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Identification of Pathogens Causing Important Diseases in Leatherleaf Fern (*Rumohra adiantiformis*) and *In Vitro* Inhibition using *Bacillus velezensis* B-27

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Abstract

Leatherleaf fern (*Rumohra adiantiformis*) is a famous ornamental-leaf plant that has been used by florist entrepreneurs around the world. It is one of the leading export commodities in Indonesia, however, currently, there are some diseases of this leaf reported in the field causing yield loss and reducing the economic value. This study aimed to identify the pathogens causing the recent 3 significant diseases of leatherleaf fern, including leaf blight, leaf tip rot, and post-harvest leaf rot, and *in vitro* analysis of beneficial bacteria, *Bacillus velezensis* B-27, against the pathogens. The methods used in this study were isolation, pathogenicity test, morphological observation, molecular identification of pathogens, and poisoned food technique of *B. velezensis* against those pathogens compared to fungicides and bactericides. The results of molecular identification showed that *Neopestalotiopsis* sp. and *Pantoea ananatis* caused leaf blight, *Fusarium oxysporum* f. sp. *sesami* triggered leaf tip rot, while *Calonectria* sp. and *P. ananatis* contributed to post-harvest leaf rot. Based on *in vitro* analysis, *B. velezensis* B-27 reduced the growth of the *Neopestalotiopsis* sp. DM C with the highest inhibition of 95.6%, *Neopestalotiopsis* sp. DM B with 84.3%, *F. oxysporum* f. sp. *sesami* with 61.9%, *Calonectria* sp. with 93.4%, and inhibited the growth of *P. ananatis* by producing a clear zone. This research concludes that *B. velezensis* B-27 has the potential as a biocontrol against pathogens causing significant diseases in leatherleaf ferns due to its ability to inhibit pathogens and its advantage as a beneficial microbe that is environmentally friendly to support sustainable agriculture.

Keywords: beneficial bacteria; biocontrol; *Calonectria* sp.; *Fusarium oxysporum* f. sp. *sesami*; *Neopestalotiopsis* sp.; *Pantoea ananatis*

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INTRODUCTION

Leatherleaf fern (*Rumohra adiantiformis*, Family: Dryopteridaceae) is one of the leading national floricultural products in Indonesia both for domestic and export markets. The leaves have beautiful shapes, dark green color, and long vase life, attracting many florists worldwide to use them for decoration (Kloepper et al., 2013; Nair and Laxman, 2020). In Indonesia, centers of fern production are located in the highlands of Java Island, some of which are in Dieng (Central Java) and Sukabumi (West Java) (Hanudin et al., 2019).

Recently, some significant diseases reducing fern leaf crops were reported from a leatherleaf fern orchard in Magelang, Indonesia, which had not been described in Sumardiyono et al. (2011). Based on the preliminary field survey prior to the research, the diseases were leaf blight, leaf tip rot,

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and post-harvest leaf rot. Based on direct observation in the orchard, leaf blight disease showed symptoms as red, curly, and necrosis burning on leaves, which expanded quickly to all leaf surfaces and caused the leaves to dry. Symptom of leaf tip rot was characterized by leaf tip rotting and withering, therefore according to the producer, the leaves were rejected for sale. Post-harvest leaf rot was characterized by brown spots on the leaf surface enlarging and causing leaf rotting but commonly was noticed in the post-harvest stage. Those diseases have not been reported in the past decade. Moreover, the reports of disease on leatherleaf fern in Indonesia are extremely limited.

An important disease found in leatherleaf fern in Indonesia for more than 10 years ago was anthracnose, which was caused by *Colletotrichum* sp. (Sumardiyono et al., 2011). However, the orchard confirmed that the disease is well-managed nowadays. This condition suggests that the important diseases on plants shifted over time, and therefore research on plant diseases should always be developed. Since this commodity, such as vegetables, is specific and not commonly cultivated by Indonesian farmers, disease management for emerging diseases is not available. It raises a big concern in a plant disease problem because leatherleaf fern from Magelang is an important export commodity. Reducing the leaf crops will bring a constraint on the export capacity. Therefore, this research is conducted to properly identify pathogens using molecular analysis.

The identification of plant pathogens through molecular analysis is crucial for effective disease management, particularly for export commodities such as the leatherleaf fern. Accurate identification of pathogenic fungi and bacteria helps in devising targeted treatment strategies, preventing further spread, and minimizing economic losses. Disease identification is performed at the molecular stage based on polymerase chain reaction (PCR) to more accurately identify pathogenic fungi and bacteria in plants (Hariharan and Prasannath, 2021). As those diseases were not reported yet in Indonesia, this study is the first to report pathogens causing leaf blight, leaf tip rot, and post-harvest leaf rot disease in leatherleaf fern.

Following the process of pathogen identification, *in vitro* antagonism analysis was carried out using *Bacillus velezensis* B-27 to inhibit the growth of pathogenic fungi and bacteria. *B. velezensis* are well-known as beneficial bacteria and antagonists, which can inhibit pathogen growth and promote plant growth (Sunkad et al., 2023). *B. velezensis* B-27 has been reported to reduce the severity of twisted disease caused by *Fusarium* spp. (Rahma et al., 2020; Sundari et al., 2023). *B. velezensis* are bacteria that function as plant growth-promoting rhizobacteria (PGPR), usually used to improve plant health and maintain the soil's microbial community, supporting sustainable agriculture (Sundari et al., 2023). As a reference, another study reported that *B. velezensis* CE 100 produces chitinase and β-1,3-glucanase enzymes, which degraded fungal cell walls and efficiently suppressed the growth of *Macrophomina phaseolina* and *F. oxysporum* f. sp. *fragariae*. These pathogens are respectively responsible for charcoal rot and *Fusarium* wilt in strawberries. Using *B. velezensis* as a biological control agent presents a more sustainable and environmentally friendly approach to managing plant pests and diseases compared to fungicides (Hong et al., 2022). Fungicides contribute to issues such as residue accumulation, the development of resistance to pathogens, and various health risks for humans and other organisms (Goswami et al., 2018). The use of fungicides can influence the physiological functions and disease-causing abilities of pathogenic fungi, thereby fostering the evolution of resistance mechanisms within these fungal populations (Bastos et al., 2021). However, fungicides remain the primary option for farmers to reduce plant diseases.

