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Isolation and Identification of *Phytophthora* sp. and *Pythium* sp. from Durian Orchard in Chumphon Province, Thailand

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Abstract The major problem of durian cultivation are root and stem rot disease caused by *Phytophthora* and *Pythium* species. Therefore, the pathogens were isolated, identified, and found the virulent pathogenic fungi that isolated from soil planted to durian in Chumphon province. The pathogens were studied on morphological and molecular analysis based on internal transcribed spacer (ITS)-nrDNA sequence. Ten isolates were confirmed as *Phytophthora palmivora*, *Pythium cucurbitacearum*, *Pythium deliense*, *Pythium splendens*, *Mortierella chlamydospora*, *Mortierella capitata* and *Mortierella* sp. The pathogenicity test was done by detached leaf method in Monthong durian variety. It found that *P. palmivora* and *P. cucurbitacearum* were high pathogenic isolates. Moreover, *P. splendens* was infected and produced symptoms on leaves. It is the first report of *P. splendens* as pathogenic to durian.

Keywords: internal transcribed spacer, *Phytophthora* sp., *Pythium* sp., root and stem rot disease

Introduction

Durian is an economic fruit and famous fruit in Southeast Asia, that known as "the king of fruit". Thailand is the largest durian production in the world (Chomchalow *et al*., 2008). Moreover, Thailand is also a leading exporter for the international market (Bais, 2016; Parichatnon *et al.*, 2017). However, the major problem is root and stem rot disease which affected yeid loss of durian plantation (Lim and Chan, 1986).

Symptoms of root and stem rot disease start attacks at the root system that roots will change to brown color. Then, trunk of durian tree has a juicy spot. When open the bark for observe this wound, the inner has brown juicy that will be change to be a dark brown color. This symptom rapidly spreads which the

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leaves will fall down and the tree will die (Lin and Sangchote, 2003; Burgess *et al*., 2008; Janick and Paull, 2008).

Many researchers reported that *Phytophthora palmivora* cause root and stem rot disease in durian (Lim and Chan, 1986; Cooke *et al*., 2009; Abad and Cruz, 2012). Furthermore, a few information reported that root and stem rot disease caused by *Phytophthora cinnamomi*, *Pythium cucurbitacearum*, *Pythium vexans* and *Pythium deliense* (Lin and Sangchote, 2003; Vawdrey *et al*., 2005; Santoso *et al*., 2015). *Phytophthora* and *Pythium* belong to the Pythiaceae, Oomycetes (Singh *et al*., 2008) that both genera are plant pathogens and widely host range (Robideau *et al*., 2011). The pathogen inoculum are spreaded rapidly by wind and rain, this feature resulted plague in all area of durian orchards (Erwin *et al*., 1983).

Phytophthora and *Pythium* are considered a very difficult group for species identification because these morphology are very similar (Uzuhashi *et al*., 2010; Schroeder *et al*., 2013). Molecular techniques for identification of *Phytophthora* and *Pythium* species have higher precision and resolution (Spies *et al*., 2011). The universal primers ITS6 and ITS4 for internal transcribed spacer (ITS) of nucleus ribosomal DNA (nrDNA) were widely used for pathogen identification (Grunwald *et al*., 2011; Santoso *et al*., 2015; Olson *et al*., 2016; Oszako *et al*., 2016). Therefore, the objectives to isolate and identify *Phytophthora* sp. and *Pythium* sp. from soil planted to durian in Chumphon province.

Materials and methods

Sampling and Isolation

Soil samples were collected from durian trees showing root and stem rot disease in Khunkrating, Banna, Talesub, Chumco and Hinkaew district orchards in Chumphon province. The district and geographic location of soil samples were shown in Table 1. Soil samples were collected infected trees, at 10-15 cm depth and kept in plastic bags.

Direct soil plating and soil baiting techniques were used to isolate. For soil plate technique, the soil sample was placed on water agar (WA) and incubated at room temperature (RT) for 1-2 days. After that, cut hyphal tip under stereo microscope put on potato dextrose agar (PDA). Another method is baiting method which was modified from Jeffers and Aldwinckle (1987), Drenth and Sendall (2001) and Martin *et al*. (2012). The mature and healthy leaves of Monthong durian were used as a bait. The leaf was washed with tap water, soaked in 70% ethanol, rinsed in sterile water and cut with scissors about 1×1 cm. The pieces of leaves were floated on soil solution and incubated in the

dark for 1-2 days at 25 ℃. After that, the pieces of leaves were placed on WA then incubated at RT for 1-2 days. After incubation, hyphal tip were cut and placed on PDA. The morphology was photographed, observed sporangium shape, papilla and measured sporangium size (Drenth and Sendall, 2001).

