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RESEARCH PAPER

# Molecular identification of oomycetes related to horticultural crops in Southern Sumatera and Java, Indonesia

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## ABSTRACT

Indonesia is an agricultural country with more than 30 million farmers nationwide most of it with poor disease management. An identification of a pathogen is the first step to establish efficient management strategies for disease control. In this study, we survey the diversity of oomycetes in horticulture. Samples were collected from 19 sites around Lampung, Sumatera and Java Islands. The oomycetes were isolated from rhizosphere soils sample and from symptomatic plants tissues. One hundred and twelve isolates belonging to two *Phytophthora* spp., three *Pythium* spp., and one *Phytophythium* sp. were identified. *Phytophthora nicotianae* was a predominant species from pineapple but also found in cabbage, chilli, and chrysanthemum. *P. colocasiae* were isolated from taro in central java, *Phytopythium vexans* were isolated from potato in Central Java, while *Pythium acanthophoron*, *Py. myriotylum*, *Py. splenden*s, and *Py. catenulatum* were isolated from soil in pineapple farms.

**Key words:** DNA barcoding, *Phytophthora*, *Pythium*, *Phytopythium*, soil-borne pathogen

## INTRODUCTION

Identification is one of the first steps and plays a crucial role in disease management strategies. Giving the proper identity for the right plant pathogen could improve the disease management strategy and prevent further loss. However, identification of oomycetes morphologically is challenging due to the broad range of host and highly similar morphology.

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Oomycetes were reported to cause some of the most devastating plant diseases affecting horticultural plants, ornamental plants, and trees (Derevnina et al., 2016). The most notable species are members of the genus *Phytophthora*, such as *Phytophthora palmivora* causing cocoa black pod (Vanegtern et al., 2015), *P. nicotianae* causing black shank in tobacco (Gallup et al., 2006), *P. infestans* known for triggering the Irish potato famine (Goss et al., 2014) and *P. ramorum*, which caused sudden oak death (Grünwald et al., 2012). Previous study in identification of oomycetes in Indonesia were mostly related to estate crops such as durian (Santoso et al., 2015), cocoa (Umayah & Purwantara, 2006), coconut (Blaha et al., 1994), rubber (Berlian & Setyawan, 2017), and black pepper (Manohara et al., 2004). This research was focused on the horticultural plants as it is more vulnerable of oomycetes infection.

Several genes have been widely studied as barcoding region to identify oomycetes species such as internal transcribed spacer ribosomal nucleotide (Lévesque & De Cock, 2004), cytochrome c oxidase (Martin & Tooley, 2003), and beta tubulin (Villa et al., 2006). Each of the genes has its own advantages and disadvantages. As for internal transcribed spacer (ITS), it has been observed that 16 species of *Phytophthora* in clade 1 have identical ITS sequence. The cytochrome c oxidase subunit 1 (COI) has the highest genus-wide resolution for identification of

oomycetes. However, the polymerase chain reaction (PCR) success rate was lower than ITS. The beta tubulin gene is easy to amplify but can not distinguish 22 species of *Phytophthora* in clades 2, and 6-9 (Yang & Hong, 2018). Considering that, this study used COI gene for molecular identification of oomycetes. This study was aim to identify oomycetes that infected horticultural crops as a base for further development of disease management strategy.

## MATERIALS AND METHODS

**Research Site.** The sampling areas for this study were several horticultural farms in the Southern Sumatera and Java Island. Sample was collected from infected plant tissues, both healthy and infested soil in the area, and nearby water resources. Sample collections were made at five Provinces in Indonesia: Lampung, West Java, Central Java, Special Region of Yogyakarta, and East Java.

**Oomycete Isolation Procedure.** The oomycetes were isolated from infected plant, soil and healthy soil the near infected area using NARM media (Morita & Tojo, 2007). The media contained four types of antibiotics: nystatin, ampicillin, rifampicin, and miconazole that only allowed *Phytophthora* and *Pythium* to grow. Isolation of the pathogen from soil was done by direct isolation and baiting methods.

Direct isolation from soil was conducted by the protocols of Shew & Gallup (2015). First, the soil was diluted in sterile water, poured over the NARM media, and incubated for 48 hours. The incubated soil on the media then rinsed with tap water, any sign of hyphae that grows inside the media then transfer to the NARM media for purification and stored in the corn meal agar (CMA) media (Figure 1). The soil collection method was conducted as described in the protocol of isolation of *Phytophthora* from soil by Shew & Gallup (2015). On each field, 10 individual soil samples from infested fields and 10 soil samples from different sections of the fields were collected. Soil collection samples will be conducted by digging 6–8 inches right next to the plants that showed disease symptoms.

