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Antagonistic Effects of Bacterial Rhizosphere of Oil Palm in Biocontrol of Basal Stem Rot Disease (*Ganoderma boninense* Pat.)

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ABSTRACT

Basal stem rot disease caused by *Ganoderma boninense* is a major problem for oil palm cultivation. The research was conducted to obtain biocontrol agents from rhizosphere of oil palm to control the disease as part of sustainable pest management in oil palm plantation. Rhizosphere bacteria were isolated from rhizospheres of healthy oil palm trees. Isolation of bacteria was done using serial dilution method. The isolated bacteria were then tested for its antifungal activity against *G. boninense in vitro* using dual culture assay. The ability of the bacteria to produce antifungal compound was also determined by culturing the bacteria on ISP2 liquid media. Once the bacterial cells were removed, the crude metabolites were then tested against *G. boninense* using agar well diffusion and toothpick colonization. The result showed that several isolates demonstrated strong antifungal activity against *G. boninense*. Some isolates were also able to degrade chitin and to solubilize phosphate. Furthermore, the crude metabolites produced by the rhizosphere bacteria demonstrated the ability to inhibit the growth of *G. boninense* in the agar diffusion method. Colonization of the *G. boninense* on toothpick following soaking in the crude metabolites was also inhibited. The isolated rhizosphere bacteria (BARK7 and BARK15 in which identified as *Burkholderia* sp.) showed promising ability to be developed as biocontrol agent for basal stem rot disease of oil palm.

1. Introduction

Oil palm (*Elais guineensis* Jacq.) is one of important estate plants in Indonesia. The oil palm is one of the major contributors for the country's income following fossil oil and gas (Statistic Indonesia 2022). Indonesia is one of the largest exported of palm oil. In 2021, the number of palm oil exported from Indonesia reached 26.9 million metric ton (Statistic Indonesia 2022). However, the presence of basal stem rot disease (BSR) is causing major threats for cultivation of oil palm not only in Indonesia but also in other oil palm-growing countries. It was reported that in Indonesia, BSR can lead to the loss of up to 50% of oil palm tree population (Susanto *et al.* 2005) and can cause up to 80% yield loss worldwide (Paterson 2019).

BSR is affecting the oil palm production through the reduction of oil palm population and reduction of the fruit's weight. It was reported that fruits infected by BRS can cause yield reduction up to 43% (Haw *et al.* 2023). Initially, BSR disease was reported to affect the fruit producing-oil palm trees but not to the younger non-fruit producing trees. However, research has shown that the disease was able to cause a more severe effect on young oil palm trees (Zakaria 2023). BSR disease was developed through three phases. Infection at seedling stage which normally affect oil palm at the age of 1-4 years, basal stem rot which were affecting oil palm at the age of 6 years and above and upper stem rot which were affecting oil palm at the age of 12 years and above (Zakaria 2023). Although there are some uncertainties regarding the species *Ganoderma* as the causal agent of basal stem rot, it is believed that *G. boninense* is the principal species responsible for the decline of oil palm trees as result of BSR infection (Purba *et al.* 2019).

Early infection of *G. boninense* remains symptomless. Symptoms will only appear at a

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later infection stage. At this point, every available treatment given cannot save the plant as infection had spread throughout all the plant (Bivi *et al.* 2016). Young oil palm infected by *G. boninense* shown yellowing and will die shortly. At older oil palm, symptoms are varied which include yellowing, unopened spears, die back and collapsing fronds and basal rotting (Haw *et al.* 2023). Controlling treatments available are not able to cure the plant following pathogen infection (Susanto *et al.* 2005). The treatments are only able to slow the disease development with varying levels of success. Currently, combination of physical measures, biological control, and the use of oil palm resistant to BSR are the best approach to control *G. boninense* (Susanto *et al.* 2005; Hushiarian *et al.* 2013; Zakaria 2023).

Bacterial rhizosphere which inhabits the soil or plant rhizosphere have been identified to possess biological control traits to control the plant pathogens. Microorganisms inhabit the soil are ideal to be used as biocontrol agents of soil-borne disease. It can hinder the pathogens from infecting the root through rapid colonization of the root as well as producing secondary compounds in which able to inhibit the pathogen development (Benaissa 2024). Mechanisms by which bacterial rhizosphere inhibit the growth of phytopathogen microorganisms include direct and indirect mechanisms. Direct mechanisms provide protection by affecting the plant pathogens directly, whereas the indirect mechanisms provide protection through increasing the plant health by providing nutrients (Bonaterra *et al.* 2022).

Bacillus amyloliquefaciens L-S60, a bacterial rhizosphere of turfy grass, was also reported to have the ability in promoting the growth of cucumber seedling as well as changing the microbial structure in the soil by enriched the soil with beneficial bacteria (Qin *et al.* 2017). Furthermore, *B. amyoliquefaciens* ZM9 applied together with calcium cyanamide, and rice bran was effective to control tobacco bacterial wilt caused by *Ralstonia solanacearum* (Hu *et al.* 2021). Although many attempts have been conducted to find antagonistic agents for controlling *G. boninense* (Yurnaliza *et al.* 2020; Dermiyati *et al.* 2023; Maherani *et al.* 2024), continuous studies are still needed to be done to obtain high efficacy of *G. boninense* biocontrol agents. The research aimed to isolate antagonistic bacterial rhizosphere of healthy oil palm which has the potential to control *G. boninense*.

2. Materials and Methods

2.1. Isolation of Bacteria from Rhizosphere of Oil Palm

Rhizosphere soil was collected from the surrounding of the root of healthy oil palm trees located at PT. Condong Garut, Garut Regency, West Java Province, Indonesia. Soil was composited from several collection sites of healthy oil palm trees. The soil was air dried until reached constant weight. Isolation of bacteria was done using serial dilution method using common microbiology method (Collins *et al.* 2004). Serial dilutions were plated on tap water yeast extract agar (TWYE, yeast extract 0.25 g, K_2HPO_4 0.5 g, agar 18 g, 1 L RO wáter, pH 7.2±0.2) and mannitol soy agar (MSA, 20 g mannitol, 20 g soy flour, 20 g agar, 1 L RO water, pH 7.2±0.2). Plates were incubated at 27°C and 37°C and checked regularly for the presence of bacteria. Any bacteria with different colony morphologies were transferred to fresh half strength potato dextrose agar (HPDA, 19.5 g PDA (Oxoid), 7.5 g agar, 1 L RO water pH 7.2±0.2). The isolated bacteria were then grouped based on their morphological similarity on MSA, HPDA and ISP2 (International Streptomyces Project 2) (malt extract 10 g, yeast extract 4 g, glucose 4 g, 18 g agar, 1 L RO water, pH 7.2±0.2).