Table 1. The district and geographic location of 7 soil samples from Chumphon province

District	Geographic location	
	Latitude	Longtitude
Khunkrating	10.46492	99.10064
Banna	10.47082	99.02060
Talesub	10.73024	99.26467
Talesub	10.73565	99.26298
Talesub	10.69141	99.23689
Chumco	10.78955	99.27878
Hinkaew	10.60287	99.06753

DNA extraction

DNA extraction are modify the CTAB protocol followed Doyle and Doyle (1990). Mycelium was scraped off from the cellophane sheet and ground with liquid nitrogen by a pestle. The powder about 1-2 g. was put into 2 ml tube and add 2 µl of β-mercaptoethanol for separate phenolic compound and 700 µl of 2X CTAB buffer was added for cell extraction. The tube was incubated at 65 ℃ in water bath for 1 hour. After incubation, add 700 µl of chloroform: isoamyl alcohol (24:1, v/v) and mixed by immediately inversion. After centrifugation, the upper aqueous layer was transferred and treated with RNase for 30 minutes at 37 ℃. After that, the solution was added with 50 µl of 10% CTAB in 0.7M NaCl, precipitated with cold isopropanol and washed with 70% ethanol and absolute ethanol. DNA pellet was dried in incubator 37 ℃ before dissolved with TE buffer and stored at -20 ℃, for further use as templates for PCR amplification.

Amplification and sequence analysis

Molecular identification was performed by sequencing the internal transcribed spacer (ITS)-nrDNA region, using universal primers ITS6 (5'-GAAGGTGAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTG-ATATGC-3') (White *et al*., 1990; Cooke *et al*., 2000). DNA templates were amplified by polymerase chain reaction (PCR) which modify the condition followed Grunwald *et al*. (2011). The PCR products were performed to sequencing analysis by Bioneer Company, Korea. The sequences were

identified species by aligning the sequence from database of National Center for Biotechnology Information (NCBI) using Basic Local Alignment Search Tools (BLAST). The phylogenetic tree was established using MEGA6 program by Tamura-Nei model, Maximum-likelihood method and bootstrap was generated using 1000 replications.

Pathogenicity test

Pathogenicity test of each isolate was tested by detached leaf method (Vawdrey *et al*., 2005). Monthong durian leaf was rinsed with steriled water and surface sterilized with 70% ethanol. Each wound was punctured nine times with a sterile needle. Each durian leaf was made for four wounds as four repeating and immediately inoculated. Before inoculation, all isolates were cultured on PDA medium at 25 ℃ for 7 days. Then, mycelia were cut to discs of 0.5 mm diameter with a sterilized cork borer and were transferred to the durian leaf. The discs of PDA medium were maintained as control. After that, each leaf was incubated in moist chamber box at room temperature for 4 days. The brown rot around wound was measured diameter and photographed. Statistical analysis of pathogenicity was done with ANOVA analysis (p<0.05).

Results

Isolation and morphological identification

All isolates were identified according to their mycelium and colony morphology, sporangium shape and size, growth rate, development of zoosporangia and the process of the zoospore release which were observed on PDA medium.

The first group, the colony morphology of isolate CHP25-S08 is stellate pattern after 10 days on PDA. Sporangia are ovoid to limoniform shape and occasionally found obpyriform shape that are often papillate. The sporangia width rang from 25.33 to 45.34 μm and length rang from 26.29 to 56.74 μm. This isolate was identified as *Phytophthora palmivora* (Figure 1-4A). The colony morphology of isolate CHP14-S11, CHP14-N11 and CHP22-S05 is petaloid pattern after 7 days on PDA that the colony grow faster than the first group. Sporangia are globose to ellipsoid shape without papilla. The sporangia of isolate CHP14-S11 and CHP14-N11 which isolated from the same soil area are difference from CHP22-S05. The sporangia of isolate CHP14-S11 ranged in width from 9.91 to 17.87 μm and length from 10.14 to 19.96 μm and isolate CHP14-N11 ranged in width from 8.25 to 17.04 μm and length from 8.50 to

19.83 µm. The sporangia of isolate CHP22-S05 ranged in width from 15.63 to 20.29 µm and length from 16.91 to 20.77 µm. Three isolates were identified as *Pythium cucurbitacearum* which the morphology of isolate CHP14-S11 shown in Figure 1-4B. CHP25-S01, the colony morphology of this isolate is rose pattern on PDA after 7 days. The sporangia are round and globose shape with spine that width rang from 12.19 to 20.06 µm and length rang from 13.94 to 21.63 µm. This isolate was identified as *Pythium spinosum* (Figure 1-4C). The final group was divided into 2 subgroups based on their shape and growth pattern of colonies on PDA after 7 days and morphology of sporangia. The first subgroup (CHP17-N05, CHP18-N03 and CHP25-S10), colonies are fast-growing and white cotton candy pattern and sporangia are globose shape without papilla. The other group (CHP06-S03 and CHP33-S06), colonies are concentric pattern like a rose and sporangia are only limoniform shape without papilla. This group was identified as *Pythium* sp. which the colony and sporangia of CHP18-N03 shown in Figure 1-4D. The morphological characteristics of ten isolates as shown in Table 2.