The soil baiting methods were done by placing the bait leaf on the soil and putting the leaves showing infected signs on the NARM media. After couple days, the hyphae that grow inside the media then will be transferred to another NARM media for purification and stored in the CMA media (Figure 2).

The isolation from infected tissue was done by putting the infected tissue directly on NARM media. The hyphae that grown then store in the CMA media (Figure 3).

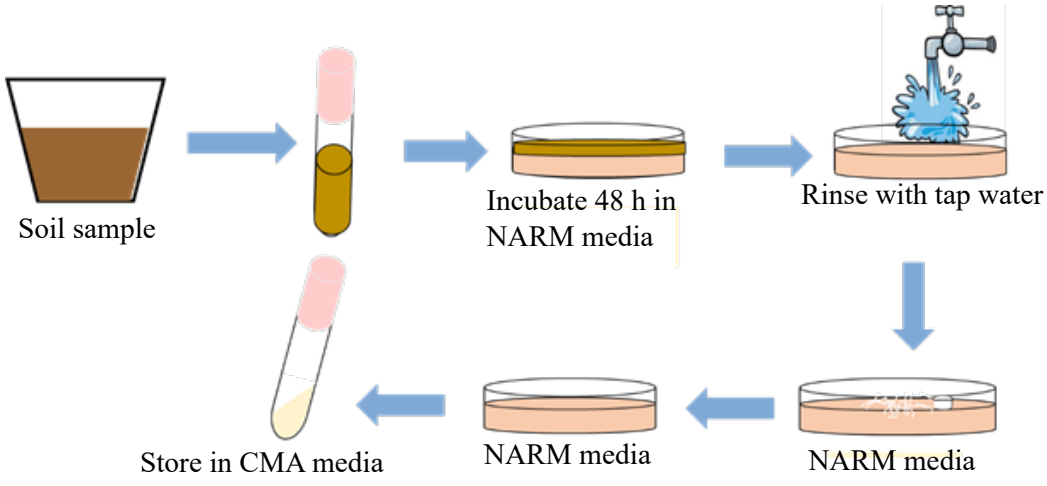


Figure 1. Methods for direct isolation of oomycetes from soil.

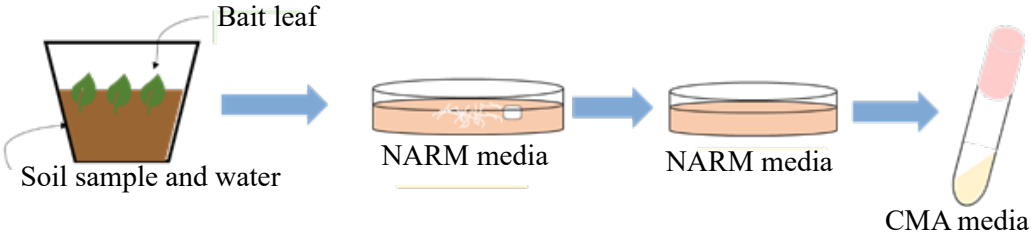


Figure 2. Methods of isolation of oomycets using bait leaf.

**Molecular Identification.** The isolates for DNA extraction was grown in the V8 media for higher production of mycelia. The DNA from mycelia then extracted using Prepman Ultra Reagent (Applied Biosystem) by following the procedure from the manufacture. A 100 µL of the reagent was put in an 1.5 mL microtube then small loopfull of hyphae was suspended in to the reagent. After that, the suspension was vortex for 10 s and incubated at 100 ºC in a heat block for 10 min. Then centrifuged the tube for 2 min at 14,000 rpm. A 50 µL of the supernatant then transferred to a new tube and stored in -20 ºC.

To obtain the identity of the species, the DNA was identified at a molecular level by sequencing of the COI gene (Robideau et al., 2011). The COI genes were amplified by PCR using primers OomCoxI-Levup (5′-TCAWCWMGATGGCTTTTTTCAAC-3′) and Fm85mod (5′-RRHWACKTGACTDATRATACC AAA-3′) modified from Martin & Tooley (2003). The 25 µl reaction mixtures contained 1 µL DNA, 2 µM of each primer, 0.4 mg/mL BSA, 0.4 mM dNTPs, 0.125 U of TaKaRa Taq DNA polymerase (Takara Bio, Kusatsu, Japan), and PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, and 1.5 mM MgCl2). The PCR reactions were carried out in a T100 DNA Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). The amplification conditions were: 94 ºC for 2 min followed by 35 cycles of 94 ºC for 1 min, 55 ºC for 30 min, and 72 ºC for 1 min, with a final extension at 72 ºC for 10 min.

