

Isolation and characterization of siderophore-producing bacteria with antagonist activity against *Ganoderma boninense* from tropical peatland ecosystems

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Abstract: The presence of diseases in oil palms is still a concern, the most important of which is infection by the fungus *Ganoderma boninense*, which can be fatal if left uncontrolled, leading to plant death. To encourage the implementation of sustainable agriculture, the role of bacteria as biological control agents is crucial, as they can reduce or replace the use of synthetic pesticides. This research focuses on obtaining bacterial isolates capable of controlling *G. boninense* infections in oil palm nurseries. This study used a descriptive exploratory method. Peat sampling as a potential source of bacteria was conducted in Teluk Bakung Village, a village in West Kalimantan Province with extensive peatlands, located in Sungai Ambawang District, Kubu Raya Regency. The sample was taken purposively, randomly based on differences in peat maturity (sapric, hemic, and fibric). This research consists of 3 stages: (1) bacterial isolation; (2) bacterial selection with hypersensitivity test on tobacco plants, siderophore production test, and antagonist test against *G. boninense in vitro* condition; (3) application of selected isolates to oil palm seedlings in peat media infected with *G. boninense*. *In vitro* antagonist testing and pot application using a completely randomized design with three replications. The results of the study showed that 12 peat samples used had a pH below 4.00 (very acidic). The sapric, hemic, and fibric samples had fiber contents ranging from 28.95% to 31.75%, 48.77% to 62.45%, and 82.02% to 83.75%, respectively. The fourteen bacterial isolates were obtained from isolation and tested for selection. The results showed that 11 isolates were negative for pathogens, and 6 isolates were positive for producing siderophores. Then, one isolate from sapric, hemic, and fibric was selected, which had the highest percentage of inhibitory power against *G. boninense*. The three isolates were S2A, H5, and F2. Based on the application test on oil palm seedlings up to week 8, it was observed that the application of all isolates was able to prevent necrosis in the leaves, in contrast to those without bacterial application, which showed quite severe necrosis symptoms.

Keywords: Peat maturity, Biocontrol, Seedling, Soil-borne disease

Abbreviation: CAS-A_chrome azurol s agar; D_diameter; Fe_ferrum; NA_Nutrient agar; NB_nutrient broth; nt_not tested; PDA_potato dextrose agar.

Introduction

Soil bacteria are important biological agents and have been extensively studied as a potential solution for sustainable agriculture. One potential source of microbes is peatland. A peatland is a tropical ecosystem that serves as a source of abundant natural microbes, particularly in several regions of Indonesia, such as West Kalimantan. Peat is considered unique because it has a very acidic pH, high organic content, and different maturity levels based on the level of microbial decomposition. It can absorb a very high amount of water but can also lose water irreversibly under certain conditions. Peat maturity is distinguished by the percentage of fiber content contained in the peat. In sapric, also known as mature peat, the fiber content is less than 33%, hemic peat (semi-mature) has a fiber content of more than 33 to 66%, while fibric peat or raw peat has the highest fiber content, more than 66% (Wiratmoko et al., 2008). The influence of organic matter content in peat is very large on the abundance and potential of microbes, making it a suitable environment for microbial isolation.

Several bacteria isolated from unique and extreme environments show effectiveness when applied as biofertilizers and biocontrols. The ability of a bacterium to produce siderophores is positively correlated with its ability to stimulate plant growth and be antagonistic to disease. Siderophores are iron ion-chelating compounds generally produced by bacteria and fungi under low-iron conditions. The presence of siderophores can make iron available to microbial cells (De Serrano, 2017). Siderophore-producing bacteria isolated from tomato plant rhizospheres produced an inhibitory zone against *Ralstonia solanacearum* (Nawangsih et al., 2017). In addition, *Bacillus siamensis* Gxun-6, isolated from the rhizosphere soil of banana plants, exhibited a potent ability to promote plant growth and displayed inhibitory activity against *Fusarium oxysporum* in both *in vitro* and *in vivo* tests (Shen et al., 2022).