Based on those problems, this study aimed to identify the pathogens causing important diseases of leatherleaf fern based on molecular analysis and observe the ability of beneficial bacteria, *B. velezensis* B-27 isolate to control the *in vitro* pathogen's growth. The results are important to figure out the potency of *B. velezensis* B-27 as biological controls of those important diseases on leatherleaf ferns in the field for further research and sustainable integrated plant health management.

MATERIALS AND METHOD

Plant materials

The plant material in this research was the leatherleaf fern with leaf blight, leaf tip rot, and post-harvest leaf rot symptoms for pathogen identification and healthy leatherleaf fern for pathogenicity test. All leaves with the disease

symptom were purposively collected from an orchard in Magelang Regency, Central Java, Indonesia (map coordinate: 110°13'04.0" E and 7°22'26.1" S) in 2023.

Sampling, isolation, and morphological observation of fungi and bacteria

Each plant showing the disease symptoms (leaf blight, leaf tip rot, and post-harvest leaf rot) characterized above was collected as a sample. The plant disease isolation process involved using 3 leaf samples for each disease symptom. Each leaf was isolated in the 3 petri dishes and 4-leaf discs were isolated in every petri dish. Plant leaves were cleaned from impurities (dust or soil) with sterile water and cut into small pieces. Afterward, those were disinfected using 1% NaOCl for 1 minute and then rinsed with distilled water for 30 seconds 2 times. The leaf pieces were placed on potato dextrose agar (PDA) medium and incubated at room temperature for 2 to 3 days. The growing mycelia were transferred to a PDA medium for fungi and a nutrient agar (NA) medium for bacteria. The fungal morphology was observed using a microscope to check the hyphae/spores produced in the culture. Bacterial characteristics are identified by colony color observation, gram test, and hypersensitivity test based on Schaad et al. (2001).

Pathogenicity test

Healthy leaves of leatherleaf fern from the field and pathogens culture were prepared for this experiment. The pathogenicity test of fungal pathogens was carried out by pricking the leaf surface with a syringe and putting a mycelial disc with a 5 mm diameter on it. Twenty-four hours afterward, the mycelial discs were taken out to let the infection progress naturally. For bacteria, bacterial suspension was prepared for density 10^8 CFU, and then approximately 1 ml of suspension was dripped into the pricked leaves. The control group was leaves treated with sterile water. All those treated leaves were placed in a 9 cm diameter petri dish with damp cotton to maintain the moist condition. Those were incubated at room temperature and observed every day until symptoms emerged.

Molecular identification

Pathogen identification in this study was conducted in molecular analysis using Sanger Sequencing method. DNA extraction of both fungal and bacterial was carried out with Mini Kit Genomic DNA Protocol for plant/fungi and bacteria, respectively (GeneAid, Taiwan), based on the protocol kit. For amplification, ITS5-4 and β-tubulin genes primer pairs were used for *Neopestalotiopsis* sp. identification, translation elongation factor (tef1-α) gene marker was used to identify *Fusarium* sp. and ITS1-4 primer set was used for morphologically unrecognized fungus. A universal primer of 16s rRNA (27F and 1492R) was used for bacteria identification. The primer sets used in this study are presented in Table 1.

PCR products were visualized using a universal BIORAD electrophoresis machine using 1% agarose gel with florosafe DNA staining at 50 V for 50 minutes. DNA bands on the agarose gel were then observed using a high-performance UV transilluminator. PCR products were then sent to Genetika Sci. Inc. for sequencing analysis.

Table 1. Primer markers used in this study

Primers	Sequence 5' – 3'	Locust/gene	Annealing temperature (°C)	Reference
ITS 1F	TCCGTAGGTGAACCTGCG	ITS	52	Aasa et al. (2022)
ITS 5F	GGAAGTAAAAGTCGTAAC AAGG		52	Singh et al. (2021)
ITS 4R	TCCTCCGCTTATTGATATGC			
TiF	AACATGCGTGAGATTGTAAGT	β-tubulin	53	Glass and Donaldson (1995)
Bt2bR	ACCCTCAGTGTAGTGACCCTT GGC			
HS392F	TCAAAATGGGTAAGGA(A/G)GA CAAGAC	Translation elongation factor	54	Lopes et al. (2021)
HS393R	GCCTGGGA(G/A)GTACCAGT (G/C)ATCATGTT			
27F	AGAGTTTGATCCTGGCTCAG	16S rRNA	52	dos Santos et al. (2019)
1492R	GGTTACCTTGTTACGACT			

The sequencing results were analyzed using MEGA XI software, with reference sequences obtained from NCBI GenBank data. The data obtained were then analyzed for similarity and a phylogenetic tree.

Inhibition test of *B. velezensis* B-27 and fungicides against fungal pathogens

The inhibition test was conducted to compare the ability of *B. velezensis* B-27 and some fungicides to reduce the growth of fungal pathogens. This study was conducted using a completely randomized design (CRD) with 3 replications for each isolate and treatment. The fungicides used in this study were selected based on several fungicides commonly applied in the leatherleaf orchard where leaf samples were collected. Those fungicides were used to compare the ability of biocontrol bacteria *B. velezensis* B-27 in reducing pathogens versus common chemical control. The treatments used in this study are presented in Table 2, with fungicide concentration referring to the recommendation on the label.

