

Potential of Endophytic Bacteria as Biological Agents for The Control of Stem Rot In Oil Palm Plants (*Elaeis Guineensis* Jacq)

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Article Info

Article history:

Received : Nov 05, 2024

Revised : Des 19, 2024

Accepted : Jan 10, 2025

Keywords:

Biocontrol agents;

G. boninense;

Oil Palm Plants.

ABSTRACT

One of the factors that limits the cultivation of oil palm plants is the presence of infection from stem rot disease (BPB). Estimated losses due to BPB can reach 80% in oil palm plantations. The cause of BPB is the *Ganoderma boninense* fungus. Several methods of controlling the *G. boninense* fungus have been carried out, including physical control through sanitation techniques and using synthetic fungicides. Long-term use of synthetic functionalities will have a negative impact on the environment. An environmentally safe control alternative to suppress *G. boninense* infection is to use biocontrol agents from the group of microorganisms. One approach that has the potential to be effective in controlling BPB is biological control. This research aims to obtain isolates of indigenous endophytic bacteria that have potential for the pathogen *G. boninense*. The research method includes taking samples of healthy oil palm plants among plants infected with *G. boninense*. Next, isolation of endophytic bacteria, screening of endophytic bacteria, macroscopic and microscopic morphological characteristics of endophytic bacteria, and antagonism test of endophytic bacteria against *G. boninense* were carried out. The results of this research obtained potential endophytic bacteria against *G. boninense*.

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1. INTRODUCTION

Oil palm (*Elaeis guineensis* jacq) is one of the plantation commodities that has an important role in economic activities in Indonesia. As the world's largest palm oil producer, Indonesia has great potential to market palm oil and palm kernel domestically and abroad. The largest production of palm oil or *Crude Palm Oil* (CPO) by province is Riau. CPO production in Riau province reaches 19% and in 2022 Riau is the province with the largest oil palm plantation area in Indonesia with an area of 2858.7 ha (Badan Pusat Statistik, 2023). The need for increasing demand for CPO requires quality oil palm plants that can produce large quantities of palm oil. However, oil palm cultivation is generally grown in monocultures which have a greater risk of being susceptible to pest epidemic disorders and plant diseases. One of the important diseases that infects oil palm plants is stem rot (Azmi et al., 2021).

Stem base rot is caused by the fungus *Ganoderma boninense*. This disease has a significant impact on oil palm production. Basal stem rot disease is an important disease in oil palm plantations in Southeast Asia which is reported to cause losses of around 50 - 80% per ha (Bharudin et al., 2022). The identification and control of this disease from the early stages plays an important role in the management of oil palm disease because this disease has not yet found a cure and treatment (Liaghat et al., 2014). For this reason, other efforts are needed that can be made in overcoming *G. boninense* disease. One of the efforts to control Basal stem rot disease is the use of endophytic bacteria.

Endophytic bacteria are potential microbes that are widely used in disease control applications in plants. Endophytic bacteria colonize plants internally without any adverse effects on their host plants. Endophytic bacteria are interesting to develop because of their potential in promoting plant growth, antagonistic effects on pathogens, reduction of abiotic stress and phytoremediation. The majority of endophytic bacteria exhibit beneficial effects such as increased nitrogen fixation, phytohormone production, phosphate solvents and inhibition of ethylene biosynthesis in response to biotic and abiotic stresses as well as biocontrol agents.

Endophytic bacteria that live in plant tissues benefit from protection from environmental stresses and competition with other microorganisms (Santoyo et al., 2016). In addition, endophytic bacteria and their secondary metabolites can directly or indirectly reduce the incidence of disease from plant disease infections by antibiotic mechanisms, ecological niche competition and lytic enzyme production (Cabanas et al., 2014). Endophytic bacteria can be isolated from the roots, leaves, stems, and fruits of different plants, such as in annuals and annuals. The population density of endophytic bacteria in woody plants varied, ranging from 10^1 - 10^6 cfu per gram of plant parts sample. The genera *Pseudomonas* and *Bacillus* are the main groups of endophytic bacterial communities, and the genera *Actinobacteria*, *Acinetobacter* and *Sphingomonas* are significant community proportions found in many woody plants (Izumi, 2011). Research on endophytic bacteria from an early stage can help find effective and competent biocontrol agents against plant pathogens. Indigenous endophytic bacteria from the Rokan Hulu oil palm plant have not been widely reported in disease control, for this reason it is necessary to explore and activate the pathogen. Based on these problems, it is necessary to explore indigenous endophytic bacteria from Rokan Hulu oil palm plants and obtain potential endophytic bacteria against the pathogen *G. boninense*.