Oil palm (*Elaeis guineensis* Jacq.) is a primary commodity on Indonesian plantations. The primary issue affecting their production is the high cost of fertilization and disease management, particularly against *Ganoderma boninense*, which can reduce productivity and even lead to plant death. Unwise and excessive application of synthetic fertilizers and pesticides can disrupt soil biology and pollute the environment. The use of microorganisms as biofertilizers and biocontrol agents in oil palm cultivation has been analyzed. Based on research by Lim et al. (2019), four soil bacterial isolates were found to have more than 50% inhibitory power against *Ganoderma boninense in vitro*. Of the four isolates, the *Pseudomonas aeruginosa* isolate had the best ability, and the crude extract from this isolate containing bioactive compounds with antifungal properties. The antagonistic activity of crude extract from *P. aeruginosa* isolates against *G. boninense* was also analyzed by Irma et al. (2018). The results also showed that fungal growth was prevented by up to 60%. In addition, isolates of the same species but originating from endophytes have also been able to inhibit fungal growth by up to 70% *in vitro* and reduce the disease infection rate by 62%–75% after 6 months of bacterial application to plants (Ramli et al., 2016). The use of bacteria as a biofertilizer and biocontrol can be a remarkable alternative for sustainable oil palm cultivation.

Results and Discussion

Characterization of bacteria isolated from peat samples

Table 1 presents the isolation results from the 12 peat samples, yielding 14 bacterial isolates characterized by their colony and cell morphology. The peat samples used as a source of bacteria comprised of two samples of sapric, eight samples of hemic, and two samples of fibric. The lower the maturity of the sample, the lower the pH, but all are classified as very acidic (pH < 4.00). The aim of using different levels of peat maturity and low-pH soil as sources of bacteria is to obtain candidate bacteria that exhibit high viability and performance, and produce secondary metabolites with potential as biofertilizers and biocontrol agents.

The difference between the three types of peat maturity is in the level of organic matter decomposition: sapric is well decomposed, hemic has moderate decomposition, and fibric has low decomposition. The abundance of bacteria in sapric and hemic peat is higher than that in fibric peat. These three types of peat produce diverse bacterial characteristics. The 14 bacterial isolates exhibited varying colony and cell morphologies.

Gram-negative bacteria have a more complex cell wall composition than gram-positive bacteria based on the morphology of their cell wall. This generally helps gram-negative bacteria become more resistant to unfavorable environments and antibacterial compounds from other microorganisms. However, the ability of a bacterium to survive is largely determined by its ability to produce compounds that are beneficial. Some Gram-positive bacteria from the soil possess superior antifungal properties because they are known to produce beneficial compounds, such as extracellular enzymes, siderophores, and antibiotics. The most common genus of soil bacteria found with potential as a biocontrol agent is the genus *Bacillus*. In addition to directly combating soil-borne diseases, the bacterium *Bacillus subtilis* has been shown to induce resistance in seedlings exposed to *G. boninense* endophytically through a mechanism that optimizes plant growth (Puspita et al., 2019).

Table 1. Data on maturity type, pH, fiber content of 12 peat samples, and characteristics of isolated bacteria.

No	Peat type	pH	Fiber content (%)	Isolate code	Colony color	Cell form	Gram
1	Sapric (1)	3.95	28.95	S1	Yellow	Coccus	Negative
2	Sapric (2)	3.84	31.25	S2A	White	Basil	Positive
				S2B	Yellow	Coccus	Negative
3	Hemic (1)	3.69	50.31	H1	Yellow	Coccus	Positive
4	Hemic (2)	3.67	61.88	H2	Yellow	Coccus	Positive
5	Hemic (3)	3.71	48.80	H3	White	Basil	Positive
6	Hemic (4)	3.73	60.24	H4	Yellow	Coccus	Positive
7	Hemic (5)	3.72	48.77	H5	White	Basil	Positive
8	Hemic (6)	3.61	62.45	H6	White	Coccus	Positive
9	Hemic (7)	3.70	57.25	H7	White	Coccus	Positive
10	Hemic (8)	3.77	60.50	H8A	White	Coccus	Negative
				H8B	Yellow	Coccus	Positive
11	Fibric (1)	3.57	82.02	F1	Yellow	Basil	Positive
12	Fibric (2)	3.64	83.75	F2	White	Basil	Positive

Source: Laboratory analysis (2024).