The inhibition test of *B. velezensis* B-27 against fungi was carried out by co-culture method referred to Jayanti and Joko (2020). The experiment was conducted in a solid PDA medium, which was poured with a mixture of 0.6%, 4 ml water agar media, and 100 µl of bacteria suspension. Afterward, a 5 mm mycelial disc was placed on the media and incubated. Then, the fungal diameter growth was measured every day. The poisoned food technique method was used for fungicide treatment based on Dahal and Shrestha (2018). One milliliter of fungicide was homogenized with 9 ml PDA medium and poured into the petri dish. After the medium was solid, a 5 mm mycelial disc was placed in the center and incubated. After that, the fungal diameter growth was measured every day. Control as reference was treated with the same method by using distilled water. The inhibition percentage was measured by Equation 1.

$$DH = \frac{a - b}{a} \times 100\% \quad (1)$$

Where, DH = inhibition percentage, a = mycelium diameter in control (distilled water), b = mycelium diameter in treatment.

Table 2. Treatments and concentration of inhibition test by *B. velezensis* B-27 and fungicides against fungal pathogens

Treatment	Concentration	Isolate/ trade name®
B. velezensis	10^8 CFU	B-27 isolate
Propineb 70%	1 g l^{-1}	Antracol
Mankozeb 80%	1 g l^{-1}	Dithane
Propinacozole and prochloraz 400 EC	1.5 ml l^{-1}	Remazol
Azoksistrobin 200 g l^{-1} and difenokazol 125 g l^{-1}	1 ml l^{-1}	Amistar top
Difenokonazol 250 g l^{-1}	1 ml l^{-1}	Remazol
Control (distilled water)	-	-

Inhibition test of *B. velezensis* B-27 and bactericides against bacterial pathogens

The experiment was conducted to compare *B. velezensis* B-27 with bactericides Copper hydroxide and Zinc thiazole to measure the inhibition growth of bacterial pathogens after treatment. This study was conducted using a CRD with 3 replications for each isolate and treatment. The bactericides concentration used referred to the label recommendation (Table 3).

The inhibition test of *B. velezensis* B-27 against bacterial pathogens was carried out with the co-culture method, by putting 1 dot of *B. velezensis* B-27 in the middle of the NA medium and incubating it for 24 hours. Afterward, the mixture of 0.6%, 4 ml water agar media, and 100 µl of bacterial pathogen suspension was poured into the media. Bactericidal treatment was conducted with agar well diffusion method (Balouiri et al., 2016). Firstly, the NA medium was homogenized with pathogenic bacterial suspension and poured into petri dish. After became solid, the center of media was perforated by a 1 cm diameter cork. After that, the 15 µl of 0.04% agarose was poured inside and was

Table 3. Treatments and concentration of inhibition test by *B. velezensis* B-27 and bactericides against bacterial pathogens

Treatment	Concentration	Isolate/ trade name®
B. velezensis	10^8 CFU	B-27 isolate
Copper hidroxide	0.6 g l^{-1}	Copcide
Zinc thiazole	3.75 ml l^{-1}	Besun elite
Control (distilled water)	-	-

awaited until dry. The 10 µl bactericidal solution was then poured into the agarose. A clear zone around the *B. velezensis* or bactericidal was observed and compared among treatments after 24 hours. With the method, data analysis for bacterial inhibition was conducted by descriptive analysis of the observed clear zone.

Data analysis

The data of the inhibition test were analyzed by analysis of variance (ANOVA) and duncan multiple range test (DMRT) at the confidence level of α 5%.

RESULTS AND DISCUSSION

Leaf blight disease

Symptoms of leaf blight on leatherleaf ferns were characterized by reddish-brown discoloration on leaves, mainly from the edge, which spread into all leaf surfaces. Late symptoms caused the leaves red, curly, burning-like, and eventually dried (Figure 1). Based on isolation, 2 fungal isolates and 1 bacterial isolate were found, identified as *Neopestalotiopsis* sp. DM B, *Neopestalotiopsis* sp. DM C, and *Pantoea ananatis* based on molecular identification.

Based on the pathogenicity test, both isolates caused reddish and blight symptoms in leaves (Figure 2). The DM B and DM C isolates morphologically have different culture shapes, however, similarly, they caused the symptoms of disease. The spores of *Neopestalotiopsis* sp. DM B were clearly identified as a Pestalotiopsis fungal group based on the microscopical characters. Pestalotiopsis fungal group, including *Neopestalotiopsis* sp., previously was known as a weak pathogenic species, but in recent years,

Figure 1. Early symptom (a) and late symptom (b) of leatherleaf fern blight disease