2.2. Screening for Potential Bacterial Rhizosphere Isolates for Antifungal Activity against *G. boninense*

Pathogenic fungi *G. boninense* was obtained from Oil Palm Research Centre Medan, North Sumatera. Screening for the potency of the isolated rhizosphere bacteria were conducted using dual culture assay. The bacteria were streaked on HPDA, after 7 days of inoculation, a 6 mm plug of *G. boninense* was placed at 3 cm at the opposite side of the bacteria. The antifungal activity was detected by the presence of *G. boninense* growth inhibition. Percentage inhibition of radial growth (PIRG) was calculated based on the reading of *G. boninense* radial colony at the direction of bacteria colony (R2) against the *G. boninense* radial colony in the control plate (R1) (Skidmore & Dickinson 1976).

$$\text{Where, PIRG} = \frac{R1 - R2}{R2} \times 100$$

Morphology of the *G. boninense* following the dual culture assay was observed under microscope. Bacterial isolates shown high antagonistic ability were subjected for molecular identification using partial 16S rRNA as target gene using pair of primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 765r (5′-CTGTTTGCTCCCCACGCTTTC-3) (Coombs & Franco 2003). The total genome extraction and PCR amplification were conducted following Han (2006). The DNA sequencing was conducted as service provided by Macrogen, Inc., Korea. The sequences were then subjected for BLAST search through the NCBI database.

2.3. Chitinase and Phosphate Solubilization Activity of the Rhizosphere Bacteria Isolates

The chitinase activities were detected by spot inoculated the bacterial isolates onto media containing 0.2% colloidal chitin, 0.05% KCl, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4$, 0.05% yeast extract, 2% agar and RO water. The media was adjusted to pH 7. Plates were then incubated for 14 days at room temperature, and chitinase activity was detected by the presence of clearing zones surrounding the colonies (Kawase *et al.* 2004). For determining the phosphate solubilization ability, GY (glucose yeast) media containing 10 g of glucose, 2 g of yeast and 2% agar in 1 L of distilled water was used. To the medium, two solutions (5 g K_2HPO_4 in 50 ml distilled water and 10 g of $CaCl_2$ in 100 ml distilled water) were added. These two solutions changed the colour of GY medium to white opaque showing the presence of insoluble calcium phosphate. The isolates were then spot inoculated onto the GY medium. The presence of a clearing area surrounding the isolates showed the ability of the isolate to solubilize phosphate (Beneduzi *et al.* 2008).

2.4. Antifungal Assay of Crude Metabolites of Rhizosphere Bacteria Isolates against *G. Boninense*

One hundred millilitres of ISP2 liquid media (malt extract 10 g, yeast extract 4 g, glucose 4 g, 1 L RO water, pH 7.2±0.2) (Kiranmayi *et al.* 2011) was prepared and poured into 250 ml Erlenmeyer flasks. It was autoclaved for 15 min at 121°C, 15 psi. Two loops full of bacterial rhizosphere isolate were inoculated into the flask and incubated at orbital shaker in room temperature at 150 rpm for 10 days. The filtrate was sterilized through centrifugation to collect the supernatant and filtration using 0.22 µm membrane filters (Wang & Ma 2011). It was then used for detecting its ability to inhibit the growth of *G. boninense*.

The antifungal activity of the metabolites was conducted on PDA media using agar well diffusion method (Balouiri *et al.* 2016). A 6 mm diameter well was made using a sterile cork borer by which 30 µL of the filtrate prepared earlier was inoculated into the well. A 6 mm plug of *G. boninense* was inoculated at 3 cm away across the well. The antifungal activity of the metabolites was detected by the growth inhibition of *G. boninense*. PIRG was calculated as mentioned above.

The effect of crude metabolites produced by bacterial rhizosphere isolates were done following modified method of Paulitz & Schroeder (2005). Wooden toothpicks (6 mm × 2 mm) were sterilized and soaked in the crude metabolites prepared as mentioned above for 14 days. The toothpick was then placed on plate containing fully grown *G. boninense*. The colonization of *G. boninense* on the toothpick was observed 14 days after the treatment under dissecting microscope. As control treatment, the toothpicks were soaked in sterile RO water.

2.5. Data Analysis

All experiments were conducted using completely randomized block design. Treatments were consisted of nine bacterial isolates and control treatment without bacterial isolate in which replicated three times. The data obtained was homogenized and normalized following data analysis using ANOVA followed by post hoc analysis of Duncan's multiple range test at $P<0.05$ using SPSS version 22 for Windows.

3. Results

3.1. Isolation and Screening of Bacteria from Rhizosphere of Oil Palm for Antifungal Activity against *G. boninense*

Not many types of bacteria were able to be isolated from TWYE and MSA media. Bacteria isolated from the media were chosen based on their morphological characteristics. As many bacteria were found to be morphologically similar, only representative of each morphological characteristic was picked and subjected for antagonistic testing. The morphological characteristics of the isolated bacteria are detailed in Table 1. Additionally, Figure 1 displays the colony

Table 1. Morphological characteristics of the bacterial rhizosphere isolates on several growing media

Isolates	MSA (mannitol soya agar)		ISP2 (International streptomyces project 2)		HPDA (Half strength Potato Dextrose Agar)		Gram staining
	Colour of colony from top	Growth	Colour of colony from top	Growth	Colour of colony from top	Growth	
BARK4	White	+++	White	+++	White	+++	-
BARK5	White	+++	Cream	+++	White	+++	-
BARK6	White	+++	White	+++	Cream	+++	-
BARK7	Brownish cream	+++	Brownish cream	+++	Cream	+++	-
BARK8	White	+++	Cream	+++	Cream	+++	-
BARK9	White	+	Brownish cream	++	White	++	+
BARK12	White	+	Brownish cream	+++	White	++	+
BARK15	Brownish yellow	+++	Purplish white	+++	Purplish white	+++	-
BARK16	White	+++	White	+++	White	+++	-

Sign (-) for no growth on media, (+) poor growth, (++) moderate growth and (+++) good growth

