



REVIEW ARTICLE

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Characterization of the Pathogenic Fungus *Phytophthora palmivora*, the Cause of Stem Rot Disease in Durian (*Durio zibethinus* Murray) in Buleleng Regency, Bali

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Abstract

Durian (*Durio zibethinus* Murray) is a horticultural commodity with high economic value in Buleleng Regency. However, its development faces obstacles from stem rot disease, which can reduce productivity and even cause plant death. This study aims to identify the pathogen causing durian stem rot through isolation of pathogenic fungi, pathogenicity tests, morphological characterization, and molecular and phylogenetic analysis. Samples were taken from symptomatic durian plants in the production center of Buleleng Regency. Six isolates were successfully isolated from plants with stem rot symptoms, namely Srt001, Srt002, Srt003, Kbt001, Bsb001, and Swn001, and then tested for pathogenicity on durian seedlings of the Otong variety. Only isolate Srt003 re-emerged with typical symptoms of reddish-brown gummosis on the stem 30 days after inoculation. Identification of morphological characters showed that isolate Srt003 formed grayish-white to transparent colonies with a stellate-radiate pattern and hyaline, coenocytic hyphae. Sporangia are ovoid to ellipsoid, some nearly globose, papillated, caducous, and measure approximately $40\text{-}60 \times 21\text{-}46 \mu\text{m}$. Sequence analysis of the rDNA Internal Transcribed Spacer (ITS) region and phylogenetic analysis confirmed that isolate Srt003 has a sequence identity of up to 99.60% with *Phytophthora palmivora*. The results showed that *P. palmivora* is a pathogen causing durian stem rot in Buleleng Regency. This study is the first report of *P. palmivora* causing stem rot in Buleleng, Bali, based on morphological and molecular approaches.

Keywords: Gummosis, ITS-rDNA, Molecular, Morphology, Phylogeny

1. Introduction

Durian (*Durio zibethinus* Murray) is a horticultural commodity with high economic value and growing market demand, particularly in Southeast Asia (ASEAN) region. This fruit is widely known not only for its distinctive taste but also for its distinctive pungent aroma. Indonesia ranks among the top four durian-producing countries in ASEAN, after Thailand, Malaysia, and Vietnam (FFTC-AP, 2023). The durian plantation area in Indonesia reaches approximately 30,000 hectares, with a total production of approximately 1.96 million tons spread across various regions (Central Statistics Agency, 2025).

Along with population growth and intensification of cultivation, problems in durian production have also begun

to emerge, one of which is the increasing incidence of plant diseases. Disease attacks, especially those caused by pathogenic fungi, have been reported to reduce productivity and even cause plant death. For example, *Phytophthora palmivora* has been reported to cause losses and control costs estimated at 20-25% of durian production. In some production centers in Southeast Asia, such as Penang, Malaysia, the attack rate can reach 30%, while in some areas of Vietnam it can cause plant mortality of up to 50%. (Drenth & Guest, 2004; Semangun, 2007).

The significant losses from this disease have prompted various studies to identify pathogens associated with the disease in durian plants. For example, Santoso et al. (2015) identified several pathogen species, such as *Pythium*,

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Phytophthium, and Phytophthora, associated with durian plants in Indonesia based on morphological characteristics and molecular analysis. However, information on the pathogens that specifically cause stem rot in durian plants in Buleleng Regency is still limited and can often lead to misidentification if only relying on the characteristics of symptoms in the field, considering that several pathogens have been reported to cause similar symptoms. Therefore, research is needed to confirm the identity of the pathogen that causes stem rot in durian plants through morphological and molecular identification so that durian stem rot control strategies can be implemented appropriately and sustainably.

2. Material and Methods

The research was conducted from September 2025 to January 2026. Sampling of plants with stem rot symptoms was carried out in durian production centers in Buleleng Regency, namely: Seririt District (8.249772° S, 114.930671° E; 401 m asl), Kubutambahan District (8.142853° S, 115.199618° E; 328 m asl), Busungbiu District (8.286250° S, 114.976536° E; 398 m asl), and Sawan District (8.122779° S, 115.168869° E; 209 m asl). Isolation, morphological and molecular identification were carried out at the Plant Disease Laboratory, Faculty of Agriculture, Udayana University. In contrast, the pathogenicity test was carried out at the Greenhouse of the Experimental Garden, Faculty of Agriculture, Udayana University.