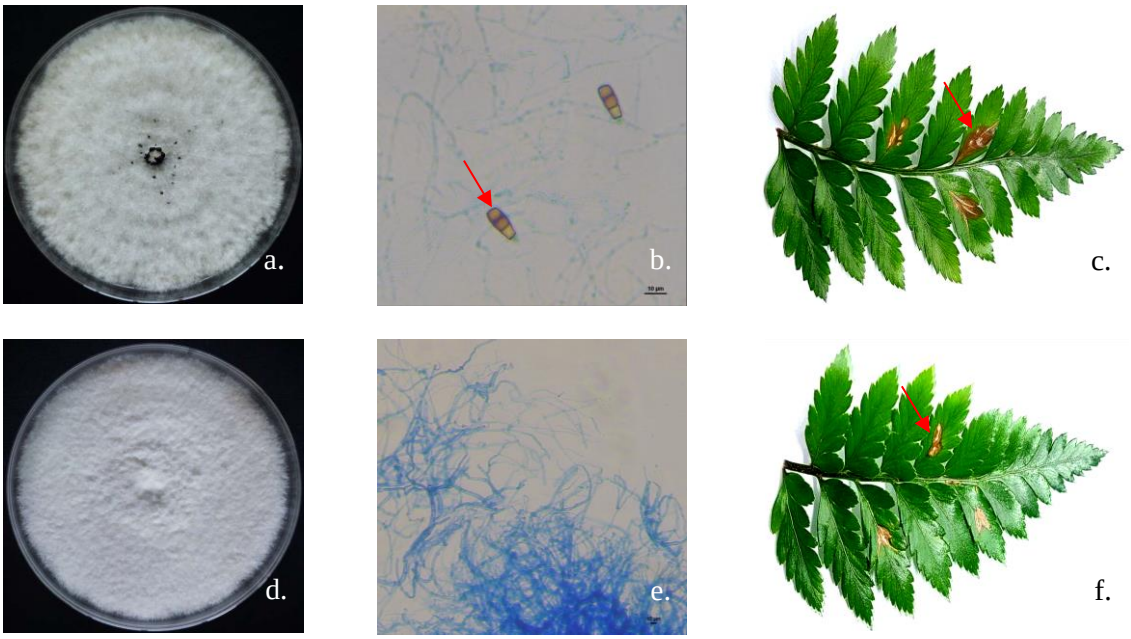


Figure 2. Culture of *Neopestalotiopsis* sp. DM B isolate (a) and DM C isolate (d); spores of the *Neopestalotiopsis* sp. DM B isolate (b); fungal hyphae *Neopestalotiopsis* sp. DM C isolate with no spores (e), symptom on leaves after pathogenicity test of *Neopestalotiopsis* sp. DM B isolate (c) and DM C isolate (f)

the fungus *Pestalotiopsis* sp. has received a lot of attention for its role as a plant pathogen and as an endophytic fungus (Maharachchikumbura et al., 2011). Meanwhile, the DM C isolate did not produce spores in its culture, and therefore, identification was done based on molecular analysis.

The isolation results also showed that 1 bacterial culture can cause a positive hypersensitive reaction and a disease symptom (Figure 3). However, based on the pathogenicity test, the symptom showed a brownish necrotic lesion rather than a reddish color. In the NA medium, it was found that the bacteria had a yellow colony color and were categorized gram-negative bacteria, characterized by the presence of mucus.

Leaf tip rot

Leaf tip rot was characterized by brown spot necrosis on the pointed tip of leaves which then developed to be rotten, and the leaf size was smaller than normal (Figure 4). Late symptoms showed that all tips of the leaves were withered, black, and rotten. The leaves then were finally rejected for sale. Based on isolation and microscopical analysis, it was known that the culture produced spores, which

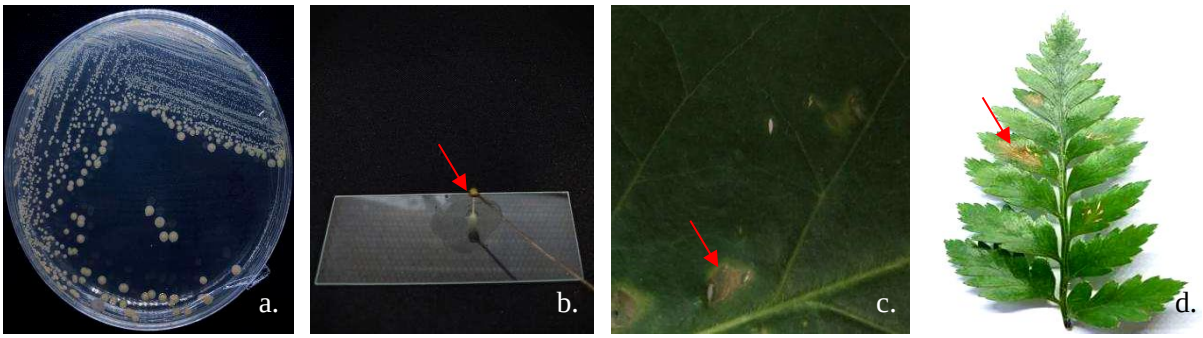


Figure 3. Colony of *P. ananatis* DM B bacteria (a); gram test based on KOH string test method of *P. ananatis* bacteria showing negative gram bacteria (b); hypersensitive reaction test of *P. ananatis* bacteria showing necrotic symptom (c); symptom resulted after pathogenicity test of *P. ananatis* bacteria DM B on fern leaves (d)

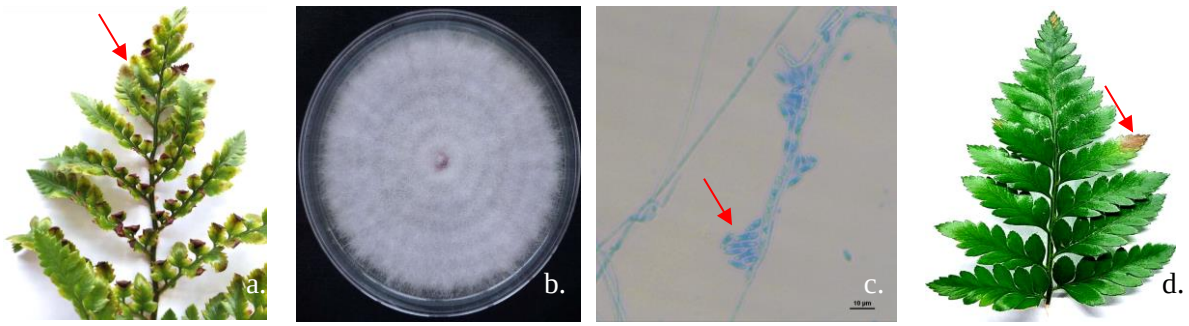


Figure 4. Symptoms of leaf tip rot (a); fungal culture (b) and spore of *Fusarium* sp. (c); symptom after pathogenicity test (d)