Hypersensitivities and Siderophore-producing

Candidate bacteria were tested for pathogenicity on tobacco leaves using the hypersensitivity method. Pathogenic bacteria on plants were observed from the death of test plant cells due to infection at the location where the bacterial culture was injected (Fig 1b). The reaction is fast and limited only to the injected part. Non-pathogenic bacteria will not cause infection or death of the test plant cells. Three bacterial isolates tested positive for plant pathogenicity and were; therefore, excluded from further testing (Table 2).

Bacteria can produce secondary metabolites to enhance their survival and adapt to their environment. Each bacterial species produces different metabolites, influenced by the availability of nutrients and environmental conditions. Extreme environments are a source of unique microbes that can create unique compounds (Mahdiyah et al., 2020).

Table 2. Results of the hypersensitivity test of 14 isolates, and the siderophore production tests of 11 non-pathogenic bacterial isolates.

No	Isolate code	Hypersensitivity	Siderophore
1	S1	Negative	Negative
2	S2A	Negative	Positive
3	S2B	Negative	Negative
4	H1	Positive	Nt
5	H2	Negative	Negative
6	H3	Negative	Negative
7	H4	Positive	Nt
8	H5	Negative	Positive
9	H6	Negative	Positive
10	H7	Negative	Positive
11	H8A	Negative	Positive
12	H8B	Negative	Negative
13	F1	Positive	Nt
14	F2	Negative	Positive

Notes: Positive: necrosis on the leaves and a halo around the colony; Negative: indicates no necrosis on the leaves and no halo formation; nt: not tested.

A siderophore is a bacterial secondary metabolite that is vital as both a biofertilizer and a biocontrol agent. CAS-A media identifies bacteria that qualitatively produce siderophores. The Fe (III) contained in the medium has been converted into Fe (II) by siderophores, which is observed as a color change or halo around the bacterial colony (Ehsan et al., 2022) (Fig 1d). The ability of bacteria to produce siderophores plays a direct or indirect role in the agricultural sector, namely in plant growth and pathogen biocontrol. One example is that its presence can alter iron uptake from the soil and stimulate the production of indole-3-acetic acid, a growth hormone (De Serrano, 2017; Nawangsih et al., 2017).

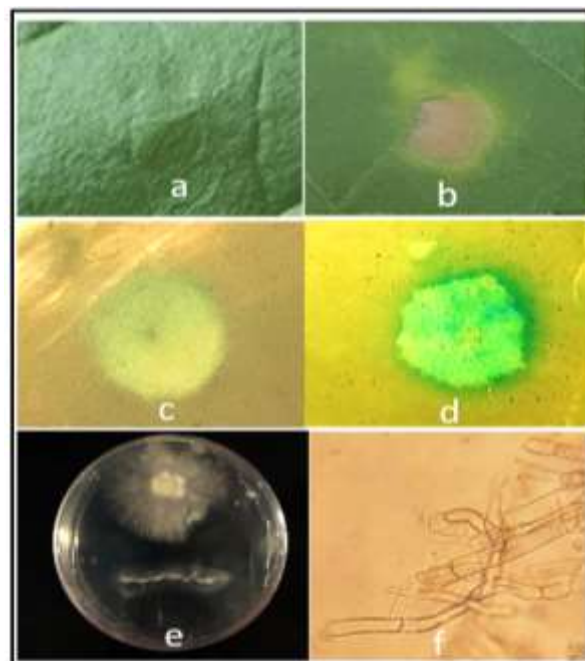


Fig 1. (a) No necrosis is observed on tobacco leaves treated with H5 isolates; (b) necrosis occurs on tobacco leaves treated with H1 isolates; (c) no halo is formed around bacterial colony H1 in CAS-A media; (d) a halo is formed around bacterial colony H5 in CAS-A media; (e) dual-culture isolates H5 and *G. boninense*; (f) microscopic image of *G. boninense* hyphae after antagonist test of isolate H5.