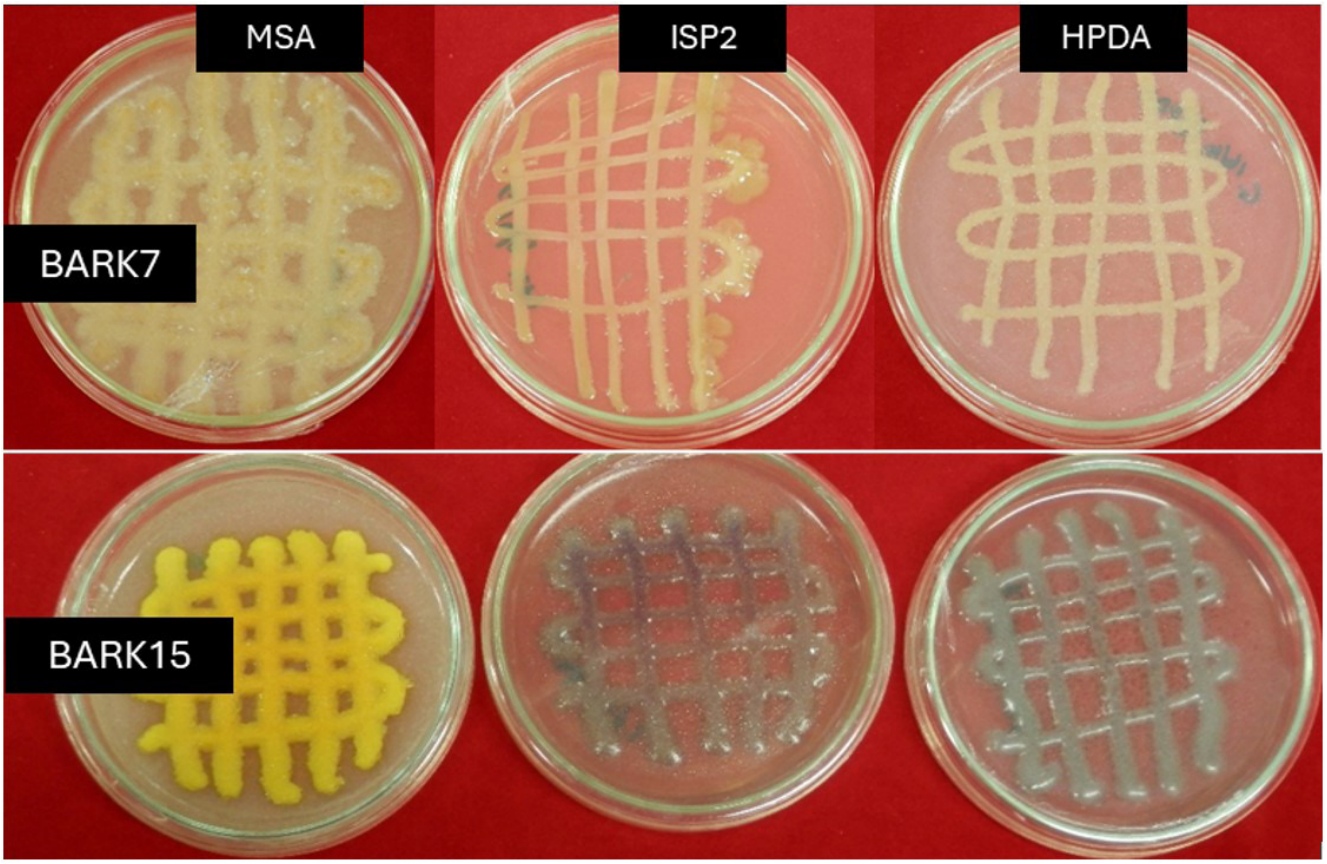

Figure 1. Comparison of morphological characteristics of BARK7 and BARK15 grown on MSA (mannitol soy agar), ISP2 (International Project Streptomyces 2) and HPDA (half strength PDA) media at 7 days after inoculation.

morphology of two bacterial isolates with notable antagonistic abilities, namely BARK7 and BARK15. The isolates demonstrated different morphological characteristics which might represent different types of bacteria.

G. boninense treated with the bacterial isolates on dual culture assay were showing significant difference of colony growth compared to control without any treatment (Table 2). The highest *G. boninese* growth inhibition was shown by BARK7 and BARK15 which inhibited up to 82.50% and 77.78%, respectively (Table 2 and Figure 2). The other isolates inhibited the growth of *G. boninense* ranging from 22.22% to 31.94%.

Table 2. Colony growth of *G. boninense* and its growth of inhibition by bacteria isolated from oil palm rhizosphere

Isolate	*G. boninense* colony width (cm)	*G. boninense* growth inhibition (%)
BARK4	4.22^{bc}±0.38	29.72
BARK5	4.67^{c}±0.12	22.22
BARK6	4.65^{c}±0.18	22.50
BARK7	1.05^{a}±0.15	82.50
BARK8	4.45^{bc}±0.36	25.83
BARK9	4.23^{b}±0.18	29.44
BARK12	4.08^{b}±0.29	31.94
BARK15	1.33^{a}±0.32	77.78
BARK16	4.57^{bc}±0.42	23.89
Control	6.00^{d}±0.00	0.00

Means following with different letters in the same column indicate significant difference at P<0.05 level by Duncan Multiple Range Test (P<0.05)

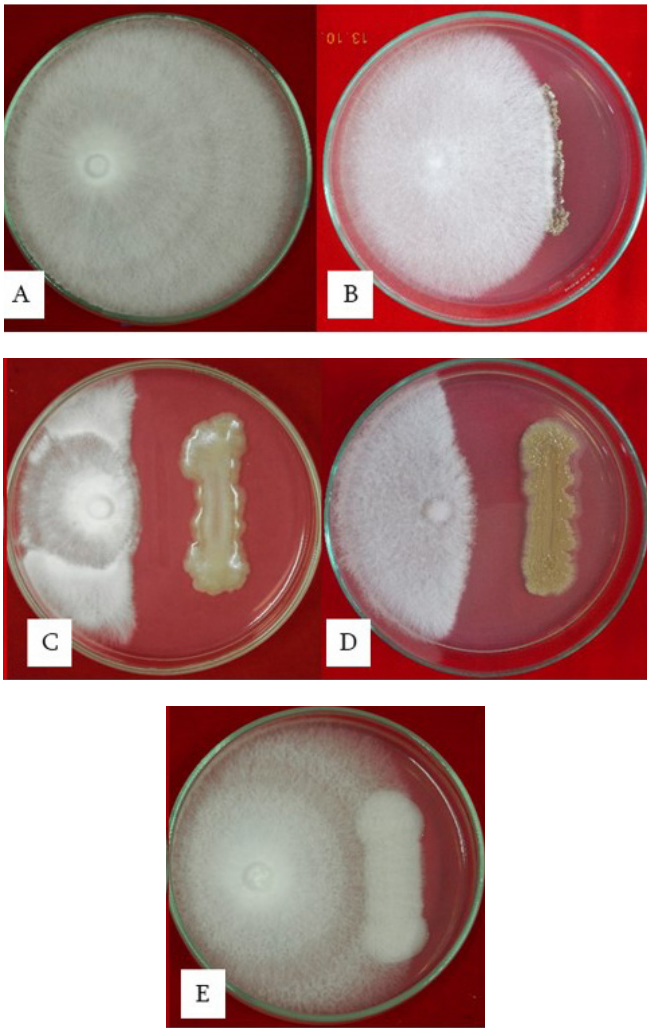

Figure 2. Comparison of *G. boninense* growth on PDA without rhizosphere bacterial isolates treatment (A) and its inhibited growth when treated with BARK4 (B), BARK7 (C), BARK15 (D), and BARK16 (E) at 7 days after inoculation

BARK7 and BARK15 showed very distinct inhibition zone compared to other isolates (Figure 2). *G. boninense* mycelia were supressed and condensed at the side facing the bacterial isolates of BARK7 and BARK15. With the rest of the isolates, the growth of *G. boninense* was not inhibited as strong as in BARK7 and BARK15. However, on dual culture treatment with BARK4, BARK5, BARK6, BARK8, BARK9, BARK9, BARK12, BARK15 AND BARK16, the mycelia of *G. boninense* showed only thinning growth. Not all the isolates were shown on Figure 2. The thinning growth represented by BARK4 (Figure 2B) and BARK16 (Figure 2E). Nevertheless, these isolates were able to suppress the growth of *G. boninense* although the percentage of inhibition was considerably low.