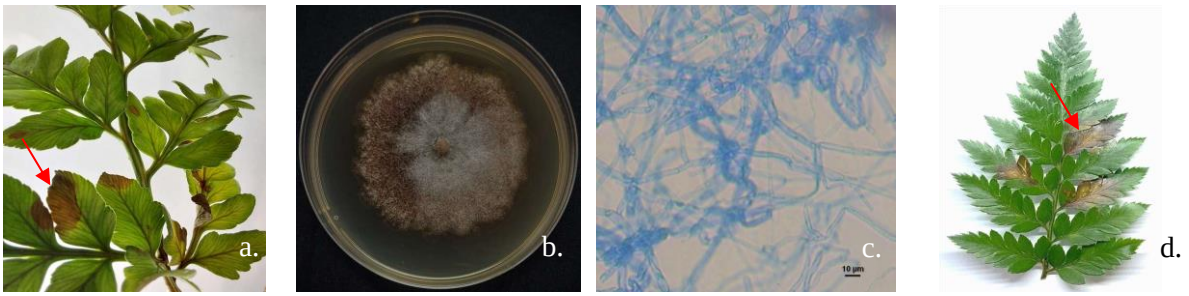


Figure 5. Symptoms of post-harvest leaf rot (a); fungal colony (b); and hyphae of *Calonectria* sp. (c); and symptom of pathogenicity test on the leatherleaf fern (d)

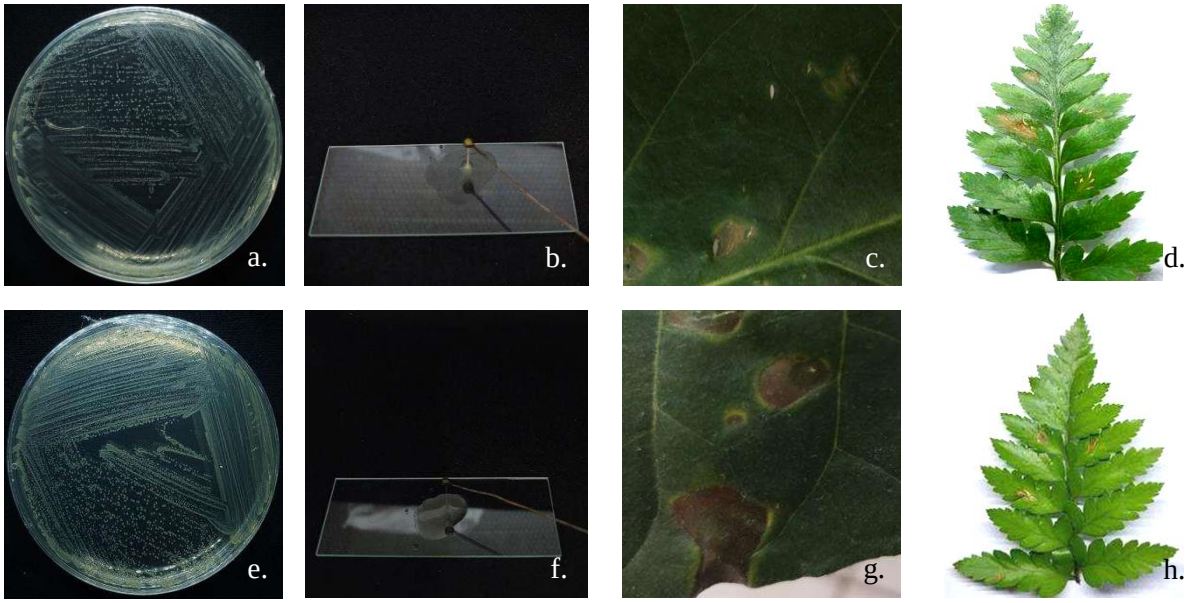


Figure 6. Colony of *P. ananatis* isolates of PH A (a) and PH C (e); gram test based on KOH string test method showed negative gram bacteria of PH A (b) and PH C (f); hypersensitive reaction test showed PH A (c) and PH C (g) were bacterial pathogens; and the result of pathogenicity test of PH A (d) and PH C (h) on leatherleaf fern

were characterized as *Fusarium* sp. (Leslie and Summerell, 2006).

Post-harvest leaf rot

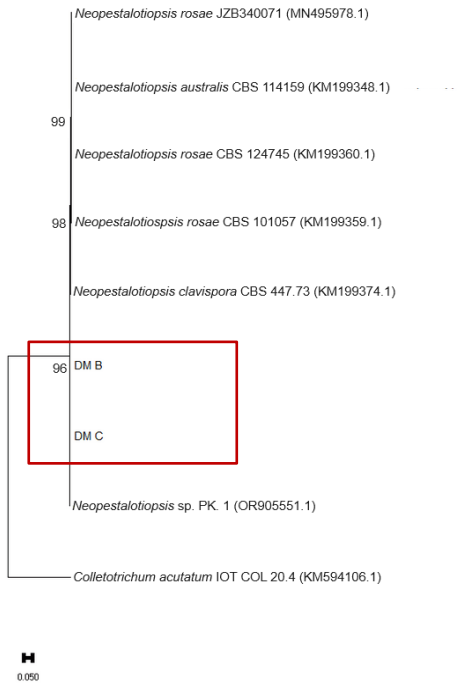
Symptoms of post-harvest leaf rot were characterized as brownish spots, especially on the lower part of leaves, then were expanded to the surrounding leaf area, rotten, and rejected for use. This symptom was commonly unseen during the harvest and sortation period but mainly received consumer complaints because it appeared when the leaves were unboxed. Based on the isolation, 1 fungal culture and 2 bacterial isolates were found, which resulted in symptoms in the pathogenicity test. The fungal pathogen has a red color in the mycelial colony, does not produce any spores, and has septate hyphae (Figure 5). Based on further molecular analysis, it was identified as *Calonectria* sp.