2. METHOD

2.1 Sampling on Healthy Oil Palm Plants

Sampling was carried out in the oil palm plantation area at the Faculty of Agriculture UPP. The oil palm plants taken are about 5-10 years old. The parts of the plant taken include the roots of the plant. The sampling technique was carried out by *purposive sampling* on oil palm plants. The sampling method was random with a single plant technique, by collecting all healthy plant root parts in the field. The collected samples are put into labeled plastic and taken to a laboratory for the process of isolation of endophytic bacteria.

2.2 Endophytic Bacteria Isolation

Isolation of endophytic bacteria is carried out using the method (Hallmann et al., 1997). Root samples of oil palm plants are surface sterilized, plant roots are washed thoroughly with running water, cut using a sterile scapel knife with a size of 1-2 cm. The roots of the plant as much as 10 g are soaked in 70% alcohol for 2 minutes, soaked in 1% NaOCl for 2 minutes, rinsed 3 times with sterile aquade. Root cuttings are dried with sterile tissue. The success of surface sterilization was tested by applying root cuttings on NA medium, incubated for 2 days. If there are no bacteria growing on the NA medium, surface sterilization is considered successful. The roots of the plant that have been sterile are macerated using a blender (the roots have a hard texture) until smooth with the addition of 1:10 (v/v) sterile aqueous. The suspension of bacteria is taken as much as 1 mL, a series dilution is carried out until a dilution of 10^{-3} . A total of 0.1 mL of suspension from each dilution was cultured on 10% TSA medium and 10% NA, incubated for 48 hours. Each colony with a different morphology is purified on NA media.

2.3 Rejuvenation of Pathogenic Fungi (*Ganoderma boninense*)

Ganoderma boninense isolate before being used as a test pathogen was first rejuvenated on potato dextrose agar (PDA) medium. The fungus *G. boninense* is rejuvenated by taking from the stock an oblique PDA culture using an ose needle. Next, it is incubated in PDA medium in petri dishes. The inoculation of *G. boninense* fungi is carried out aseptically in the laminar chamber of the air flow cabinet. Furthermore, the *G. boninense* mushroom is incubated at room temperature for 5-7 days.

2.4 Filtration of Endophytic Bacterial Isolate

2.4.1 Test Hypersensitive Response (HR)

HR testing aims to pre-select bacteria that are pathogenic or non-pathogenic to plants. HR testing was carried out on all endophytic bacterial isolates obtained. The endophytic bacterial isolate was grown on NB medium for 12 h in a swaying incubator at 120 rpm. Furthermore, a suspension of bacteria of 2 mL is infiltrated on the axial side of the tobacco plant leaf until it wets the space between cells. Observation was carried out after 24-48 hours of treatment. The positive reaction is the appearance of necrotic symptoms in the inoculated tobacco leaves, and the negative reaction is the absence of necrotic symptoms. Necrotic symptoms indicate the potential for endophytic bacteria to be pathogens of plants. Bacterial isolates with positive reactions are not used in subsequent tests (Schaad et al., 2001).

2.4.2 Morphology Bacteria Endophyte

The endophytic bacteria that were successfully isolated were then killed macroscopically and microscopically. Macroscopic observations are carried out visually using the eye directly while microscopic observations are carried out by the wet preparation method by placing bacterial colonies on objects that have been dripped with aquades and lactophenols. After that it is covered with a cover glass and observed with a binocular microscope with weak magnification (10x10), medium magnification (10x40) and high magnification (10x100) (Schaad et al., 2001).