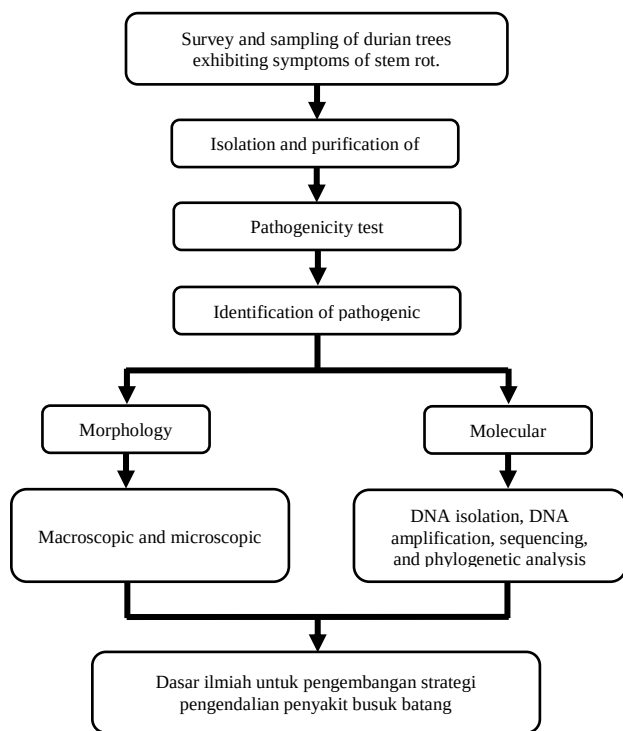


Figure 1. Research Flow Diagram

2.1. Pathogen Isolation

Durian plant stems with stem rot symptoms were cleaned with running water to remove dirt attached to the stem surface. Plant parts showing symptoms of disease and healthy parts were cut to a size of approximately 1cm x 1cm, then sterilized by immersion in 70% alcohol for 1 minute, then rinsed three times with sterile water. After drying, the sample pieces were then planted on *Potato Dextrose Agar* / PDA media (250 g of potato, 20 g of dextrose, 20 g of colorless agar, and 1 liter of sterile water), then incubated at room temperature (25-30 ° C) for 3-5 days. The isolated pathogenic fungal mycelium was then purified on new PDA media.

2.2. Pathogenicity Test

Pathogenicity testing was conducted to ensure that the isolated pathogenic fungus was the pathogen that caused durian stem rot disease, with symptoms consistent with those observed in the field. Pieces of PDA media infested with the pathogenic fungus were attached to previously wounded stems of Otong cultivar durian seedlings to facilitate infection. The inoculated stem sections were wrapped with cotton and plastic wrap to maintain humidity. Observations were made daily until 30 days after inoculation (dsi). We compared the symptoms with those observed in the field. We re-isolated infected pathogens with the same symptoms for further identification.

2.3. Morphological Identification

Pure cultures of pathogens resulting from pathogenicity tests were observed macroscopically and microscopically. The morphology observed included colony shape, colony color, hyphae, spore shape, spore size, and hyphae of the pathogenic fungus. For sporangium observation, the pathogenic fungus was grown on agar media mixed with carrot extract (carrot agar media). The microscopic identification results were then compared with the CMI book *Descriptions of Pathogenic Fungi and Bacteria* (Commonwealth Mycological Institute, 1966) and other supporting journals.

2.4. Molecular Identification

Molecular identification involves several stages, such as: pathogen DNA extraction, DNA amplification, DNA visualization, DNA sequence analysis, and phylogenetic analysis. DNA extraction was carried out following the procedure of Doyle & Doyle (1987), namely 0.2 g of pathogenic fungal mycelium was ground with liquid nitrogen in a mortar until it became a powder. The ground material was transferred to an Eppendorf tube, then 500 µL of CTAB buffer and 5 µL of β-mercaptoethanol were added, and the mixture was vortexed until homogeneous. Cell wall lysis was carried out by heating at 70°C for 60 minutes, and to accelerate the lysis process, inversion was carried out every 10 minutes. After lysis was complete, the mixture was cooled to room temperature. A total of 500 µL