Effectiveness of the antagonistic ability of bacterial isolates

The inhibitory effect of bacteria as an antifungal was seen in PDA media inoculated with a dual culture. Isolates originating from hemic and fibric peat showed inhibition of more than 50% (Table 3).

Table 3. Data on the percentage of inhibitory power against *G. boninense* by 6 bacterial isolates that produce siderophores.

Peat source	Isolate Code	% Inhibition
Sapric	S2A	14.81
Hemic	H5	53.70
Hemic	H6	16.67
Hemic	H7	31.46
Hemic	H8A	46.30
Fibric	F2	55.56

Source: Laboratory analysis (2024). Notes: The isolates in bold are selected isolates from each level of peat maturity for further testing.

This inhibitory ability can also be caused by the excretion of catalytic enzymes that can break down hyphae. Discontinuous hyphal conditions were observed after testing with isolate H5 (Fig 1f). It has also been discovered that soil bacteria have the ability to produce siderophores and act antagonistically (Kalyan et al., 2022). Four isolates from Calcareous Soils in Tamil Nadu, identified as *Bacillus licheniformis*, *Bacillus subtilis*, and *Ochrobactrum grignonense*, were tested for antagonistic activity against *Sclerotium rolfsii*. All four were able to reduce their mycelial growth by 48–76%.

The higher the ability to produce various secondary metabolites of a bacterium, the better its antagonistic ability. The natural habitat of the bacteria greatly influences this ability. Bacteria isolated from hemic and fibric have better ability than bacteria isolated from sapric. At the maturity level of fibric, the materials are more complex than those of hemic, and hemic is more complicated than those of sapric, owing to incomplete decomposition factors, which have been shown to have high fiber content. Therefore, when bacteria live in environmental conditions with low nutrient availability, they produce secondary metabolites to decompose or break down complex materials into smaller ones so they are easier to absorb. Diverse microbes with different peat maturities can be found in the peat area of Teluk Bakung Village. Hemic peat has a greater number of microbes than fibric and sapric. However, the number of cellulose-degrading microbes, especially fungi, in hemic and sapric, is not considerably different (Khotimah et al., 2021). A higher number of decomposer microbes causes the nutrients in such soils to be simpler and more readily available to microbes than in fibric peat. Therefore, isolate F2, derived from fibric, may produce more potent secondary metabolites than other isolates.

One isolate from each peat maturity level was selected for the application test on oil palm seedlings exposed to *G. boninense* in pots, namely isolates S2A, H5, and F2. In this study, observations were conducted eight weeks after bacterial application to determine its effect. The results showed that no necrosis was observed in the leaves of oil palm seedlings after the application of S2A, H5, and F2 bacteria. However, necrosis symptoms did appear in seedlings that were not treated with bacteria, as shown in Figure 2. One of the early symptoms of *G. boninense* infection in oil palm seedlings is leaf necrosis. Further symptoms include stem rot and the appearance of *G. boninense* bodies. Although *in vitro* S2A bacteria have a low ability to inhibit the growth of *G. boninense*, the presence of S2A in the growth medium of oil palm seedlings infected with *G. boninense* can increase the resistance of seedlings to disease attacks. This may be due to other factors, such as the biofertilizer ability of the S2A isolate, which enables it to induce better seedling growth, thereby indirectly allowing the seedlings to survive disease attacks.



Fig 2. Plants infected with *G. boninense*; the presence of symptoms of necrosis on leaves without bacterial application (1); there was no necrosis on the leaves where isolates H5 (2), F2 (3), and S2A (4) were applied to the growing media after 8 weeks.

Materials and Methods

Soil sampling

Bacteria were obtained from peatland in Teluk Bakung Village, Sungai Ambawang District, Kubu Raya Regency, West Kalimantan (0° 00'31.0" N, 109° 48'35.7" E). The sampling method used was purposeful random sampling based on the peat maturity level (sapric, hemic, and fibric). This study was conducted in a laboratory and greenhouse at the Pontianak State Polytechnic, West Kalimantan, Indonesia. This study was conducted from January to September 2024.