Microscopic observation was conducted on the two highest antagonistic activities (Figure 3). It shown that BARK7 and BARK15 caused mycelium alteration, whereas mycelium of *G. boninense* on control plate appeared to be normal (Figure 3A). Clamp connection (red arrow) (Figure 3B) and intertwined mass (Figure 3C) of *G. boninense* mycelia were found on plates treated with BARK7. While segmented (Figure 3D) and lysed mycelia (Figure 3E) were found on plates treated with BARK15.

Molecular identification of BARK7 and BARK15 suggested that these isolates were belonged to different species of bacteria. Approximately 700 bp 16S rRNA gene fragment was amplified using PCR method from the two isolates (Figure 4). The partial 16S rRNA sequencing following BLAST search through the NCBI database demonstrated that both BARK7 and BARK15 identified as *Burkholderia* sp., although there are species identification differences (Table 3).

3.2. Chitinase and Phosphate Solubilization Activity of the Rhizosphere Bacteria Isolates

Table 4 represents the ability of the bacterial isolates to degrade chitin and to solubilize phosphate. Only three isolates (BARK5, BARK12 and BARK 16) were able to hydrolyse chitin. Biocontrol agents' function through one or more mode of actions such as competition, parasitism, antibiosis, and induced resistance. Although only three isolates demonstrated the ability of degrading chitin, this does not mean that the rest of the isolates do not possess biocontrol properties. The isolates also demonstrated the ability to solubilize phosphate. All isolates, except for BARK5, produced clearing zone when grew on insoluble phosphate-containing media. This showed

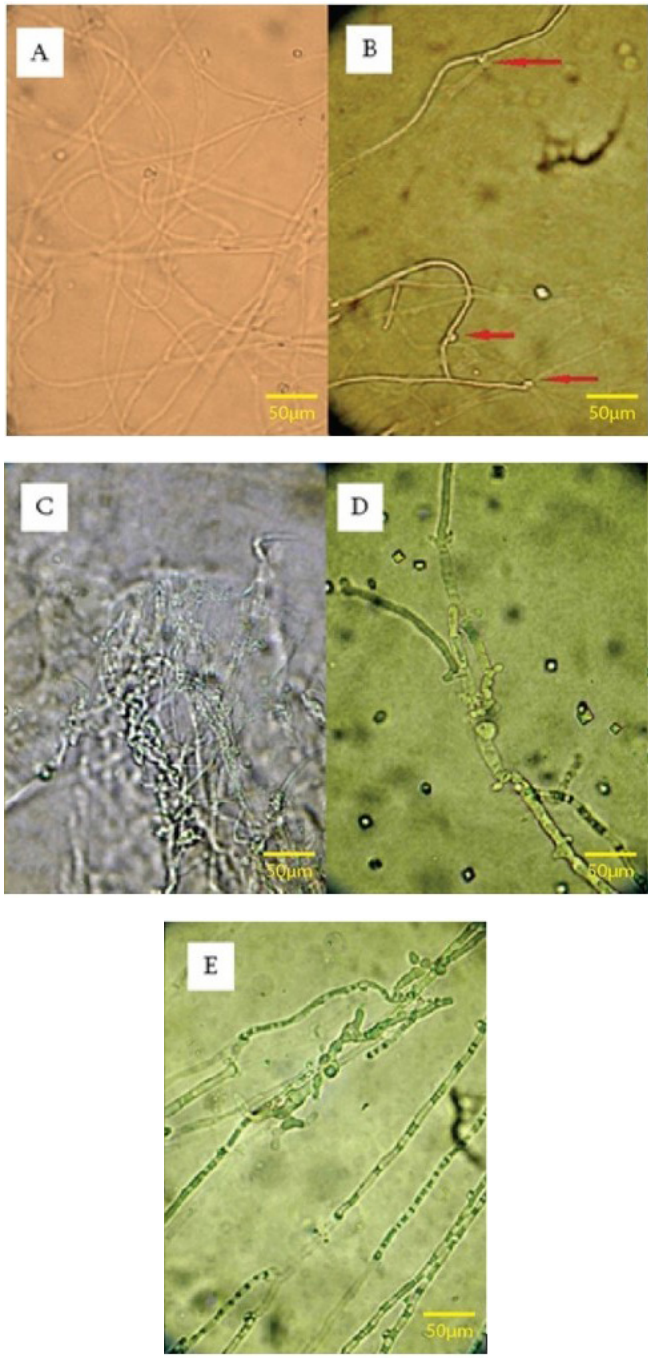

Figure 3. Mycelia of *G. boninense* on control treatment (A) and mycelia *G. boninense* alteration found on plates treated with BARK7 (B, C) and BARK15 (D, E)

that the isolates were able to solubilize the insoluble phosphate added to the media.

3.3. Antifungal Assay of Crude Metabolites of Rhizosphere Bacteria against *G. boninense*

The rhizosphere bacterial isolates were demonstrated potency to produce bioactive crude

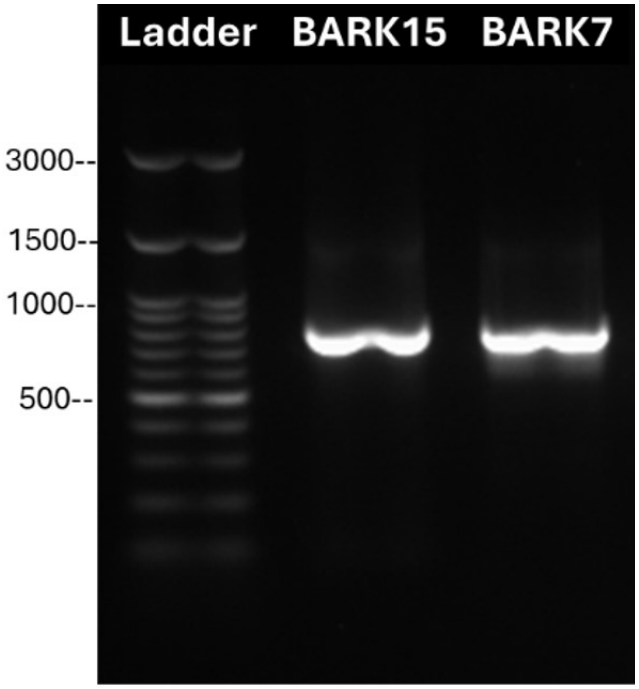

Figure 4. Visualization of the 16S rRNA gene PCR amplification products from the two selected bacterial isolates (BARK7 dan BARK15) which has high antagonistic activity