of chloroform isoamyl alcohol (24:1) was added to the tube, then mixed using a vortex until homogeneous and centrifuged for 15 minutes at 12,000 rpm. Next, transfer the supernatant to a new Eppendorf tube and add sodium acetate up to 1/10 of the total supernatant volume; vortex until homogeneous. Next, isopropanol was added up to 2/3 of the total volume, or absolute ethanol up to 2.5 times the total volume, and then vortexed until homogeneous. Next, store the sample at -20°C overnight, or insert the Eppendorf tube into liquid nitrogen. The sample was then centrifuged at 12,000 rpm for 10 minutes. The supernatant was discarded, the pellet was washed using ethanol (70%), then centrifuged for 5 minutes at 8,000 rpm. Next, the ethanol was discarded, and the pellet was dried. The pellet was resuspended with 25-50 µL of TE buffer or *nuclease-free water* and then stored at -20°C for further use in the DNA amplification process.

We performed DNA amplification using universal primers to detect the ITS region of ribosomal DNA (rDNA). The primer pairs used were *forward primer ITS1* (5'-TCCGTAGGTGAACCTGCGG-3') and *reverse primer ITS4* (5'-TCCTCCGCTTATTGATATGC-3'), with a target amplification size ranging from 500 – 700 base pairs (bp) (White et al., 1990). The DNA amplification reaction was performed with a total volume of 20 µL consisting of 1 µL of template DNA, 1 µL for each *forward* and *reverse primer*, 10 µL of Taq DNA (10 units/µL), and 7 µL of *nuclease-free water*. Amplification was divided into several stages: predenaturation at 95°C for 1 minute, followed by 35 amplification cycles, each consisting of DNA strand separation (denaturation) at 95°C for 1 minute, primer annealing at 58°C for 30 seconds, and DNA synthesis at

72°C for 1 minute. Specifically, for the final cycle, a synthesis stage of 72°C for 5 minutes is added, then the cycle ends at 4°C.

The amplified PCR products were analyzed with 1% agarose (20 mL of 0.5× Tris-Borate EDTA/TBE mixed with 20 g of agarose). Electrophoresis was carried out for 30 minutes at 100 volts. The gel that had been stained with FluoroVue™ (Smobio, Taiwan) as much as 5 µl was visualized with a UV *transilluminator*, and the results were documented with a digital camera. We sequenced the samples to determine the nucleotide sequence at First Base, Malaysia, through PT. Genetika Science, Jakarta.

The sequencing results were manually edited using BioEdit version 7.2.5 software to align the forward and reverse sequences before further analysis for initial identification using the *Basic Local Alignment Search Tool* (BLAST). The results of the sequence alignment with the comparison sequences in GenBank were further analyzed using MEGA 11 version 11.0.13 software to determine the percentage of sequence identity and the construction of a phylogenetic tree.

3. Results and Discussion

3.1. Isolation of Pathogenic Fungi and Pathogenicity Testing

We successfully isolated six fungal isolates from durian stem samples showing stem rot symptoms. The six isolates (Srt001, Srt002, Srt003, Kbt001, Bsb001, and Swn001) were then tested for pathogenicity to confirm their ability to cause stem rot in durian plants in Buleleng Regency.