All PCR products were checked for successful amplification by electrophoresis in 2% (w/v) agarose gels (TAKARA L03 agarose, Takara Bio). The PCR products were purified using the ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific) and sequencing was performed using the BigDye Terminator v3.1 cycle sequencing kit (Thermo Fisher Scientific) and the manufacturer's instructions. The sequencing products were purified by ethanol precipitation and analyzed using an ABI 3100 DNA sequencer (Thermo Fisher Scientifc). The sequences were then edited using Bioedit.

All obtained sequences were compared to other nucleotide sequences deposited in the NCBI database (www.ncbi.nlm.nih.gov) using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). To determine the phylogenetic relationships of sampled isolates and their relationships to the reference species, COI regions were aligned and phylogenetic analysis was performed. Sequence alignment and phylogenetic tree of maximum likelihood then constructed using MEGA 11 (Tamura et al., 2021).

## RESULTS AND DISCUSSION

Oomycetes are important pathogens in a wide variety of crops. Some of these are facultative biotrophs, such as most of *Phytophthora* and *Pythium*. The facultative biotroph or hemibiotroph were characterised by an initial biotrophic infection, followed by necrotrophy on killed host tissue. Many horticultural plants are affected by oomycetes. As soil and water-borne pathogens, it can easily spread due to trade or transporting the planting materials (Afandi et al., 2018). However, the report on the pathogen infection in horticultural plants in Indonesia was still limited.

This study collected 112 isolates from 13 locations at 5 provinces in Java and Southern Sumatera (Table 1). Most of the isolates were collected from infected plant tissue and soil surrounding the plant showing infection symptoms (Figure 4). In total, 98 isolates were collected from pineapple, while the rest were collected from orchid, chili, tobacco, taro, cabbage, and potato (Table 1).

Identification by BLAST search against worldwide database showed identity with reference sequences of representative isolates. Out of 112 isolates, 102 isolates were identified as *Phytophthora nicotianae*, 4 isolates was identified as *Py. periilum*, 1 isolate of *Py. splendends*, 2 isolates of *Py. acantophoron*, 1 isolate of *Py. sp. cal* 2011, 1 isolate of *Py. ultimum*, and 1 isolate of *Phy. vexans*.

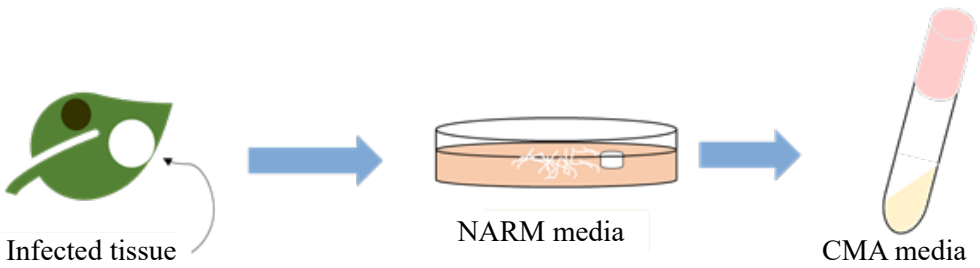


Figure 3. Methods for direct isolation of oomycetes from infected tissue.

The phylogenetic analysis was constructed in MEGA X using maximum parsimony algorithm using reference strains including the ex-type culture to assure the identity of the species. The phylogenetic tree was showed 4 major clusters (Figure 5). The first cluster consisted of members of *Phytophthora* species clade 1 (Martin & Tooley, 2003), including collected *P. nicotianae* isolates obtained from the horticultural plants used in this study. Clade 1 contains *Phytophthora* species that has papillate or semipapilate sporangia. Most of the species in clade 1 has multiple host and heterothallic (Kroon et al., 2012). In Indonesia, *P. nicotianae* was previously reported in tobacco (Agustina et al., 2013) and citrus (Marpaung et al., 2010).

On the second cluster, the isolate TAROKDT L was grouped with other clade 2 *Phytophthora* species (Martin & Tooley, 2003) and shared the same branch with *P. colocasiae* P6290 from World Phytophthora Genetic Resource Collection indicating same identity.