2.4.3 Entrophic Bacterial Antagonism Test Against *Ganoderma boninense*

Before the antagonism test is carried out, the test pathogen isolate, namely *G. boninense*, is rejuvenated on PDA media. The *G. boninense* mushroom is an isolate collected by the Faculty of Agriculture UNRI. The antagonism test of endophytic bacteria against *G. boninense* used the cross-streak method (Velho-Pereira and Kamat, 2011), namely by scratching the test endophytic bacteria thoroughly on one-third of the diameter of the \pm 3 cm wide dish. Next, the cup is incubated for 3 days. *G. boninense* was scratched perpendicular to the endophytic bacterial scratch at a distance of \pm 0.5 cm from the edge of the endophytic bacterial scratch. The stroke is in the form of a single line and is made as many as four strokes with a stroke length of \pm 3 cm and a width of \pm 0.2 cm. This inoculation method is carried out in the center in the form of a single stroke 7 cm long and 0.5 cm wide.

2.5 Observation Parameters

2.5.1 Characteristic of macroscopic bacteria endophyte

Observation of macroscopic characteristics was carried out on *sodium agar* (NA) medium cultures in petri dishes that had been incubated 3-5 days after incubation which included: colony color (observation of colony color observed whether it was cloudy white, clear white or yellow) and colony shape (colonies were observed to be round, flat edges, shiny convex and slimy color).

2.5.2 Characteristic Microcopy of bacteria endophyte

Observation of bacterial characteristics was carried out in NA medium at the age of 3 days after incubation or 3 x 24 hours with the wet preparation method using a microscope including: bacterial shape, bacteria were observed whether they were elliptical, spherical, rod and spiral.

2.5.3 Growth Rate of Pathogenic Fungi (*Ganoderma boninense*)

The growth rate of pathogenic fungi is carried out the day after incubation until the pathogenic fungi fill the petri dish on the control. The growth rate is calculated by the formula: (Crueger et al., 2017)

$$\mu = \frac{X}{T} \quad (1)$$

with μ , growth rate (cm/day); X colony diameter (cm), T observation time (days).

2.5.4 Entrophic Bacterial Antagonism Test

Observation of the inhibition of endophytic bacteria against *G. boninense* was carried out by measuring the colony growth diameter of *G. boninense* using a ruler. Measurements are taken if the growth on the control has covered the entire surface of the PDA media. The calculation of the effectiveness of the inhibition force is carried out using the formula (Dwiastuti et al., 2015)

$$\text{EDH (\%)} = \frac{R_1 - R_2}{R_1} \times 100\% \quad (2)$$

with EDH, the effectiveness of the inhibition (%); R1, control diameter (cm); R2, treatment diameter (cm).

3. RESULTS AND DISCUSSION

3.1 Results of Bacterial Exploration from Oil Palm Plant Roots

The results of the exploration of endophytic bacteria from oil palm plantations were obtained by several biological agent candidates. Endophytic bacteria isolated from the roots of oil palm plants

were obtained as many as 18 bacterial isolates. Sampling of oil palm plant roots can be seen in Figure 1.



Figure 1. The Location of the Root Collection of Oil Palm Plants, SP (the Location of the Oil Palm Root Sample in Front of Faperta UPP), SK (the Location of the Oil Palm Root Sample Behind Faperta UPP), ST (the Location of the Oil Palm Root Sample Behind the FKIP UPP).

The results of endophytic bacterial isolation from oil palm plant roots were obtained as many as 18 bacterial isolates. A total of 5 bacteria were obtained from the SP location, as many as 6 bacteria were obtained from the SK location and as many as 7 bacteria were obtained from the ST location. Endophytic bacterial isolates from the roots of oil palm plants can be seen in Figure 2. According to (Utami, 2023) the root part, the plant has the potential for a greater number of endophytic bacteria than other parts of the plant because the root is a place where nutrients enter the plant tissue. This is supported by (Serdani et al., 2018) the study of the percentage of endophytic bacterial isolates obtained in root tissue of 37.5%, stems by 22.2% and leaves by 25%.