A total of ten isolates were phenotypically identified as *Phytophthora palmivora* and *Pythium* sp. (*Pythium cucurbitacearum*, *Pythium spinosum* and *Pythium* sp.). However, some isolates can not be identified to species with traditional morphological observations. So, it is necessary to confirm by the molecular technique for complete identification.

Table 2. The morphological characteristics of ten isolates

Isolate	Colony pattern	Sporangia			
		Shape	Width (µm)	Length (µm)	Papilla
CHP25-S08	stellate	Obpyriform ovoid limoniform	25.33-45.34	26.29-56.74	+
CHP14-S11	petaloid	globose ellipsoid	9.91-17.87	10.14-19.96	-
CHP14-N11	cottony with slightly petaloid	globose ellipsoid	8.25-17.04	8.50-19.83	-
CHP22-S05	petaloid	globose ellipsoid	15.63-20.29	16.91-20.77	-
CHP25-S01	rose	globose	12.19-20.06	13.94-21.63	-
CHP17-N05	cotton candy	Globose	11.51-20.18	11.56-22.53	-
CHP18-N03	cotton candy	Globose	27.72-42.62	27.77-42.70	-
CHP25-S10	cotton candy	globose	9.89-18.21	10.44-19.02	-
CHP06-S03	rose	Limoniform	5.85-11.43	8.11-14.99	-
CHP33-S06	rose	Limoniform	7.55-10.10	10.00-12.82	-