The third cluster consisted of *Pythium* clade B species (Lévesque & de Cock, 2004) including 7 isolates collected from soils in pineapple farms. The clade B consists almost entirely of species with

Table 1. BLAST search results of isolates collected in this study

| No. | Isolate | Collection Site | Host | Species | Strain | Ident (%) |
|---|---|---|---|---|---|---|
| 1 | AA 129D 2 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 2 | AA 129D 1 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 3 | AA 129D4 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 4 | AA 71A S1 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 5 | AA 71A S2 | L | Pineapple | *Pythium periilum* | | |
| 6 | AA71A 3 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 7 | aa 71 a 2 b | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 8 | AA 71A 2 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 9 | AA 71A 3 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 10 | AA 114K HS 2 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 11 | AA 114K HS 1 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 12 | AA 114K HS 3 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 13 | AA 114K HS 4 | L | Pineapple | *Pythium periilum* | CBS16968 | |
| 14 | AA 114K S1 | L | Pineapple | *Pythium acanthophoro* | CBS 337.29 | |
| 15 | AA 114K S3 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 16 | AA 114K S2 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 17 | AA 114 K | L | Pineapple | *Pythium splendens* | CBS 462.48 | 99 |
| 18 | AA 35A1 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 19 | AA 35AS1 | L | Pineapple | *Pythium catenulatum* | CBS46175 | |
| 20 | AA 35A S3 | L | Pineapple | *Pythium myriotylum* | | |
| 21 | AA NIA S | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 22 | AA 36G 1A | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 23 | ORC GOD | SRY | Orchid | *Phytophthora nicotianae* | P10381 | 99 |
| 24 | CHL KDT A | CJ | Chili | *Phytophthora nicotianae* | P10381 | 99 |
| 25 | CHL S A2 | CJ | Chili | *Phytophthora nicotianae* | P10381 | 99 |
| 26 | AA 51402R01 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 27 | CS GH2 2 | SRY | Chrysanthemum | *Phytophthora nicotianae* | P10381 | 99 |
| 28 | CS GH3 1 | SRY | Chrysanthemum | *Phytophthora nicotianae* | P10381 | 99 |
| 29 | CBG DIENG 1-1 | CJ | Cabbage | *Phytophthora nicotianae* | P10381 | 99 |
| 30 | 51402R2b | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |

L= Lampung; WJ= West Java; CJ= Central Java; SRY: Special Region of Yogyakarta; EJ: East Java.

Table 1. Continued. BLAST search results of isolates collected in this study

| No. | Isolate | Collection Site | Host | Species | Strain | Ident (%) |
|---|---|---|---|---|---|---|
| 31 | AA83607 HS | L | Pineapple | *Pythium acanthophoron* | CBS 337.29 | |
| 32 | aa 25a06 pt1 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 33 | AA 36G 2A | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 34 | AA 36G 2B | L | Pineapple | *Pythium periilum* | CBS16968 | 94 |
| 35 | 31423 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 36 | 155A 3 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 37 | B156 C3 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 38 | P31C | CJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 39 | 155 A 2 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 40 | PUNG 36 2 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 41 | B 151 B3 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 42 | B 156 B2 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 43 | B 159 C | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 44 | S 69 | WJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 45 | B 156 C | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 46 | S 63 | WJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 47 | PUNG 152 2 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 48 | B 150 A | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 49 | B 154 B1 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 50 | B 156 A2 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 51 | B 157 A 2 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 52 | PUNG 182 N | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 53 | B 156 C2 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 54 | 1725A3 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 55 | 17124C3 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 56 | 1725A06 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 57 | 17114K2 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 58 | 1736G2 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 59 | 129D1 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 60 | 17124D2 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 61 | 17514042 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 62 | P32B | CJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 63 | B156B3 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 64 | 159C2 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 65 | B156C2 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 66 | 151A1 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 67 | 155A1 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 68 | B157B3 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 69 | 153B1 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 70 | 159C2 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 71 | 153A2 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |

L= Lampung; WJ= West Java; CJ= Central Java; SRY: Special Region of Yogyakarta; EJ: East Java.