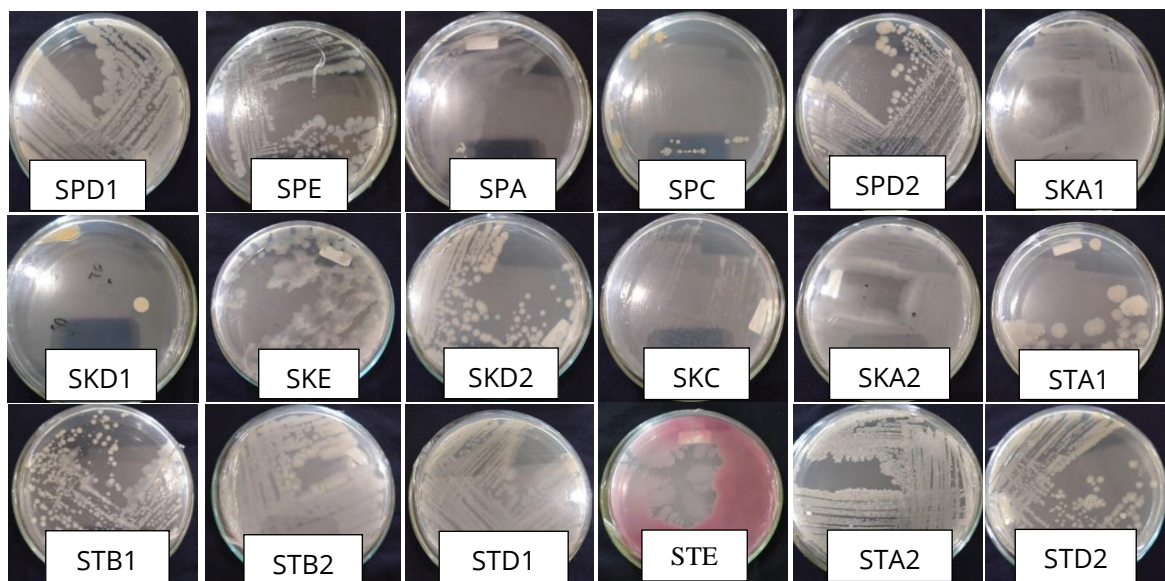


Figure 2. Endophytic Bacterial Isolate from Oil Palm Plant Roots

The hypersensitivity response test is one of the screenings using tobacco plants as an indicator of the pathogen of a bacterium. If the leaves of the plant show necrotic symptoms on the leaves of the tobacco plant after bacterial inoculation then the bacteria are pathogens in the plant, on the other hand, if the leaves of the tobacco plant do not occur necrotic, then the bacteria are not pathogens in the plant.

Table 1. Results of Hypersensitivity Test of Endophytic Bacterial Isolate Isolated from The Roots of Oil Palm Plants

No	Isolation code	Hypersensitivity test
1	SPD1	Positive
2	SPE	Negative
3	SPA	Negative
4	SPC	Positive
5	SPD2	Positive
6	SKA1	Negative

7	SKD1	Positive
8	SKE	Negative
9	SKD2	Positive
10	SKC	Positive
11	SKA2	Negative
12	STA1	Negative
13	STB1	Positive
14	STB2	Positive
15	STD1	Positive
16	STE	Positive
17	STA2	Negative
18	STD2	Positive



Figure 3. Visualization of Hypersensitive Reaction Test on Tobacco Plant Leaves

Based on hypersensitivity tests on tobacco plants on 18 endophytic bacterial isolates, 7 bacterial isolates were produced that showed negative reactions (Table 1 and Figure 3). The negative reaction produced in the hypersensitive test showed that the bacteria did not have the potential to cause disease or pathogens for plants. This can be seen whether there is necrosis in the tobacco leaves that have been injected using an endophytic bacterial suspension while the appearance of necrosis symptoms in tobacco shows that there are 11 bacterial isolates which are bacteria that are plant pathogens. According to (Seung et al., 2015) the hypersensitivity test on tobacco plants carried out to determine the pathogenic character of the isolate to the plant, the hypersensitivity response in the plant is indicated by yellow or brown spots.

3.2 Macroscopic and Microscopic Characteristics of Endophytic Bacteria

Based on the endophytic bacterial antagonism test against *G. boninense*, two potential isolates that can inhibit the growth of *G. boninense* were obtained, namely STA2 bacterial isolate and SKE bacteria. The results of macroscopic and microscopic observations can be seen in Table 2.