Chemicals

Nutrient broth (NB) (composed of 15 g/L peptone, 3 g/L yeast extract, 6 g/L sodium chloride, and 1 g/L dextrose) from HIMEDIA, Chrome Azurol S (CAS), hexadecyltrimethylammonium bromide (HDTMA) from Sigma, Gram stain reagent (crystal violet, Lugol's iodine, alcohol, and safranin), iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), and potato dextrose broth (composed of 20 g/L dextrose and 4 g/L potato infusion) from Merck.

Bacterial isolation

The peat samples were diluted in stages using sterile distilled water. A total of 100 μL of suspension from dilutions 10^{-5} and 10^{-8} was inoculated into a Petri dish containing nutrient broth and agar medium and incubated at 37°C . The bacterial isolate was purified by repeated culture and rejuvenation until a single colony was obtained. The obtained bacteria were then tested for the identification of the colony and cell morphology.

Hypersensitivity test

The isolated bacterial isolates were tested for their pathogenicity on plants by conducting a hypersensitivity test on the leaves of 3-month-old tobacco plants. A total of 1 mL of bacterial isolate in NB media was inserted into the leaf tissue and observed for 24 hours. Isolates producing necrosis around the injection area within 24 h were eliminated as pathogenic candidates (Nawangsih et al., 2017).

Siderophore-producing bacteria test

The media used herein consisted of two layers. The bottom of the Petri dish was filled with 10 mL of CAS-blue agar medium. After the medium had solidified, 6 mL of nutrient agar (NA) was added and incubated at 32°C for 2 days (Hu and Xu, 2011). Bacterial isolates were inoculated on media and incubated for 7 days. If a halo formed around the bacterial colony, the positive isolate produced siderophores.

Antagonist test against *G. boninense* in vitro

Bacterial candidate antagonist tests were performed using the double culture method (Lim et al., 2019). PDA media was prepared, and then *G. boninense* was inoculated on one side of the petri dish. The dish was then kept in an incubator for 2 days. Subsequently, the bacterial isolate was inoculated at a distance of 3 cm from the center of *G. boninense*. The diameter of *G. boninense* in the plate was measured after seven days of incubation at room temperature ($24\text{--}29^\circ\text{C}$). The antagonistic ability of bacteria was measured using the following formula:

$$\text{Percent inhibition (\%)} = \frac{(D1-D2)}{D1} \times 100\%.$$

D1 = the diameter of *G. boninense* in the control. D2 = the diameter of *G. boninense* in each treatment.

Effectiveness test by artificially inoculating oil palm seedlings

The *G. boninense* inoculant was incubated for 6 weeks in a sterile rubber block measuring 6 cm \times 6 cm \times 6 cm. Hemic peat media was placed in the pot. Rubber blocks were inserted into the soil media at a depth of 5 cm below the soil surface. The 2-month-old seedlings were transplanted into pots. Symptoms appeared on the leaves of oil palm seedlings. The presence of necrosis on the leaves indicates early signs of disease in the seedlings (Lo et al., 2023; Supriyanto et al., 2024).

Conclusion

A total of 14 bacterial isolates were obtained from 12 peat samples with varying maturity levels. Eleven of these isolates were non-pathogenic, and 6 isolates were positive for siderophore production. Isolates S2A, H5, and F2 exhibited *in vitro* inhibitory effects against *G. boninense* of 14.82, 53.70, and 55.56%, respectively. Application tests on oil palm seedlings up to 8 weeks of age showed that application of all isolates prevented leaf necrosis. In contrast, oil palm seedlings without bacterial application showed quite severe necrosis symptoms.

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References

- De Serrano LO (2017) 'Biotechnology of siderophores in high-impact scientific fields', *Biomolecular Concepts* 8(3-4): 169-178. <https://doi.org/10.1515/bmc-2017-0016>
- Ehsan S, Riaz A, Qureshi MA, Ali A, Saleem I, Aftab M, Mehmood K, Mujeeb F, Ali MA, Javed H, Ijaz F, Haq A, Rehman K & Saleem MU (2022) 'Isolation, purification and application of siderophore producing bacteria to improve wheat growth', *Pakistan Journal of Agricultural Research* 35(2): 449-459. <https://doi.org/10.17582/journal.pjar/2022/35.2.449.459>
- Hu Q & Xu J (2011) 'A simple double-layered chrome azurol S agar (SD-CASA) plate assay to optimize the production of siderophores by a potential biocontrol agent Bacillus', *African Journal of Microbiology Research* 5(25). <https://doi.org/10.5897/AJMR11.238>