Table 1. Continued. BLAST search results of isolates collected in this study

| No. | Isolate | Collection Site | Host | Species | Strain | Ident (%) |
|---|---|---|---|---|---|---|
| 72 | 159A1 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 73 | 151C | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 74 | 157A1 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 75 | 156C3 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 76 | 155A1 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 77 | B154C2 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 78 | B156C1 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 79 | 157A2 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 80 | 155A3 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 81 | B157B2 | EJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 82 | 1771A1 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 83 | P3B2 | CJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 84 | 1736G3 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 85 | S51 | WJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 86 | 1736G1B | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 87 | 1725A062 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 88 | P31B | CJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 89 | S33A | WJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 90 | S1R2 | WJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 91 | P31E | CJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 92 | 1710A7 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 93 | S1R1 | WJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 94 | P34E | CJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 95 | P33F | CJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 96 | S71 | WJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 97 | S63 | WJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 98 | P33C | CJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 99 | S62 | WJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 100 | 1736B | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 101 | S1R3 | WJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 102 | 1710A1 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 103 | AA 36G PT 1 | L | Pineapple | *Pythium sp. Cal 2011c* | ADC9966 | |
| 104 | AA 36G | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 105 | AA 36G HS A-2 | L | Pineapple | *Pythium sp. CAL-2011c* | ADC9966 | |
| 106 | TBC GTS | CJ | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 107 | TBC GTS 4 | CJ | Pineapple | *Phytophthora nicotianae* | P10381 | 100 |
| 108 | AA 10A 1 | L | Pineapple | *Phytophthora nicotianae* | P10381 | 99 |
| 109 | AA 10A HS 1 | L | Pineapple | *Pythium periilum* | CBS16968 | |
| 110 | TARO KDT L | CJ | Taro | *Phytophthora colocasiae* | P6290 | |
| 111 | TOM GH 1 | CJ | Tomato | *Pythium ultimum* | | |
| 112 | POTO2 STEM | CJ | Potato | *Phytopythium vexans* | | |

L= Lampung; WJ= West Java; CJ= Central Java; SRY: Special Region of Yogyakarta; EJ: East Java.

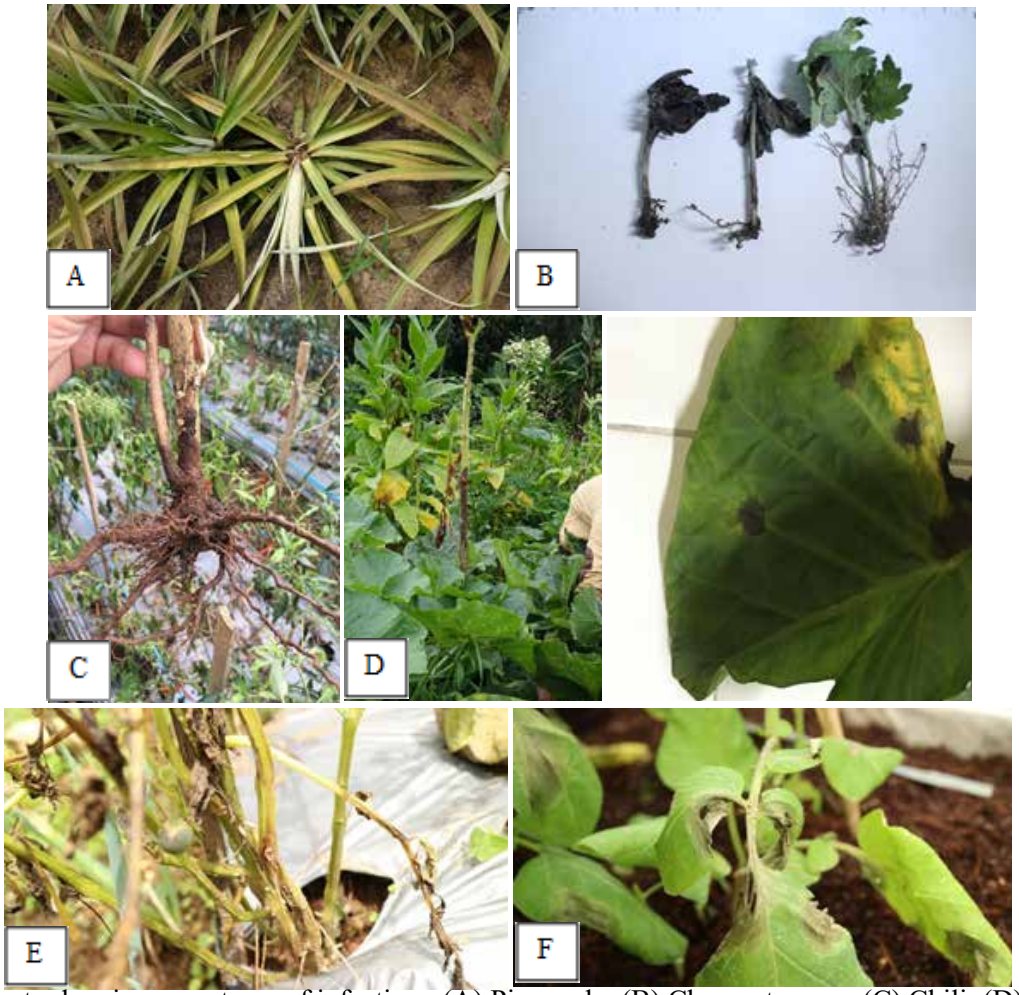


Figure 4. Plants showing symptoms of infection. (A) Pineapple; (B) Chrysantemum; (C) Chili; (D) Tobacco; (E) Taro; (F) Tomato; (G) Potato.

