66%-35.21%). The highest rate of inhibition was observed in treatment S1, which involved the application of a symbiotic bacteria isolate coded P2, with a cellulolytic index of 1.26. The microscopic observation revealed that the hyphae of *G. boninense* appeared abnormal after treatment with the symbiotic bacteria; they became curled, twisted, branched, and swollen.

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