- Irma A, Meryandini A & Rupaedah B (2018) 'Biofungicide producing bacteria: an in vitro inhibitor of *Ganoderma boninense*', *Hayati Journal of Biosciences* 25(4): 151–156. <https://doi.org/10.4308/hjb.25.4.151>
- Kalyan VSRK, Meena S, Karthikeyan S & Jawahar D (2022) 'Isolation, screening, characterization and optimization of bacteria isolated from calcareous soils for siderophore production', *Archives of Microbiology* 204(12): 721. <https://doi.org/10.1007/s00203-022-03322-w>
- Khotimah S, Suharjono S, Ardyati T & Nuraini Y (2021) 'The potential of cellulose-degrading fungi at various peat maturities in Teluk Bakung peat area, Kubu Raya district, Indonesia', *Biodiversitas Journal of Biological Diversity* 22(4). <https://doi.org/10.13057/biodiv/d220446>
- Lim PH, Gansau JA & Chong KP (2019) 'Biocontrol of basal stem rot pathogen *Ganoderma boninense* by *Pseudomonas aeruginosa*', *Bangladesh Journal of Botany* 48(2): 209–215. <https://doi.org/10.3329/bjb.v48i2.47494>
- Lo ML, Thanh TAV, Midot F, Lau SYL, Wong WC, Tung HJ, Jee MS, Chin MY & Melling L (2023) 'Comparison of *Ganoderma boninense* isolate's aggressiveness using infected oil palm seedlings', *Journal of Microbiology* 61(4): 449–459. <https://doi.org/10.1007/s12275-023-00040-w>
- Mahdiyah D, Farida H, Riwanto I, Mustofa M, Wahjono H, Laksana Nugroho T & Reki W (2020) 'Screening of Indonesian peat soil bacteria producing antimicrobial compounds', *Saudi Journal of Biological Sciences* 27(10): 2604–2611. <https://doi.org/10.1016/j.sjbs.2020.05.033>
- Nawangsih AA, Parida I, Wiyono S & Kartika JG (2017) 'Selection and characterization of siderophore-producing rhizobacteria and potential antagonistic activity toward *Ralstonia solanacearum*', *Biotropia* 24(2): 85–93. <https://doi.org/10.11598/btb.2017.24.2.406>
- Puspita F, Hadiwiyono, Poromarto SH & Roslim DI (2019) 'Induced resistance by *Bacillus subtilis* on oil palm seedling infected by *Ganoderma boninense*', *Biodiversitas Journal of Biological Diversity* 21(1). <https://doi.org/10.13057/biodiv/d210105>
- Ramli NR, Mohamed MS, Seman IA, Zairun MA & Mohamad N (2016) 'The potential of endophytic bacteria as a biological control agent for *Ganoderma* disease in oil palm' 45: 401–409.
- Shen N, Li S, Li S, Zhang H & Jiang M (2022) 'The siderophore-producing bacterium, *Bacillus siamensis* Gxun-6, has an antifungal activity against *Fusarium oxysporum* and promotes the growth of banana', *Egyptian Journal of Biological Pest Control* 32(1): 34. <https://doi.org/10.1186/s41938-022-00533-7>
- Supriyanto S, Purwanto P, Poromarto SH & Supyani S (2024) 'The effectiveness of acid-tolerant antagonists in the control of oil palm basal stem rot disease caused by *Ganoderma* sp. in peat soils', *Asian Journal of Agriculture* 8(2). <https://doi.org/10.13057/asianjagric/g080208>
- Wiratmoko D, Rahutomo S & Santoso H (2008) 'Karakteristik gambut topogen dan ombrogen di Kabupaten Labuhan Batu Sumatera Utara untuk budidaya tanaman kelapa sawit', *Jurnal Penelitian Kelapa Sawit* 16(3): 119–126.