Two isolates of bacteria were found, named PH A and PH C, resulting in necrosis in the hypersensitive reaction test and causing symptoms in the leatherleaf ferns (Figure 6). PH A and PH C isolates showed a yellowish color in their colony, and based on further molecular analysis, both isolates were known as *P. ananatis.*

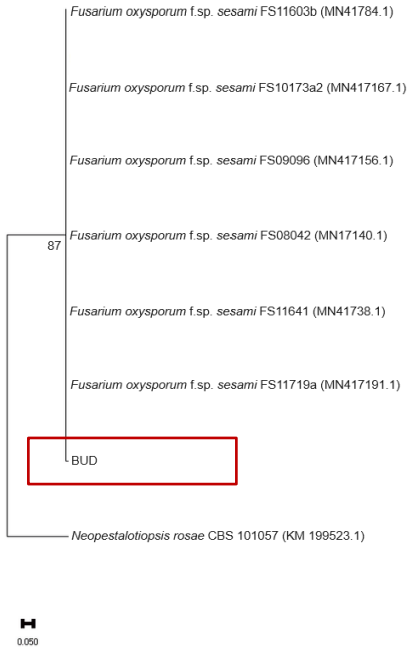
Molecular identification of pathogens

This molecular analysis was conducted to identify the pathogens causing all those diseases in the leatherleaf fern because morphological observation did not support the identification process. After all sequences were aligned with the reference isolates in GenBank, the phylogenetic tree was constructed. Figure 7 presents that leaf blight on leatherleaf fern was caused by *Neopestalotiopsis* sp. and bacteria *P. ananatis*, leaf tip rot was triggered by *F. oxysporum* f. sp. *sesame,* and post-harvest leaf rot was attributed to *Calonectria* sp. and *P. ananatis.*

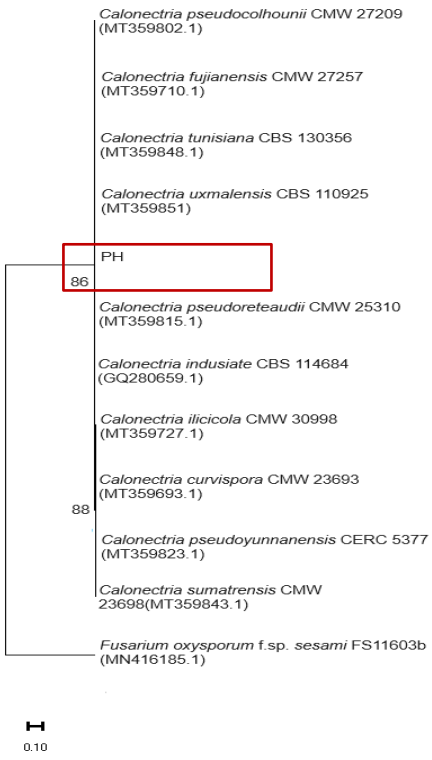
Neopestalotiopsis leaf blight was currently reported as an emerging disease on leatherleaf fern (Widiastuti et al., 2024). Recently, the status of *Neopestalotiopsis* sp. as a pathogen gained significance such as it caused an outbreak of fruit rot and leaf spot of strawberry plants in Florida (Baggio and Peres, 2020; Baggio et al., 2021) and in Turkey (Erdurmuş et al., 2023). This study revealed that not only *Neopestalotiopsis* caused leatherleaf fern leaf blight, but also *P. ananatis.* Meanwhile, *Fusarium* sp. causing leaf tip rot was known to be closely related to *F. oxysporum* f. sp. *sesami.* This fungal species is an important pathogen causing sesame (*Sesamum indicum*) wilt (Duan et al., 2020; Hassan et al., 2021). Besides wilt disease, *F. oxysporum* was reported to cause tomato fruit rot, characterized by white lesions and fruit softening (Safari et al., 2021), and as a crown rot pathogen in asparagus (Elmer, 2015). The fungus *F. oxysporum* f. sp. *radicis-lycopersici* causes crown and root rot disease was reported not only in tomato as the main host, but also in pepper, eggplant, and a number of common weeds (Marissa et al., 2021). This result indicates



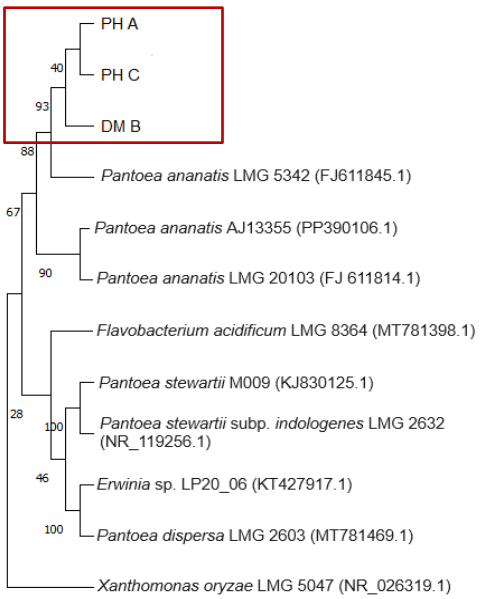
a.



b.



c.



d.