filamentous-inflated sporangia. The isolate AA114K were identified as *Py. splendens*. It was collected from infected pineapple tissue in Central Lampung. The pathogen was previously reported as a root parasite or pseudomycorrhizal on a wide variety of plants including pineapple (Waterhouse & Waterson, 1966). In Indonesia, *Py. splendens* was reported as a causal agent of wilt and root rot in Chinese cabbage (Vermeulen & Bustamam, 1977) and dying off of apple tree (Lestari et al., 2013). The other isolates grouped in cluster 3 were AA114KHS4 and AA83607 identified as *Py. acanthophoron*. These isolates were collected from healthy soil around the pineapple farms in Central Lampung. The *Py. acanthophoron* report in Indonesia was still limited and further exploration was needed to study the pathogen. Another *Pythium* isolates collected from soil around pineapple farms were *Py. myriotylum* (Isolate AA35AS3), *Py. catenulatum* (isolate AA35AS1), and *Py. periilum* (Isolate AA36G2 and AA71S2).

The last cluster was genus *Phytopythium*. The genera was previously classified as *Pythium* clade K (Lévesque & de Cock, 2004) and described as a new genera with *Phy. sindhum* as type species by Bala et al. (2010). The *Phytopythium* can produce papillate, internally proliferating sporangia that make it distinct from *Phytophthora* and *Pythium*. This study collected a species of *Phy. vexans* from infected potato tissue in Dieng, Central Java. Previously *Phy. vexans* was reported causing dying off of durian (Santoso et al., 2015).

## CONCLUSION

Overall, this study has shown that eight different species of oomycetes were isolated from horticultural farms around Southern Sumatera and Java. The isolates were identified as *P. nicotianae*, *P. colocasiae*, *Py. splendens*, *Py. myriotylum*, *Py. catenulatum*, *Py. periilum*, *Py. sp. cal* 2011, and *Phy. vexans*.

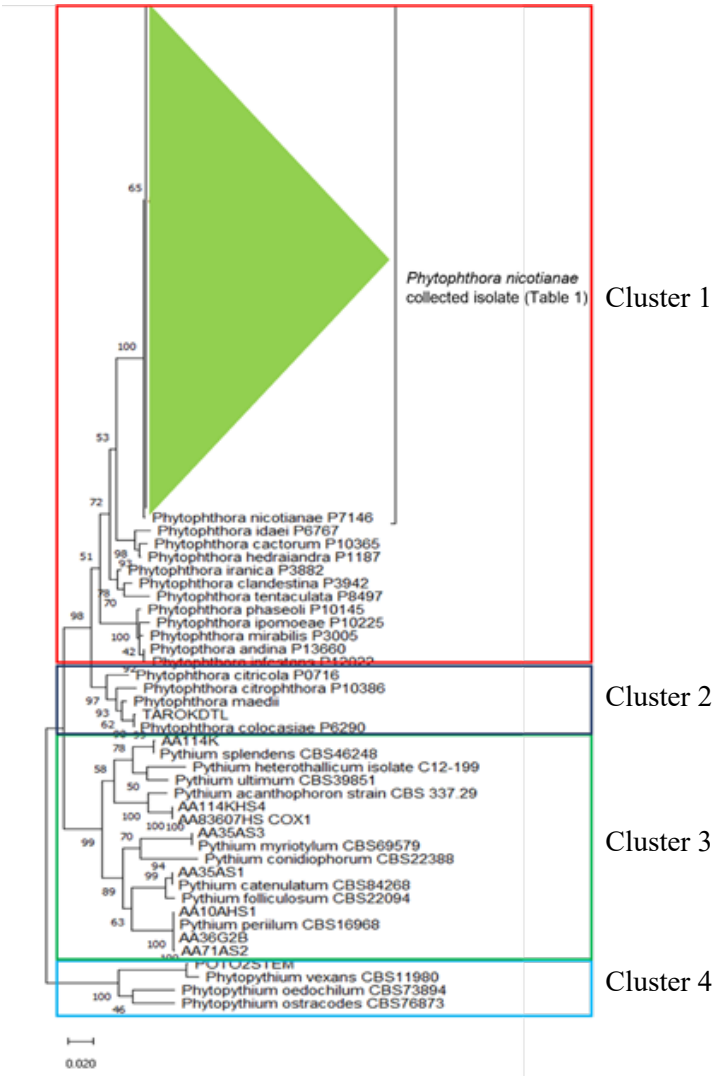


Figure 5. Phylogenetic tree showing the genetic relationship among different oomycetes isolates based on COI sequences constructed in MEGA X using maximum likelihood algorithm. Numbers on the branches represent bootstrap value (percentage of 1000 replications). Branch lengths are proportional to genetic distance which is indicated by a bar at the lower left.