Table 3. NCBI nucleotide BLAST search result of the BARK7 and BARK15 nucleotide sequences

Isolate	Scientific name	Query cover (%)	e-value	Percent identity (%)
BARK7	*Burkholderia* sp. CRM109	62	$1e^{-161}$	89.19
	Burkholderia sp. CRM199	62	$1e^{-161}$	89.19
	Burkholderia sp. CRM81	62	$1e^{-161}$	89.19
BARK15	*Burkholderia cenocepacia*	91	0.0	98.13
	Burkholderia sp. NU93	91	0.0	97.99
	Burkholderia sp. MZ02	91	0.0	97.99

metabolites although the colony radial growth of *G. boninense* was not significantly different compared to control (Table 5). The level of the growth inhibition of *G. boninense* colony was also relatively low. However, under the microscope, it can be seen that the crude metabolites were able to cause the mycelia of *G. boninense* alteration (Figure 5). Thinning, and limited colonization of the *G. boninense* mycelia was also observed on wooden toothpicks soaked on the crude metabolites (Figure 6).

Table 4. The rhizosphere bacteria isolate ability in degrading chitin and solubilizing phosphate demonstrated by the presence of clearing zone on media

Isolate	Chitin hydrolysis Clearing zone (mm)	Phosphate solubilisation Clearing zone (mm)
BARK4	0.00±0.00[a]	3.23±0.18[b]
BARK5	4.16±0.05[b]	0.00±0.00[a]
BARK6	0.00±0.00[a]	2.86±0.07[b]
BARK7	0.00±0.00[a]	3.26±0.12[b]
BARK8	0,00±0.00[a]	4.10±0.07[c]
BARK9	0.00±0.00[a]	4.56±0.08[c]
BARK12	5.80±0.31[c]	5.13±0.06[d]
BARK15	0.00±0.00[a]	5.96±0.30[e]
BARK16	5.66±0.26[c]	5.30±0.22[d]

Means following with different letters in the same column indicate significant difference at P<0.05 level by Duncan Multiple Range Test (P< 0.05)

Table 5. Inhibition growth of *G. boninense* colony (%) following bacterial crude metabolite treatments

Treatment	Colony radial growth of *G. boninense* (cm)	Inhibition growth of *G. boninense* (%)
BARK4	4.75[a]±0.25	10.38
BARK5	4.67[a]±0.68	11.89
BARK6	5.20[a]±0.10	1.87
BARK7	4.97[a]±0.06	6.29
BARK8	4.95[a]±0.55	6.66
BARK9	4.90[a]±1.13	7.55
BARK12	4.70[a]±0.12	11.32
BARK15	4.87[a]±0.59	8.18
BARK16	4.80[a]±0.65	9.43
Control	5.30[a]±0.35	0.00

Means following with different letters in the same column indicate significant difference at P<0.05 level by Duncan Multiple Range Test (P< 0.05)

The colonization of *G. boninense* on wooden toothpicks was categorized into 4 groups. They were no colonization (group I), less than 20% colonization (group II), >20-40% colonization (group III) and 100% colonization (group IV) (Figure 6). Colonization of more than 40% to less than 100% was not detected. The result demonstrated that the crude metabolites produced by the bacterial isolates on ISP2 liquid media affect the growth of *G. boninense*. Wooden toothpicks treated with crude metabolites were not fully colonized by *G. boninense* as in control treatment where the wooden toothpicks were only treated with sterile water (Figure 6A and E).

The wooden toothpicks on the control treatment changed their color. It became whitish and looked paler compared to the other treatments as it fully covered (100% colonization, group IV) with mycelia of *G. boninense*. The growth of *G. boninense* on control toothpicks were observed denser than mycelia on wooden toothpicks treated with crude metabolites. However, colonization of *G. boninense* was not found on the wooden toothpicks treated with crude metabolite produced by BARK7 and BARK8 which then categorized into group I (Figure 6B). Less than 10% colonization (Group II) of *G. boninense* was found on BARK5, BARK9, BARK12 and BARK15 (Figure 6C). On those treatments, mycelia of *G. boninense* were unable to cover the wooden surface, it was only found on the edge of the wooden toothpicks. Furthermore, group III colonization with 20-40% mycelia covering the wooden surface were detected

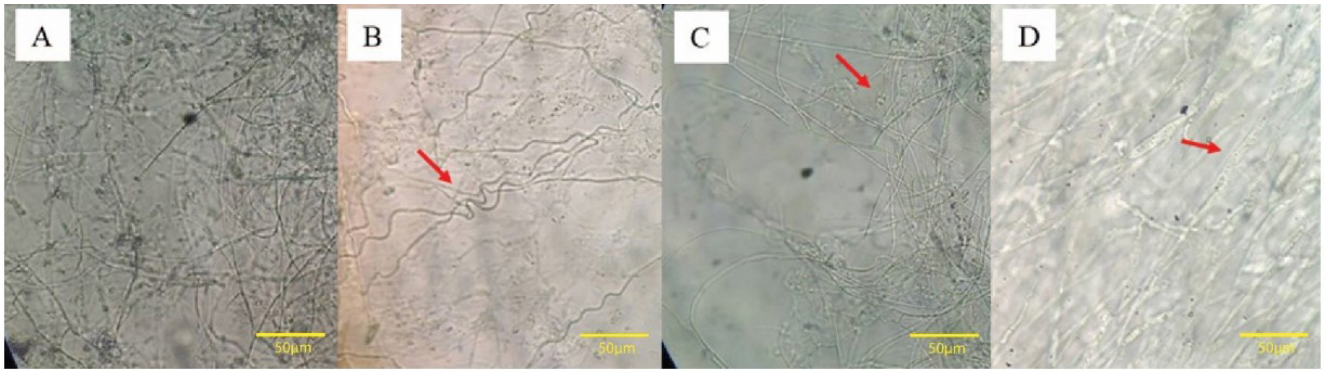

Figure 5.Effect of crude metabolites produced by rhizosphere bacterial isolates on ISP2 liquid media on the mycelia of *G. boninense* using the well diffusion method. (A) Normal hypae (control and BARK9), (B) curling (BARK4, BARAK5, BARK6, BARK8, BARK12 and BARK15), (C) lysed (BARK16), and (D) swelling (BARK7)