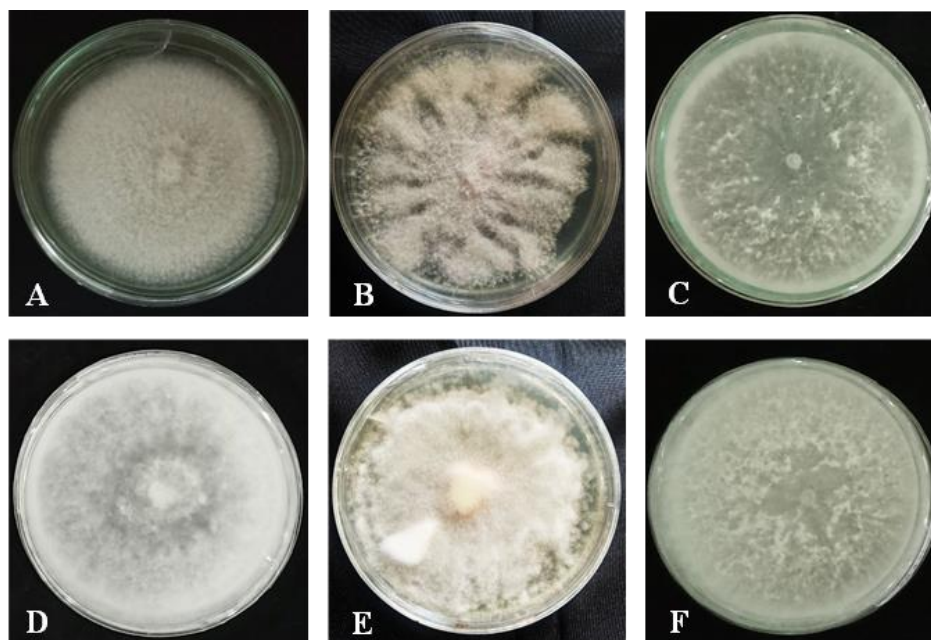


Figure 2. Macroscopic morphology of isolate colonies isolated from durian plant samples with stem rot symptoms on PDA medium. (AC) Isolates Srt001 and Srt002; Srt003 from Seririt District, (D) isolate Kbt001 from Kubutambahan District, (E) isolate Bsb001 from Busungbiu District, and (F) isolate Swn001 from Sawan District.

The results of pathogenicity test observations at 30 days after planting of each isolate Srt001, Srt002, Srt003, Kbt001, Bsb001, and Swn001 on Otong durian seedlings aged about one year showed that only isolate Srt003 showed symptoms of stem rot disease characterized by the discharge of *gummosis fluid*, similar to the symptoms of stem rot disease found at the sampling location (Figure 3). Meanwhile, in durian seedlings inoculated with isolates Srt001, Srt002, Kbt001, Bsb001, Swn001, and PDA medium (control) showed no symptoms of stem rot disease. We isolated and purified pathogenic fungi from durian seedlings again for macroscopic and microscopic morphological characterization.



Figure 3. Pathogenicity test of pathogenic fungal isolates on durian seedlings. (A) Durian seedlings inoculated with PDA medium alone (control), (B) durian seedlings inoculated with isolate Srt003, (C) no symptoms in the control treatment, and (D) stem rot symptoms in the Srt003 isolate treatment.

3.2. Macroscopic and Microscopic Morphological Characteristics of Pathogenic Fungi

The pathogenic fungal isolate Srt003 associated with stem rot disease in durian plants has colony characteristics similar to the macroscopic morphology of the *Phytophthora* sp. fungus, namely having white colony characteristics with a colony growth pattern and shape that spreads radially from the inoculation point and forms star-like or root-like branches (*stellate-radiate*), as well as an aerial mycelium texture that is cotton-like and *fluffy* (Figure 4).

The macroscopic morphological characteristics of the pathogenic fungi in this study are in accordance with the findings of Santoso et al. (2015) and Santoso (2016), who

reported that *P. palmivora* isolates associated with durian plants in Indonesia generally form colonies with a *stellate pattern*. However, sometimes it can vary between isolates. André and Sendall (2001) and Abad et al. (2023a) reported that the main characteristics of *P. palmivora* include colonies that lack a typical pattern, with variations in growth patterns such as uniform, radial, stellate, chrysanthemum, and rosette. In addition, variations in colony shape that appear in isolates can be influenced by environmental temperature and the transparency or thickness of the media used (Van der Plaats-Niterink, 1981).

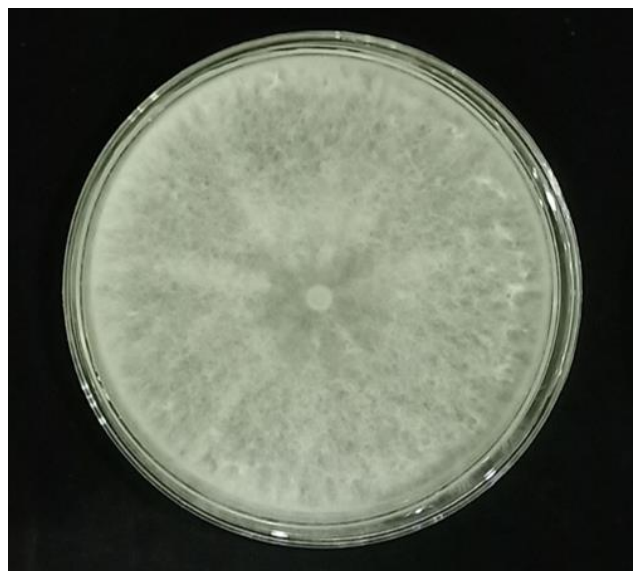


Figure 4. Pathogenic fungal isolates isolated from durian plant seeds in the pathogenicity test.