Results from this study can be referenced for further pathogenicity investigation.

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## AUTHORS' CONTRIBUTIONS

AA was the main contributor for this manuscript. AA, SS, and KK was concepting the experiment. AA, M, AW, SL, and A, was carried out the isolation and pathogen sampling. AA, AH, and KO was performed the molecular analysis. AA prepared the manuscript. All authors provided response and comments on the research flow. All authors was read and approved the

final manuscript.

## COMPETING INTEREST

Authors declare that there is no competing interest regarding the publication of this manuscript.

## REFERENCES

Afandi A, Murayama E, Yin-Ling, Hieno A, & Kageyama K. 2018. Population structures of the water-borne plant pathogen *Phytopythium helicoides* reveal its possible origins and transmission modes in Japan. *PLoS ONE*. 13(12): e0209667. https://doi.org/10.1371/journal.pone.0209667

Agustina I, Pinem MI, & Zahara F. 2013. Uji efektivitas jamur antagonis *Trichoderma* sp. dan *Gliocladium* sp. untuk mengendalikan penyakit lanas (*Phytophthora nicotianae*) pada tanaman tembakau deli (*Nicotiana tabaccum* L.) [The test of the effectiviveness of *Trichoderma* sp. and *Gliocladium* sp. antagonism fungi is aimed to control lanas disease (*Phytophthora nicotianae*) in deli tobacco plants (*Nicotiana tabaccum* L.)]. *Jurnal Online Agroekoteknologi*. 1(4): 1130−1142.

Bala K, Robideau GP, Désaulniers N, de Cock AWAM, & Lévesque CA. 2010. Taxonomy, DNA barcoding and phylogeny of three new species of *Pythium* from Canada. *Persoonia.* 25: 22– 31. https://doi.org/10.3767/003158510X524754

Berlian I & Setyawan B. 2017. Gugur daun tanaman karet akibat penyakit *Phytophthora* spp. di perkebunan karet Jember, Jawa Timur [Incidence of leaf fall caused by *Phytophthora* spp. on rubber tree in Jember, East Java]. *Warta Perkaretan.* 36(2): 121–136. https://doi.org/10.22302/ppk.wp.v36i2.366

Blaha G, Hall G, Warokka JS, Concibido E, Ortiz-Garcia C. 1994. Phytophthora isolates from coconut plantations in Indonesia and Ivory Coast: characterization and identification by morphology and isozymes analysis. *Mycol. Res*. 98: 1379–1389. https://doi.org/10.1016/S0953-7562(09)81067-8

Derevnina L, Petre B, Kellner R, Dagdas YF, Sarowar MN, Giannakopoulu A, De la Concepcion JC, Chaparro-Garcia A, Pennington HG, van West P, & Kamoun S. 2016. Emerging oomycete threats to plants and animals. *Phil. Trans R. Soc. B.* 371(1709): 20150459. https://doi.org/10.1098/rstb.2015.0459

Gallup CA, Sullivan MJ, & Shew HD. 2006. *Black Shank of Tobacco. The Plant Health Instructor*. https://doi.org/10.1094/PHI-I-2006-0717-01

Goss EM, Tabima JF, Cooke DEL, Restrepo S, Fry WE, Forbes GA, Fieland VJ, Cardenas M, & Grünwald NJ. 2014. The Irish potato famine pathogen *Phytophthora infestans* originated in central Mexico rather than the Andes. *PNAS.* 111(24): 8791−8796. https://doi.org/10.1073/pnas.1401884111

Grünwald NJ, Garbelotto M, Goss EM, Heungens K, & Prospero S. 2012. Emergence of the sudden oak death pathogen *Phytophthora ramorum*. *Trends Microbiol*. 20(3): 131−138. https://doi.org/10.1016/j.tim.2011.12.006