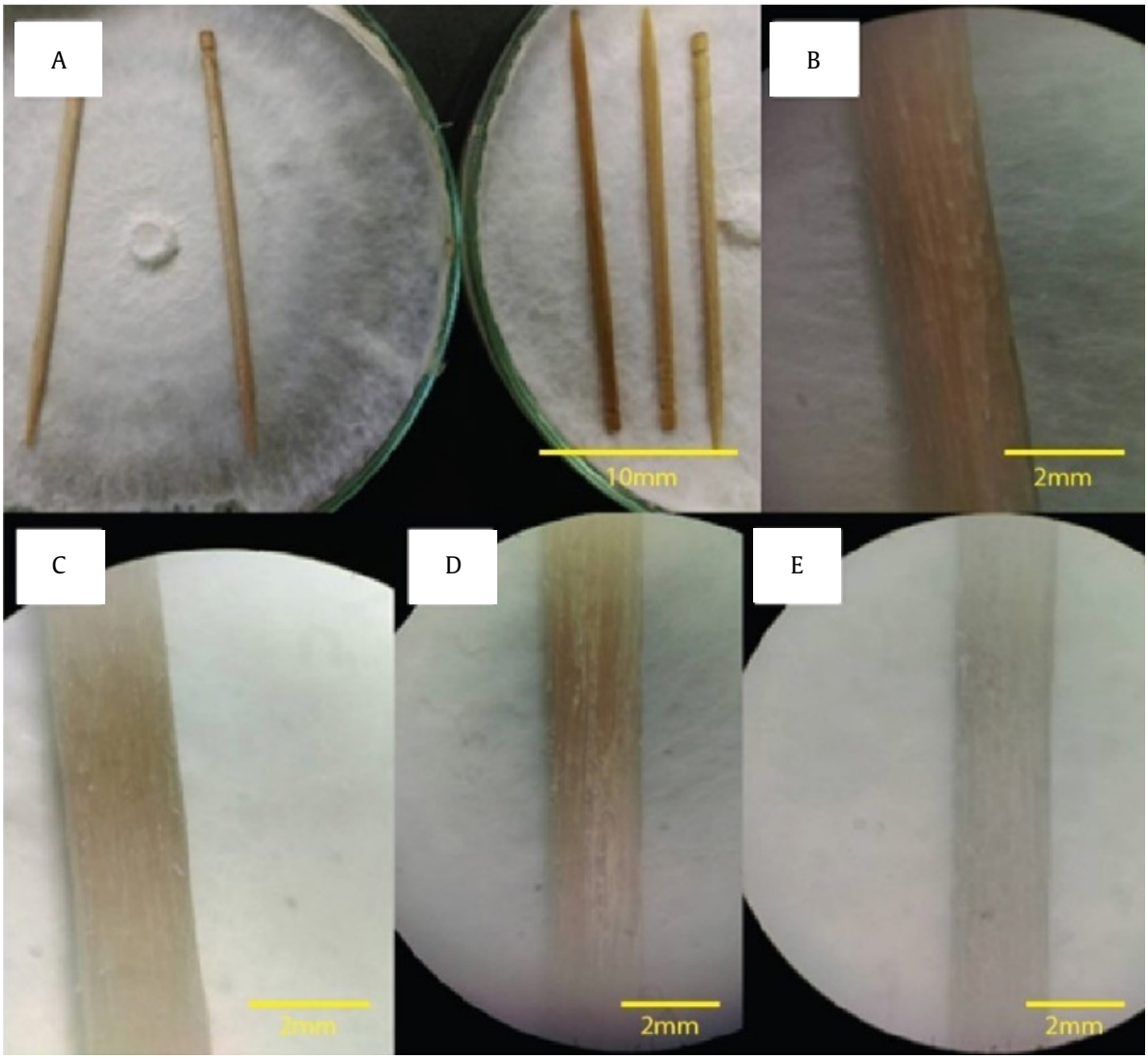

Figure 6. Colonization of *G. boninense* on wooden toothpicks. Comparison of wooden toothpicks with (right) and without (left) crude metabolite treatment (A), no colonization (group I) (B), less than 20% colonization (group II) (C), 20-40% colonization (group III) (D), and 100% colonization (group IV) (E)

on BARK4, BARK6 and BARK16 crude treatments (Figure 6D). *G. boninense* colonization of more than 40% was not found.

4. Discussion

Two rhizosphere bacterial isolates (BARK 7 and BARK15), identified as *Burkholderia* sp., demonstrated potential ability to be developed as biocontrol agents to control *G. boninense*. These isolates showed high antagonistic activity on the growth of *G. boninense* and able to cause alteration of the mycelia. The alteration in hyphal morphology can contribute to the loss of fungal pathogen's virulence. Hyphae plays important role in the plant pathogen fungi penetration processes into plant tissues. *G. boninense* infects the oil palm by forming needle like structure form its hyphae to puncture the plant root (Bharudin *et al.* 2022).

Numerous studies have documented the antagonistic activities of microorganisms isolated from the rhizosphere of oil palm trees, aiming to develop sustainable methods for controlling basal stem rot disease. Potential biocontrol agents have been reported to be isolated from various niches of oil palm with Burkholderia found to be the dominant bacterial genus (Yurnaliza *et al.* 2020). *Burkholderia* is a diazotrophic bacterial genus that can be found in diverse environments including plant rhizosphere

(Company *et al.* 2008). Although the genus also known as plant pathogenic member, it also contains species that are posses biocontrol properties. *B. cepacia, B. contaminans, B. metallica*, and *B. stagnalis* were reported to have high antagonistic activity against *G. boninense*, able to produce antifungal properties as well as hydrolytic enzymes (Yurnaliza *et al.* 2020). Furthermore, the main mechanism actions of bacterial biocontrol agents are those that directly affecting the pathogen. To control the pathogens, biocontrol agents can employ both direct and indirect mechanisms. Direct mechanisms are involving the antibiosis activity in which the biocontrol agents can secrete inhibitory compounds, competition where the agents compete for nutrition and space with the pathogen, and mycoparasitism in which the biocontrol agents taking nutrients from the pathogen (Thambulaga *et al.* 2020; Bonaterra *et al.* 2022). Whereas the indirect mechanisms involve in strengthening the plant resistant through increasing the nutrients uptake as well as inducing the plant resistance (Bonaterra *et al.* 2022).

Chitin is the major component of fungal cell wall. Degradation of chitin can cause severe damage and lead to the death of fungal pathogen (Veliz *et al.* 2017). Therefore, the ability to degrade chitin plays an important role in biocontrol agent. BARK5, BARK 12 and BARK15 were detected able to degrade chitin in which one of biocontrol mechanisms possessed by the isolates. *G. boninense* is a hemi biotrophic fungal pathogen that infects its host through two phases, biotrophic phases and necrotrophic phase (Ramzi *et al.* 2019). The biotrophic phase initiates by the contact of mycelium with the root. This phase is shown by the presence of white hyphae on the infected root. The necrotrophic phase starts by the production of cell wall degrading enzymes such as lignin peroxidase to destroy the plant cell wall (Faizah *et al.* 2022). Therefore, if a biocontrol agents have the ability to degrade chitin of the pathogenic fungi, it can inhibit or slow down the infection rate of the pathogen.