Figure 7. Phylogenetic tree of *Neopestalotiopsis* sp. DM B and DM C isolates using ITS primer and β-tub (a); *F. oxysporum* f. sp. *sesami* using tef-α primer (b); *Calonectria* sp. using ITS primer (c) and *P. ananatis* DM B, PH A, and PH C isolates using 16s rRNA (d)

Note: The tree was constructed by using Neighbor-Joining method with bootstrap analysis and Kimura-2 Model Parameters. Basic nucleotide data information was aligned using the CLUSTAL W Program

that a *formae speciales* of fungi could also infect several hosts. However, the factor contributing to the presence of *F. oxysporum* f. sp. *sesami* in the leatherleaf fern was not identified. One possibility that could explain this result is that the leatherleaf fern seedlings were currently imported, and therefore, the pathogen might contaminate them and develop in the orchard. Therefore, further research about leaf tip rot is needed in the future. *Calonectria* sp. was already reported to cause many significant diseases in a wide range of hosts, such as in eucalyptus, groundnut, and some other crops (Zhang et al., 2022). In Indonesia, *Calonectria* sp. has been reported to cause late blight in eucalyptus (Tarigan et al., 2023). This study is the first to report *Calonectria* sp. as a fungal pathogen in the leatherleaf fern and further research on this pathogen is important to conduct, to study the pathogen status in Indonesia.

Recently, *P. ananatis* was reported as a bacterial pathogen in many important crops such as strawberries in Egypt (Abdel-gaied et al., 2022) and in China (Song et al., 2023), and caused leaf blight in shallot in Indonesia (Asrul, 2020). All the bacterial pathogens isolated in this study were found to have a close relation with *P. ananatis* (Figure 7d). It brings insight that the status of *P. ananatis* needs attention as an important plant pathogen in Indonesia.

Inhibition test of *B. velezensis* B-27 and chemicals against fungal and bacterial pathogens

Based on the results of the inhibition test, *B. velezensis* B-27 mainly had a high *in vitro* inhibition percentage against fungi. The results showed that the fungicide Propinacozole-Prochloraz with a concentration of 1.5 ml l^{-1} gave the best inhibition percentage against all fungal pathogens. However, *B. velezensis* B-27 gave a similar, not significant result compared to Propinacozole-Prochloraz, in inhibition against *Neopestalotiopsis* sp. DM C and *Calonectria* sp. *B. velezensis* B-27 also had a slight lower inhibition below the fungicide for *Neopestalotiopsis* sp. DM B and *F. oxysporum* f. sp. *sesami* (Table 4).

Other than Propinacozole-Prochloraz fungicide, *B. velezensis* B-27 had a higher, significant inhibition percentage than other fungicides tested in this experiment. Figure 8 also showed that the fungus did not grow well when co-cultured with *B. velezensis* B-27, similarly with Propinacozole-Prochloraz treatment. Based on the data, *B. velezensis* B-27 gave either the second or the best results in inhibition percentage of fungal growth. Therefore, it is a good candidate for fungal control in the field, by considering environmental safety and a healthy ecosystem for sustainable agriculture. According to research by Abdelkhalek et al. (2020), *B. velezensis* PEA 1 contains compounds, which can penetrate plant cells and play an important role in systemic acquired resistance (SAR) by activating gene excretion and enzyme activity related to resistance, inhibiting infection and strong antifungals against *F. oxysporum*. *B. velezensis* strains BIB0110 have the potential as biocontrol agents. These bacteria can inhibit the growth of fungal mycelium *Botrytis cinerea* and *Calonectria gracilis* as well as reduce the incidence of disease severity caused by the pathogens in eucalyptus plants (Agent et al., 2023). *B. velezensis* can produce cellulase and protease enzymes that function to destroy the

Table 4. Inhibition test of *B. velezensis* B-27 and fungicides against fungi causing disease in leatherleaf fern

Treatment	*Neopestalotiopsis* sp. DM B (%)	*Neopestalotiosis* sp. DM C (%)	*F. oxysporum* f. sp. *sesame* (%)	*Calonectria* sp. (%)
B. velezensis B-27	84.3^{d}	95.6^{e}	61.9^{d}	93.4^{d}
Propineb	50.8^{b}	44.1^{bc}	63.8^{d}	93.2^{d}
Mankozeb	62.4^{c}	48.0^{c}	49.6^{b}	73.1^{b}
Propinacozole-Prochloraz	97.6^{e}	100.0^{e}	100.0^{f}	100.0^{d}
Azoksistrobin-Difenokazol	83.7^{d}	77.8^{d}	55.4^{c}	78.1^{bc}
Difenokonazol	65.0^{c}	40.9^{b}	76.9^{e}	83.5^{c}
Control	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}

Note: The same letters within the column show no significant difference based on the DMRT analysis with α 0.5%