Kroon LPNM, Brouwer H, de Cock AWAM, & Govers F. 2012. The Genus *Phytophthora* Anno 2012. *Phytopathology.* 102(4): 348−364. https://doi.org/10.1094/PHYTO-01-11-0025

Lestari FW, Suharjono, & Arumingtyas EL. 2013. Phylogenetic identification of pathogenic fungi from apple in Batu City, Malang, Indonesia. *Adv. Microbiol*. 3(1): 69−75. https://doi.org/10.4236/aim.2013.31011

Lévesque CA & De Cock AWAM. 2004. Molecular phylogeny and taxonomy of the genus *Pythium*. *Mycol. Res*. 108(12): 1363–1383. https://doi.org/10.1017/S0953756204001431

Manohara D, Mulya K, Purwantara A, & Wahyuno D. 2004. *Phytophthora capsici* on Black Pepper in Indonesia. In: Drenth A & Guest DI (eds). *Diversity and Management of Phytophthora in Southeast Asia*. *ACIAR Monograph 114*. Australian Center for International Agricultural Research. Carnberra

Marpaung AE, Silalahi FE, & Purba EIY. 2010. Identifikasi patogen penyebab busuk pangkal batang pada tanaman jeruk di Tanah Karo [Identification of pathogens causing stem rot on citrus plants in Tanah Karo]. *J. Hort.* 20(3): 262–273.

Martin FN & Tooley PW. 2003. Phylogenetic relationships among *Phytophthora* species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I

and II genes. *Mycologia*. 95(2): 269–284. https://doi.org/10.1080/15572536.2004.11833112

Morita Y & Tojo M. 2007. Modifications of PARP medium using fluazinam, miconazole, and nystatin for detection of *Pythium* spp. in soil. *Plant Disease* : *The American Phytopathological Society.* 91(12): 1591–1599. https://doi.org/10.1094/PDIS-91-12-1591

Robideau GP, De Cock AWAM, Coffey MD, Voglmayr H, Brouwer H, Bala K, Chitty DW, Désaulniers N, Eggertson QA, Gachon CMM, Hu CH, Küpper FC, Rintoul TL, Sarhan E, Verstappen ECP, Zhang Y, Bonants PJM, Ristaino JB, & Lévesque CA. 2011. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. *Mol. Ecol. Res.* 11(6): 1002–1011. https://doi.org/10.1111/j.1755-0998.2011.03041.x

Santoso PJ, Aryantha INP, Pancoro A, & Suhandono S. 2015. Identification of *Pythium* and *Phytophthora* associated with durian (*Durio* sp.) in Indonesia: their molecular and morphological characteristics and distribution. *Asian J. Plant Pathol.* 9(2): 59–71. https://doi.org/10.3923/ajppaj.2015.59.71

Shew D & Gallup C. 2015. PROTOCOL 01-05.1: Isolation of *Phytophthora nicotianae* from soil. In: Ivors KL (Ed.). *Laboratory Protocols for Phytophthora Species*. pp. 1–3. APS PRESS. https://doi.org/10.1094/9780890544969.01.05.1

Tamura K, Stecher G, & Kumar S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol*. 38(7): 3022–3027. https://doi.org/10.1093/molbev/msab120

Umayah A & Purwantara A. 2006. Identifikasi isolat *Phytophthora* asal kakao [Identification of isolates of *Phytophthora* from cocoa]. *Menara Perkebunan.* 74(2): 75–85. https://doi.org/10.22302/ppbbi.jur.mp.v74i2.108

Vanegtern B, Rogers M, & Nelson S. 2015. *Black Pod Rot of Cacao Caused by Phytophthora palmivora*. *UH-CTHAR*. PD–108. University of Hawaii. Hawaii

Vermeulen H & Bustamam M. 1977. Association of *Pythium splendens* with wilting and root rot of Chinese cabbage in Indonesia. *Bulletin Penelitian Hortikultura*. 13–17.

Villa NO, Kageyama K, Asano T, & Suga H. 2006. Phylogenetic relationships of *Pythium* and *Phytophthora* species based on ITS rDNA, cytochrome oxidase II and β-tubulin gene sequences. *Mycologia*. 98(3): 410–422. https://doi.org/10.1080/15572536.2006.11832676

Waterhouse GM & Waterston JM. 1966. *Pythium splendens*. [Descriptions of Fungi and Bacteria]. CABI International, Wallingford. https://doi.org/10.1079/DFB/20056400120

Yang X & Hong C. 2018. Differential usefulness of nine commonly used genetic markers for identifying *Phytophthora* species. *Front. Microbiol*. 03 October 2018. https://doi.org/10.3389/fmicb.2018.02334