Naturally *G. boninense* can infect wooden toothpicks as it can degrade cellulose and lignin as food source (Tan *et al.* 2018). In this study, inhibition of *G. boninense* colonization were shown on the wooden toothpicks treated with the bacterial crude metabolite. The rhizosphere bacteria isolates were showing potency to produce some antifungal compounds. However, the production of the compounds needs to be optimized. Antimicrobial compounds can affect the phytopathogenic fungi through several mechanisms which include fungistatic induction, inhibition of spore germination, lysis of fungal mycelia, or by exerting fungicidal effects. Many antibiotics have been reported to be produced by rhizobacteria which are then able to inhibit the growth of pathogens including from *Burkholderia*. Prihatna *et al.* (2022) reported that antifungal peptides produced by *Burkholderia* sp. strain CP01 showed the ability to supress the basal stem rot disease on oil palm seedling.

Biocontrol agents that own several pathogen inhibition mechanisms have great potency to be successful in supressing the disease. Most of the rhizosphere bacterial isolates were also found able to solubilize phosphate except for BARK5. Phosphorus is one of the key elements needed for plant growth. However, its availability in the ground is limited for the plant uptake. Phosphorus is available on the soil in the form of insoluble iron and aluminium phosphate in soil with pH lower than 5 or in the form of insoluble calcium phosphate in soil with pH above 7 (Penn and Camberato 2019). Oil palm trees need large quantity of nutrients including phosphorus (Arifin *et al.* 2022). Therefore, the presence of microorganisms with ability to solubilize phosphate gives advantages to the plant by enhancing plant phosphorus acquisition. Biocontrol agents with phosphate solubilisation ability can benefit the plant by providing protection to the plant pathogens as well as aid the plant growth and plant health (Alori *et al.* 2017).

In conclusion, rhizospheric bacteria isolates, especially BARK 7 and BARK15, showed promising ability to be developed as biocontrol agents for basal stem rot disease of oil palm. They were able to inihibit the growth of *G. boninense* and trigger morphological changes of the *G. boninense* mycelia. Some rhizospheric bacterial isolates were able to degrade chitin and most of the isolates were phosphate solubilization bacteria. The crude metabolites produced by the isolates on ISP2 liquid media demonstrated inhibition activity toward the growth of *G. boninense* which also causing mycelial alteration although the inhibition level was relatively low. In addition, inhibition of *G. boninense* colonization on wooden toothpicks soaked with the metabolites were also detected.

Conflict of Interest

The authors declare that there is no conflict of interest.

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References

Alori, E.T., Glick, B.R., Babalola, O.O., 2017. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Front. Microbiol.* 8 , 971. https://doi.org/10.3389/fmicb.2017.00971

Arifin, I., Hanafi, M.M., Roslan, I., Ubaydah, M.U., Karim, Y.A., Tui, L.C., Hamzah, S., 2022. Responses of irrigated oil palm to nitrogen, phosphorus and potassium fertlizers on clayey soil. *Agricultural Water Management*. 274, 107922 .https://doi.org/10.1016/j.agwat.2022.107922

Balouiri, M., Sadiki, M., Ibnsouda, SK., 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. *J. Pharm. Anal.* 6, 71-9. https://doi.org/10.1016/j.jpha.2015.11.005

Benaissa, A., 2024. Rhizosphere: Role of bacteria to manage plant diseases and sustainable agriculture–a review. *J. Basic. Microbiol.* 64, 2300361. https://doi.org/10.1002/jobm.202300361

Beneduzi, A., Peres, D., Vargas, L.K., Bodanese-Zanettini, M.H., Passaglia, L.M.P., 2008. Evaluation of genetic diversity and plant growth promoting activities of nitrogen-fixing bacilli isolated from rice fields in South Brazil. *Appl. Soil. Ecol.* 39, 311-20. https://doi.org/10.1016/j.apsoil.2008.01.006

Bharudin, I., Ab Wahab, A.F.F., Smad, M.A.A., Yie, N.X., Zairun, M.A., Bakar, F.D.A., Murad, A.M.A., 2022. Review update on the life cycle, plant-microbe interaction, genomics detection and control strategies of the oil palm pathogen *Ganoderma boninense*. *Biology*. 11, 251. https://doi.org/10.3390/biology11020251

Bivi, M.S.H.R., Paiko, A.S., Khairulmazmi, A., Akhtar, M.S., Idris, A.S., 2016. Control of basal stem rot disease in oil palm by supplementation of calcium, copper, and salicylic acid. *Plant. Pathol. J.* 32, 396-406. https://doi.org/10.5423/PPJ.OA.03.2016.0052

Bonaterra, A., Badosa, E., Daranas, N., Frances, J., Rosello, G., Montesinos, E., 2022. Bacteria as biological control agents of plant diseases. *Microorganisms.* 10, 1759. https://doi.org/10.3390/microorganisms10091759

Collins, C.H., Lyne, P.M., Grange, J.M., Falkinham, J.O., 2004. *Microbiological Methods*. Hodder Education Publishers, London.

Company, S., Nowak, J., Coenye, T., Clemet, C., Barka, E.A., 2008. Diversity and occurrence of *Burkholderia* spp. in the natural environment. *FEMS Microbiol. Rev.* 32, 607-626. DOI: 10.1111/j.1574-6976.2008.00113.x

Coombs, J.T., Franco, C.M.M., 2003. Isolation and identification of actinobacteria from surface-sterilized wheat roots. *Appl. Environ. Microbiol.* 69, 5603–5608. DOI: 10.1128/AEM.69.9.5603-5608.2003

Dermiyati, Suharjo, R., Telaumbanua, M., Yosita, R., Sari, A.W., Andayani, A.P., 2023. Antagonist and plant growth promoting potential of indigenous bacteria isolated from oil palm empty fruit bunches. *Biodiversitas.* 24, 1136-1142. DOI:10.13057/biodiv/d240253

Faizah, R., Putranto, R.A., Raharti, V.R., Supena, N., Sukma, D., Budiani, A., Wening, S., Sudarsono, S., 2022. Defense response changes in roots of oil palm (*Elaeis guineensis* Jacq.) seedlings after internal symptoms of *Ganoderma boninense* Pat. infection. *BMC Plant Biol.* 22, 139. https://doi.org/10.1186/s12870-022-03493-0

Han, X.Y., 2006. Bacterial identification based on 16S ribosomal RNA gene sequence analysis, in: Tang, Y.W., Stratton, S.W. (Eds.), *Advance Techniques in Diagnostic Microbiology*, New York: Springer Science+Media Business, LLC, pp. 323-332.