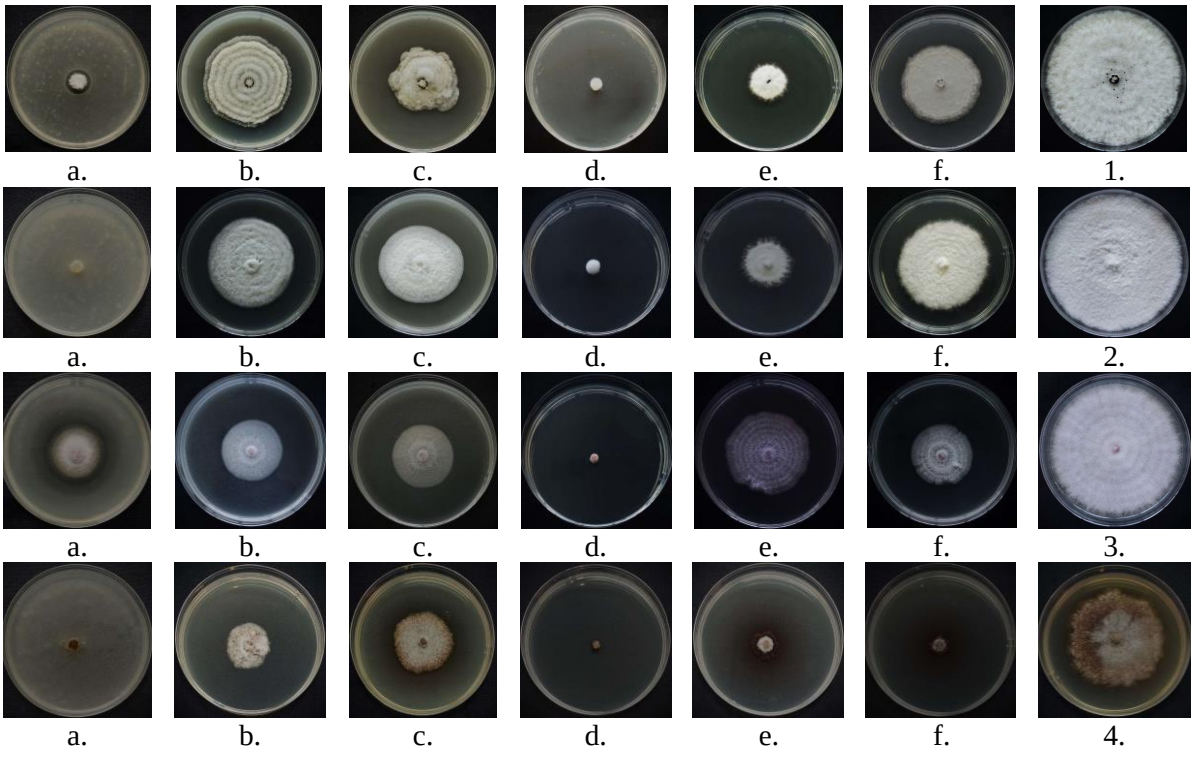


Figure 8. *In vitro* inhibition test of *B. velezensis* B-27 and fungicides against fungi. (1) *Neopestalotiopsis* sp. DM B isolate; (2) *Neopestalotiopsis* sp. DM C isolate; (3) *F. oxysporum* f. sp. *sesami* and (4) *Calonectria* sp. (a) *B. velezensis* B-27; (b) Propineb 70%; (c) Mancozeb 80%; (d) Propinacozole and Prochloraz 400 EC; (e) Azoxystrobin 200 g l^{-1} and Diphenoazole 125 g l^{-1}, and (f) Diphenoconazole

Table 5. Test of inhibitory power of *B. velezensis* bactericidal against bacteria that cause fern plant disease

Treatment	*P. ananatis* DM B	*P. ananatis* PH A	*P. ananatis* PH B
Cooper hidroxide	+	+	+
Zinc thiazol	-	-	-
B. velezensis B-27	+	+	+
Control	-	-	-

Note: (+) Clear zone formation, (-) No clear zone formation

Figure 9. Inhibition test of *B. velezensis* B-27 and bactericides against *P. ananatis*. (a) *B. velezensis* B-27; (b) copper hydroxide; (c) zinc thiazole; and (d) control. Clear zone was performed inside the red circle

cell wall of the fungus *Apiospora arundinis* to inhibit *in vitro* fungal growth (Liao et al., 2023).

Based on bacterial inhibition percentage data in Table 5, *B. velezensis* B-27 could inhibit the growth of *P. ananatis* and cooper hidroxide. Moreover, Figure 9 shows that a clear zone produced by *B. velezensis* B-27 was identified, while zinc thiazol could not inhibit the growth

of *P. ananatis.* The possible explanation is that the label states that zinc thiazol is effective against *Xanthomonas oryzae* pv. *oryzae.* A study by Jin et al. (2020) reported that a clear zone formed by *B. velezensis* indicated that the bacteria have an anti-bacterial compound of C_{15} surfatin, which can induce rice resistance against bacterial leaf blight disease. *B. velezensis* BR-01 isolated from Tubeimu plant (*Rhizoma Bolbostemmatis*) was reported to inhibit *in vitro* fungal and bacterial pathogens causing disease in rice (Zhou et al., 2022). This study showed that *B. velezensis* B-27 has potency as a biocontrol agent against both *P. ananatis* bacterial and fungal pathogens in leatherleaf fern due to its ability in pathogen inhibition and its advantage as a beneficial microbe that is environmentally friendly to support sustainable agriculture.

CONCLUSIONS

This study showed that important diseases and pathogens in leatherleaf fern were leaf blight caused by *Neopestalotiopsis* sp. and bacteria *P. ananatis*, leaf tip rot attributed to *F. oxysporum* f. sp. *sesami*, and post-harvest leaf rot triggered by *Calonectria* sp. and *P. ananatis*. *B. velezensis* B-27 inhibited the growth of *Neopestalotiopsis* sp. DM C isolate and *Calonectria* sp. similarly as Propinacozole-Prochloraz, which achieved the highest inhibition percentage. *B. velezensis* B-27 also produced a clear zone to inhibit all *P. ananatis*, similar to cooper hidroxide. *B. velezensis* B-27 showed its potential as a biocontrol agent against pathogens causing vital disease in leatherleaf ferns. Further research in plants or fields is needed to confirm the *in vitro* result.

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