Other microscopic morphological characteristics observed on PDA media indicate that the hyphae of the pathogenic fungus are non-septate (coenocytic) and transparent or hyaline. Observations were made at 400x magnification using a light microscope (Figure 5.A). The non-septate and transparent hyphae are generally found in both *Phytophthora* and *Pythium*. (Ho, 2018) . The sporangium of isolate Srt003 is ovoid to ellipsoid in shape, with some sporangia almost globose and papillae at the apex (Figure 5.B). The size of the sporangia in this study ranged from 40-60 x 21-46 μm , a larger range than the size of 28-51 x 19-39 μm reported by Santoso (2016). Other reports also state that *P. palmivora* sporangia can be globose, ovoid, obpyriform, ellipsoid, or irregular, and are generally papillate and have short pedicels (Pongpisutta and Sangchote, 2004; Latifah et al., 2018; Abad et al., 2023b).

In general, based on macroscopic and microscopic morphological identification of isolate Srt003, the identification results show that the pathogenic fungal isolate Srt003 has a close relationship with fungi from the *Phytophthora* sp. group.

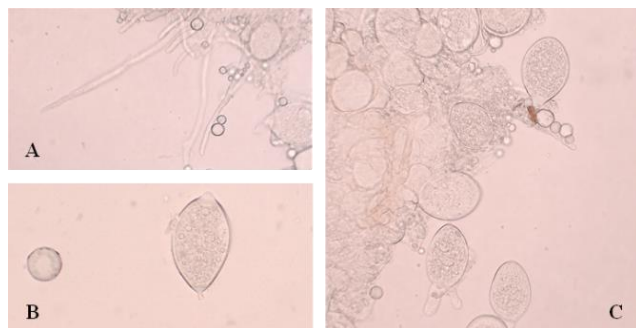


Figure 5. Observation results: Microscopic view of isolate Srt003. (A) Hyaline and non-septate hyphae, (B) sporangium ellipsoid to ovoid in shape.

3.3. Molecular Characteristics and Phylogenetic Analysis

DNA amplification of the pathogenic fungus Srt003 targeting the ITS region was successfully carried out using a pair of primers ITS1 and ITS4 with a target amplicon of approximately 600 base pairs (Figure 6).

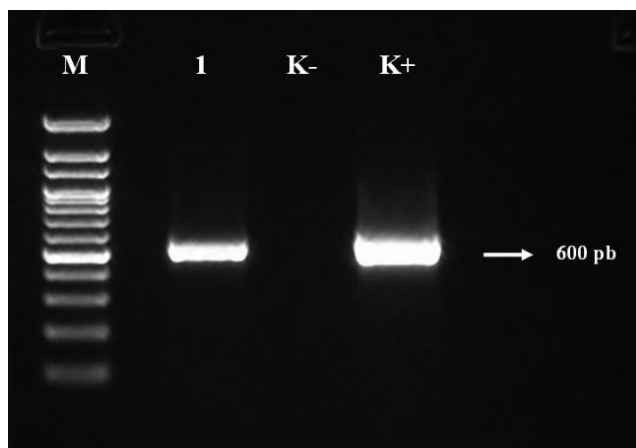


Figure 6. Visualization of DNA amplification of the pathogenic fungus Srt003 using primers ITS 1 (F) and ITS 4 (R). (M) 100 bp marker, (1) Srt003 isolate, (K-) negative control, and (K+) positive control.

The amplicon size of approximately 600 base pairs in the electrophoresis visualization results is within the general range of the length of the ITS region (ITS1 – 5.8S – ITS2) in most plant pathogenic fungi, which generally ranges from 500 – 700 base pairs depending on the genus and species (White et al., 1990; Schoch et al., 2012). White et al. (1990) first designed ITS1 and ITS4 as a universal primer pair that binds to highly conserved regions, namely the *small subunit* (18S)/SSU and *large subunit* (28S)/LSU genes flanking the ITS1, 5.8S rDNA, and ITS2 regions. Because it targets highly conserved *flanking regions*, this primer pair can amplify almost all members of the Kingdom Fungi, making it the most extensive primer set for identification and phylogenetics, as well as universal DNA barcoding for fungi (Schoch et al., 2012).