Table 2. Results of Observation of Endophytic Bacteria that Have Inhibitory Power Against *G. boninense*

Isolation code	Morphological characterization	
	Macroscopic	Microscopic
STA2	Colony Shape: Round Color: White Elevation: Embossed Edge: Choppy	Trunk
SKE	Colony Shape: Round Color: White Elevation: Flat Edge: Smooth	Trunk

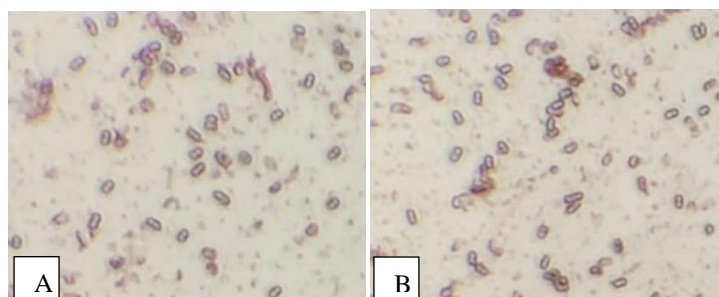


Figure 4. Microscopic Endophytic Bacteria, A (STA2 Bacteria) and B (SKE Bacteria)

Based on table 2 and figure 4, it shows that endophytic bacteria are suspected to be a group from the genus *Bacillus bacteria*. This can be seen from macroscopic and microscopic observations of bacteria. The morphological characteristics of *Bacillus* bacteria have a whitish color with the shape of a round colony. In line with the results of the study, bacterial (Sidhiawan et al., 2023) colonies from

Bacillus sp. are generally round with a white or beige color on agar media, with a slippery texture, not slippery or rough. According to (Seung et al., 2015) the state, rhizosphere bacteria in oil palm plants consist of the genera *Pseudomonas*, *Bacillus* and *Burkholderia*.

3.3 Growth Rate of *Ganoderma boninense*

The results of the observation of the growth rate of *Ganoderma boninense* can be seen in figures 5 and 6. Observation of the growth rate is carried out after 1 day of days after incubation until the fungus fills the petri dish. Growth rate *G. boninense* showed an average increase in mycelium in PDA media of 1 cm per day. The peak phase of growth *G. boninense* is available on day 2 of days after incubation. On the 8th day of the observation of the growth rate of *G. boninense* shows a declining growth phase until the growth of mycelium *G. Boninense* fills the Petri cup.

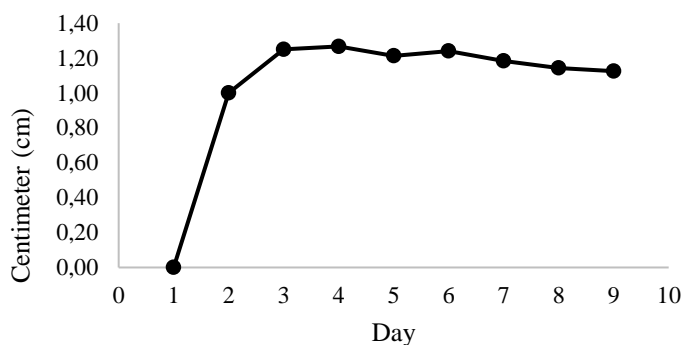


Figure 5. Growth Rate Graph of *G. boninense*

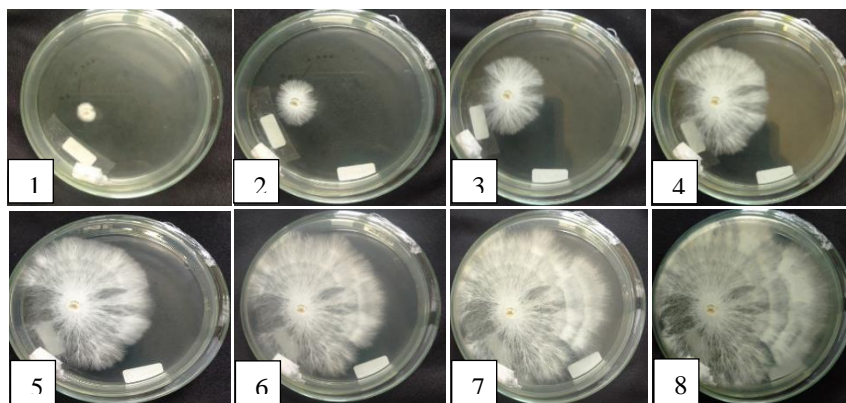


Figure 6. Visualization of The Growth Rate of *G. boninense*, Days (1, 2, 3, 4, 5, 6, 7, and 8)