Haw, Y.H., Lai, K.W., Chuah, J. H., Bejo, S.K., Husin, N.A., Hum, Y.C., Yee, P.L., Tee, C.A.T.H., Ye, X., Wu, X., 2023. Classification of basal stem rot using deep learning: a review of digital data collection and palm disease classification methods. *PeerJ. Comput. Sci.* 9, e1325. DOI: 10.7717/peerj-cs.1325

Hu, Y., Li, Y., Yang, X., Li, C., Wang, L., Feng, J., Chen, S., Li, X., Yang, Y., 2021. Effects of integrated biocontrol on bacterial wilt and rhizosphere bacterial community of tobacco. *Sci. Rep.* 11, 2653. https://doi.org/10.1038/s41598-021-82060-3

Hushiarian, R., Yusof, N.A., Dutse, S.W., 2013. Detection and control of Ganoderma boninense: strategies and perspectives. *SpringerPlus.* 2, 555. https://doi.org/10.1186/2193-1801-2-555

Kawase, T., Saito, A., Sato, T., Kanai, R., Fujii, T., Nikaidou, N., Miyashita, K., Watanabe, T., 2004. Distribution and phylogenetic analysis of family 19 chitinases in Actinobacteria. *Appl. Environ. Microbiol.* 70, 1135-44. https://doi/org/10.1128/AEM.70.2.1135-1144.2004

Kiranmayi, M.U., Sudhakar, P., Sreenivasulu, K., Vijayalakshmi, M., 2011. Optimization of culturing conditions for improved production of bioactive metabolites by Pseudonocardia sp. VUK-10. *Mycobiology*. 39, 174-81. https://doi.org/ 10.5941/MYCO.2011.39.3.174

Maherani, V.F.A., Mubarik, N.R., Priyatno, J.A., Putra, I.P., 2024. Biosurfactant activity of Bacillus strain LP04 isolate and its antifungal potency against *Ganoderma boninese* and *Fusarium* sp. *Hayati J. Biosci.* 31, 725-732. DOI: 10.4308/hjb.31.4.725-732

Paulitz, T.C., Schroeder, K.L., 2005. A new method for the quantification of *Rhizoctonia solani* and *R. oryzae* from soil. *Plant Dis.* 89, 767-72. https://doi.org/ 10.1094/PD-89-0767

Penn, C.J., Camberato, J.J., 2019. A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. *Agriculture.* 19, 120. DOI:10.3390/agriculture9060120

Paterson, R.R.M., 2019. *Ganoderma boninense* disease of oil palm to significantly reduce production after 2050 in Sumatra if projected climate change occurs. *Microorganisms*. 7, 24. https://doi.org/10.3390/microorganisms7010024

Prihatna, C., Pramudito, T.E., Arifin, A.R., Nguyen, T.K.N., Purnamasari, M.I., Suwanto, A., 2022. Antifungal peptides from a *Burkholderia strain* suppress basal stem rot disease of oil palm. *Phytopatol.* 112, 238-248. https://doi.org/10.1094/PHYTO-11-20-0529-R

Purba, A., Basyuni, M., Putri, L., Chalil, D., Hayati, R., Arifiyanto, D., Syahputra, I., 2019. Sequence analysis of *Ganoderma boninense* isolates from oil palm. *IOP Conference Series: Earth and Environmental Science.* 260, 012172.

Qin, Y., Shang, Q., Zhang, Y., Li, P., Chai, Y., 2017. Bacillus amyloliquefaciens L-S60 reforms the rhizosphere bacterial community and improves growth conditions in cucumber plug seedling. *Front. Microbiol.* 8, 02620. https://doi.org/10.3389/fmicb.2017.02620

Ramzi, A.B., Me, M.L.C., Ruslan, U.S., Baharum, S.N., Muhammad, N.A.N., 2019. Insight into plant cell wall degradation and pathogenesis of *Ganoderma boninense* via comparative genome analysis. *PeerJ*. 7, e80065. https://doi.org/ 10.7717/peerj.8065

Skidmore, A.M., Dickinson, C.H., 1976. Colony interactions and hyphal interference between *Septoria nodorum* and phylloplane fungi. *Transactions of the British Mycological Society.* 66, 57-64. https://doi.org/ 10.1016/S0007-1536(76)80092-7

Statistic Indonesia. 2022. Exports of Palm Oil by Major Countries of Destination, 2012-2021. Available at: https://www.bps.go.id/statictable/2014/09/08/1026/ekspor-minyak-kelapa-sawit-menurut-negara-tujuan-utama-2012-2021.html. [Date accessed: 12 June 2023]

Susanto, A., Sudharto, P.S., Purba, R.Y., 2005. Enhancing biological control of basal stem rot disease (*Ganoderma boninese*) in oil palm plantation. *Mycopathologia.* 159, 153-157. https://doi.org/ 10.1007/s11046-004-4438-0

Tan, J.S., Lee, Y.P., Sulaiman, S., Camus-Kulandaivelu, L., Klopp, C., Mercière, M., Breton, F., Durand-Gasselin, T., Syed Alwee, S.S.R., 2018. The route to the development of basal stem rot resistance in oil palm (*Elaeis guineensis*) via the discovery of lignin degradation process in the pathogen Ganoderma boninense. *Acta Hortic.* 1205, 359-370. https://doi.org/10.17660/ActaHortic.2018.1205.42

Thambulaga K.M., Daranagama D., Phillips A.J.L., Kannangara S.D., Promputtha I., 2020. Fungi vs fungi in biocontrol: An overview of fungal antagonists applied against fungal plant pathogens. *Front. Cell. Infect. Microbiol.* 10, 604923. https://doi.org/10.3389/fcimb.2020.604923

Veliz, E.A., Martinez-Hidalgo, P., Hirsch, A.M., 2017. Chitinase-producing bacteria and their role in biocontrol. *AIMS Microbiol.* 3, 689-705. https://doi.org/ 10.3934/microbiol.2017.3.689

Wang, M., Ma, Q., 2011. Antagonistic Actinomycete XN-1 from phyllosphere microorganisms of cucumber to control *Corynespora cassiicola. Cucurbit Genetics Cooperative Report.* 33-34, 17-21.

Yurnaliza, Y., Rambe, D.I., Sarimunggu, L., Purba, M., Nurwahyuni, I., Lenny, S., Lutfia, A., Hartanto, A., 2020. Screening of *Burkholderia* spp. from oil palm plantation with antagonistic properties against Ganoderma boninense. *Biodiversitas.* 21, 3431-3437. DOI:10.13057/biodiv/d210803

Zakaria, L., 2023. Basal stem rot of oil palm: the pathogen, disease incidence, and control methods. *Plant Dis.* 107, 603-615. https://doi.org/10.1094/PDIS-02-22-0358-FE