The results of the sequence analysis that has been combined between the *forward* and *reverse DNA sequences* (*contig*) of the Srt003 isolate obtained a target sequence with a length of ± 582 bases. This target sequence was then compared with the organism encoded with the same DNA target in the National Center for Biotechnology Information (NCBI) database using the BLAST feature. The results of the organism search showed that the pathogenic fungal isolate Srt003 had the same percentage of identity (*percent identity*) in the BLAST results with the pathogenic fungus from the *P. palmivora* species.

Table 1. Percentage of sequence identity of isolate Srt003 with comparison sequences based on *identity matrix analysis*

Species and Origin of Comparison Isolates	Accession Number	Sequence Identity with Isolate Srt003 (%)
<i>Phytophthora palmivora</i> (CRI)	MG865560	99.00
<i>Phytophthora palmivora</i> (IND)	MG865559	99.00
<i>Phytophthora palmivora</i> (IDN)	MG865561	99.00
<i>Phytophthora palmivora</i> (THA)	OR655080	99.60
<i>Phytophthora palmivora</i> (VNM)	MN750015	99.60
<i>Phytophthora palmivora</i> (MYS)	OR051912	99.60
<i>Phytophthora megakarya</i> (NGA)	MG865533	90.50
<i>Phytophthora megakarya</i> (CMR)	MG865534	90.50
<i>Phytophthora capsici</i> (USA)	MG865467	90.20
<i>Phytophthora nicotianae</i> (IDN)	MG865550	87.70
<i>Phytophthora vexans</i> (TUR)	PV354106	66.20

The sequence of isolate Srt003 has sequence identity ranging from 87.70–99.60% with comparison isolates from the genus *Phytophthora*. More specifically, the highest percentage of identity is between isolate Srt003 and *P. palmivora* (99.00–99.60%). The high percentage of sequence identity of isolate Srt003 with several comparison sequences of *P. palmivora* indicates that isolate Srt003 is

genetically close to *P. palmivora*. In contrast, the sequence of *Phytophthora vexans* (PV354106) used as an *outgroup* shows the lowest percentage of identity to isolate Srt003, which is 66.20%.

To determine the molecular characteristics of the Srt003 isolate, we performed a phylogenetic analysis by constructing a phylogenetic tree between the Srt003 isolate

and reference sequences deposited in the NCBI database. We constructed the phylogenetic tree using the Maximum Likelihood method with 1,000 bootstrap replicates. The best nucleotide substitution model based on the best-fit model analysis was the Hasegawa–Kishino–Yano model with a Gamma distribution (HKY+G) with the lowest BIC value of 3595.803. The lowest BIC value was chosen because it is the optimal model in constructing a phylogenetic tree using the Maximum Likelihood method in MEGA 11 software (Tamura et al., 2011; Tamura et al., 2021).

Maximum Likelihood method with a 1,000-fold bootstrap is in line with the approach used in recent *P. palmivora* research. Neeraja et al. (2025) used ITS sequences and the Maximum Likelihood method with a 1,000-fold bootstrap to confirm the identity of *P. palmivora* in cocoa, while Jami et al. (2026) used Maximum Likelihood with a 1,000-fold bootstrap in the analysis of several loci for the identification of *P. palmivora*.

The analysis results showed that isolate Srt003 was in the same group as all the comparison sequences of *P. palmivora*, thus supporting the results of the molecular identification of isolate Srt003 as *P. palmivora* (Figure 7). Isolate Srt003 formed a subgroup with *P. palmivora* (MN750015) from Vietnam, (OR051912) from Malaysia, and (OR655080) from Thailand with a bootstrap value of 82%. This value indicates strong support for the grouping of these four sequences. This subgroup was further joined by the comparison sequences of *P. palmivora* (MG865560) from Costa Rica, (MG865559) from India, and (MG865561) from Indonesia into a single *P. palmivora* group with 76%

bootstrap support. This grouping indicates that isolate Srt003 has a close phylogenetic relationship with the comparison *P. palmivora* sequences and is consistently placed within this species group. IDphy also identified MG865559, MG865560, and MG865561 as authenticated ITS sequences, and their positions play an important role in molecular identification of the genus *Phytophthora* (Abad et al., 2023a).