The results of the observation of the growth rate of *G. boninense* in Figures 5 and 6 show that the fungus *G. boninense* has a different growth rate every day. The growth rate of *G. boninense* on the second day after incubation showed the highest growth rate with growth reaching 1.27 cm. while the growth rate of *G. boninense* on the first day after incubation showed that there was no growth with growth still zero, this was due to the lag phase in fungal growth. According to (Willey et al., 2019) the lag phase, it is the phase of mold adjustment to a new environment. The duration of the lag phase in fungi varies greatly, depending on the composition of the medium, pH, temperature, aeration, the number of cells in the initial inoculum and the physiological properties of the microorganisms in the previous medium. Then there is the log/exponential phase, which is a period of rapid growth. The variation in the degree of fungal growth in this exponential phase is greatly influenced by the genetic traits it inherits. This phase occurs on the 2nd to 5th day after incubation. The stationary phase occurs on the 6th to 10th day where the fungal growth is relatively fixed, the fungal growth is balanced by the number of dead cells. In the stationary phase, even though carbon as an important source of energy or nutrients has been used up, it does not mean that growth has stopped, this is due to lysis in dead cells and used as a source of nutrients (Santoyo et al., 2016)

3.4 Entrophic Bacterial Antagonism Test Against *Ganoderma boninense*

The results of the antagonism test of seven endophytic bacteria against *Ganoderma boninense* showed that the seven isolates, namely SKA1, STA2, STA1, SPE, SKE, SPA and SKA2 with the greatest inhibitory power were found in the isolates of STA2 and SKE bacteria that were able to

inhibit *G. boninense*. This shows the suppression power of endophytic bacteria against the fungus *G. boninense* (Table 3 and Figure 7).

Table 3. Results of the endophytic bacterial antagonism test against *G. boninense*

Isolate	Drag (%)
Control	0
SKA1	0
STA2	47,78
STA1	0
SPE	0
SKE	41,11
SPA	8,33
SKA2	6,67

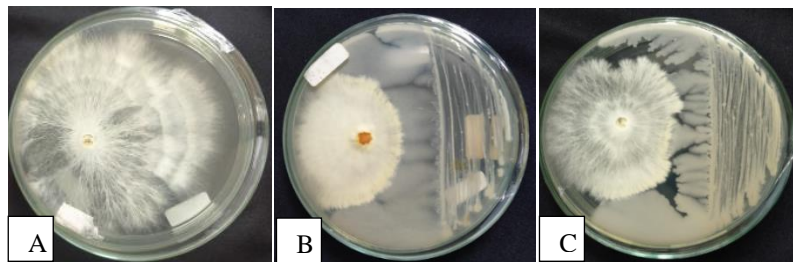


Figure 7. Entrophic Bacterial Antagonism Test Against *G. boninense*, A (*G. boninense* control), B (STA2 Endophytic Bacteria Against *G. boninense*), C (SKE Endophytic Bacteria Against *G. boninense*)

The ability of endophytic bacteria from the roots of oil palm plants in suppressing the growth of *G. boninense* shows that some isolates are able to inhibit the growth of pathogenic fungi through antimicrobial compounds. Although the percentage of inhibition is not too high, when compared to the control, it has a greater growth of mycelium *G. boninensise*. According to (Santoyo et al., 2016) endophytic bacteria do many things in biological control, such as secreting antimicrobial compounds, competing in space and nutrients, competing for micronutrients such as iron and siderophore production and being able to create resistance in plants.

4. CONCLUSION

Endophytic bacteria from the root rhizosphere of palm plants can inhibit the growth of *Ganoderma Boninense*. Potential endophytic bacteria are STA2 endophytic bacteria and SKE endophytic bacteria. It is recommended that further studies be carried out on the molecular identification of STA2 and SKE endophytic bacteria and STA2 and SKE endophyte tests on plants.

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