Outside the *P. palmivora* group, two *P. megakarya* sequences, namely (MG865533) from Nigeria and (MG865534) from Cameroon, formed a separate group with a bootstrap value of 100%, indicating very strong support for the separation of these two sequences from the *P. palmivora* group. Furthermore, *P. nicotianae* (MG865550) and *P. capsici* (MG865467) formed a group with a bootstrap value of 72%. The branching relationship involving the *P. megakarya* group with *P. nicotianae* and *P. capsici* received lower bootstrap support, 57%. This further emphasizes the separation of the *P. palmivora* group from other *Phytophthora* species, consistent with isolate Srt003 remaining in the *P. palmivora* group. This result is also supported by the level of sequence identity of isolate Srt003 with *P. palmivora*, which reached 99.60%. Dalila (2023) reported that *P. palmivora* isolates causing durian stem cancer in Malaysia had a sequence similarity level of 99–100% with the *P. palmivora* reference sequence. Phylogenetic analysis in this study also placed the *P. palmivora* isolates in one group with a bootstrap value of 99% based on the ITS region and 76% based on the COX region.

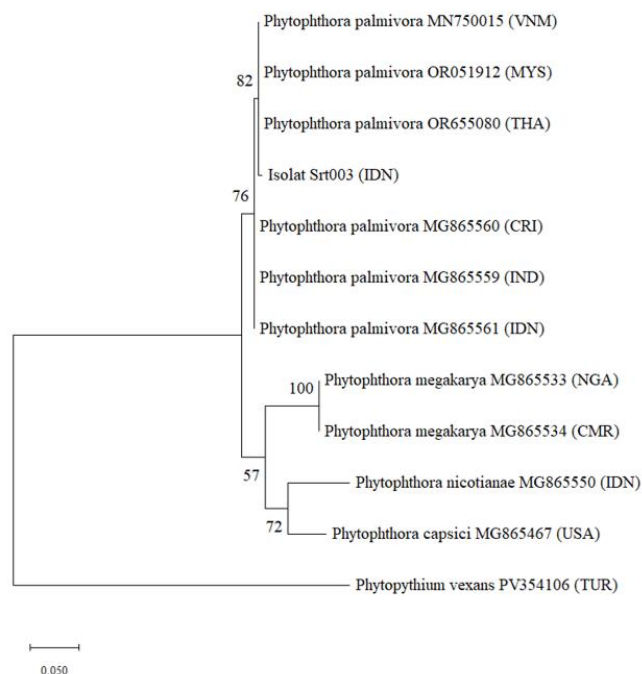


Figure 7. Phylogenetic analysis of isolate Srt003 based on ITS sequences and using the Maximum Likelihood method with the HKY+G model and 1,000-fold bootstrap. *Phytophthora vexans* was used as the outgroup.

The position of *Phytophthora vexans* (PV354106), which is located at the outermost position and separated from all *Phytophthora* species, is in accordance with its use as an outgroup. *P. vexans* has also been used as an outgroup in the broader phylogeny of the genus *Phytophthora*, including in the developed phylogeny (Yang et al., 2017).

Overall, the phylogenetic tree shows a clear separation between the *P. palmivora* group and the other comparison species. The position of isolate Srt003 within the *P. palmivora* group, particularly its formation of a subgroup with isolates from Vietnam, Malaysia, and Thailand with 82% bootstrap support, strengthens the identification of the isolate as *P. palmivora*. The tendency of isolate Srt003 to

cluster with several *P. palmivora* isolates from Southeast Asia cannot yet be used to directly infer geographic relationships or the origins of the organisms.

4. Conclusion

The pathogenic fungus associated with durian stem rot disease in Buleleng Regency was identified as *P. palmivora* based on the results of macroscopic and microscopic morphological characterization, as well as molecular characteristics from the ITS gene sequence. This result is the first report of the association between the pathogen *P. palmivora* and durian stem rot disease in Buleleng Regency, so it can be used as a reference in selecting optimal control strategies.

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