+: papillated sporangium, -: non papillate sporangium

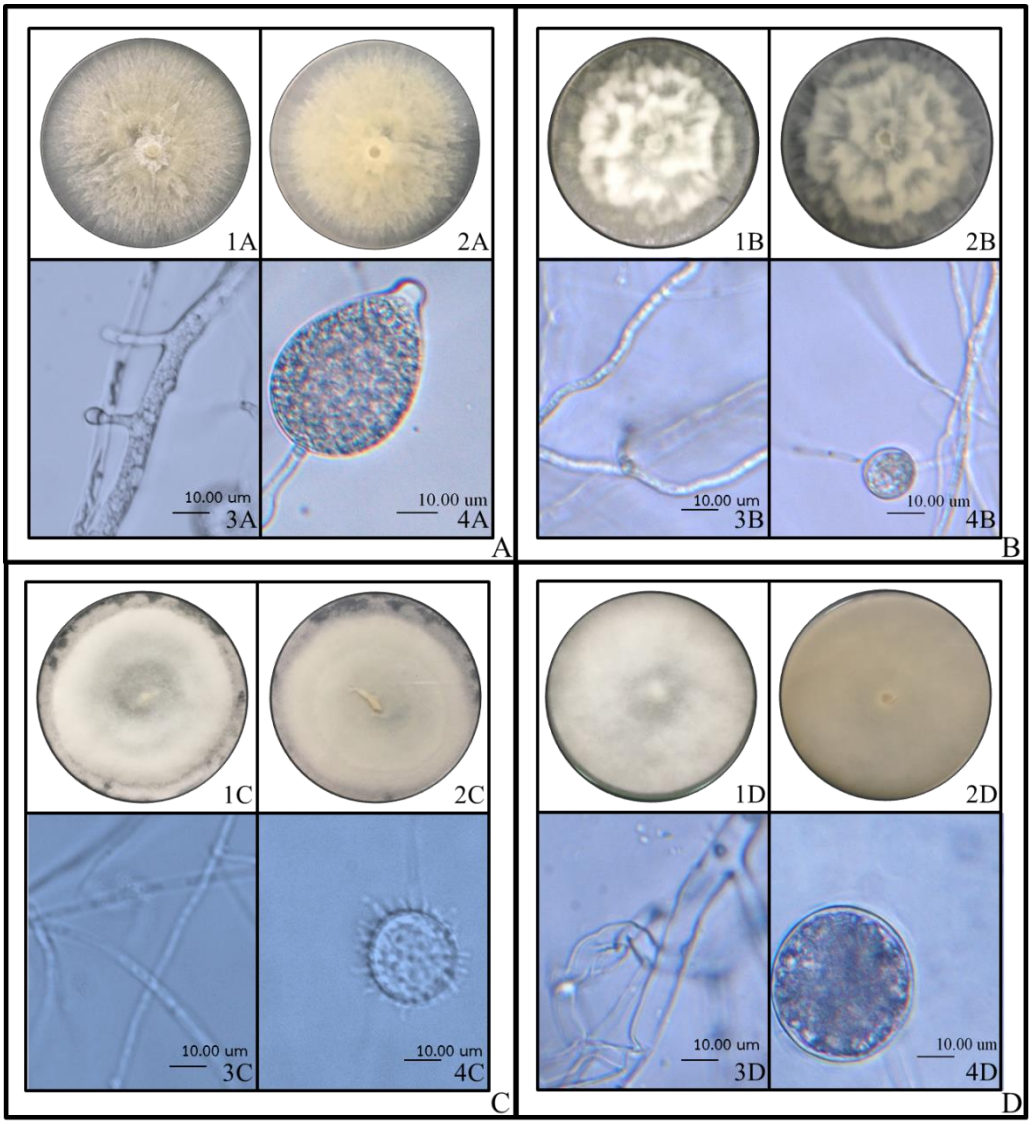

Figure 1. Colonies and sporangia morphology of *Phytophthora* and *Pythium* on PDA: colony on the top of the Petri dish (1), colony on the bottom of the Petri dish (2), hypha (3) and sporangium (4) of the CHP25-S08 (A), CHP14-S11 (B), CHP25-S01 (C) and CHP18-N03 (D)

Molecular identification and sequence analysis

The nrDNA-ITS sequences of each sample which amplified with ITS6 and ITS4 primers were sequenced and compared with known DNA sequences in the NCBI databases. The nucleotide sequences in all isolates are ranged in size from 634 to 924 (Table 3). The isolate CHP25-S08 was identified as *Phytophthora palmivora* based on molecular analysis, corroborating the above morphological identification. BLAST analysis of this isolate revealed 99% identity with reference sequences of *P. palmivora* in GenBank. CHP14-S11, CHP14-N11 and CHP22-S05 were identified as *Pythium cucurbitacearum* which showed 94-99% identity. The isolate CHP25-S01 was assumed to belong to *P. spinosum* based on the morphological identification of its sporangia and its gross colony appearance. However, nucleotide sequence analysis of this isolate was most closely related to *Mortierella chlamydospora* (JX975927) showed 98% identity. Five isolates were identified as *Pythium* sp. CHP17-N05 and CHP18-N03 were identified to *Pythium splendens* which a sequence identity higher than 85%. CHP25-S10 sequence was more closely related to *Pythium deliense*, showed 99% identity. Moreover, in CHP06-S03 and CHP33-S06 showed 92% and 82% identity with the *Mortierella capitata* (MF115611) *Mortierella hyaline* (KC009040), respectively. Sequence comparing between morphology and molecular identification showed result in Table 3.

Table 3. Identification comparing between morphology and molecular

Isolate	Morphology	Sequence length (bp)	Identification		
			Molecular	Accession number	% Identity
CHP25-S08	*Phytophthora palmivora*	895	*P. palmivora*	KP183963	99
CHP14-S11	*Pythium cucurbitacearum*	924	*P. cucurbitacearum*	KP183959	98
CHP14-N11	*Pythium cucurbitacearum*	924	*P. cucurbitacearum*	KP183959	94
CHP22-S05	*Pythium cucurbitacearum*	924	*P. cucurbitacearum*	KP183959	99
CHP17-N05	*Pythium* sp.	920	*P. splendens*	AB780581	87
CHP18-N03	*Pythium* sp.	920	*P. splendens*	AB780605	86
CHP25-S10	*Pythium* sp.	885	*P. deliense*	KP183964	99
CHP25-S01	*Pythium spinosum*	723	*M. chlamydospora*	JX975927	98
CHP06-S03	*Pythium* sp.	674	*M. capitata*	MF115611	92
CHP33-S06	*Pythium* sp.	634	*M. hyalina*	KC009040	82

The DNA sequences were analyzed for phylogenetic relationships using MEGA 6 software. All sequences were compared with the sequences in GenBank. Bootstrap analysis was performed with 1,000 replications to determine the support for each group. The phylogenetic tree shows in Figure 2 clearly separate to two major groups consist of genus *Phytophthora*, *Pythium* and *Mortierella*. The tree shows that the three isolates of *P. cucurbitacearum* (CHP22-S05, CHP14-N11 and CHP14-S11) was well embedded within *P.*

palmivora with strong support (92%) and demonstrated the close relationships between *P. splendens* (CHP17-N05 and CHP18-N03) and *P. deliense* (CHP25-S10). Including, phylogenetic analysis confirmed the relationships in genus *Mortierella*. However, the CHP33-S06 shown to be less closely related to *M. hyalina*.

Pathogenicity test

Pathogenicity test of each isolate which the detached leaf bioassay was used to evaluate on Monthong durian leaf after 4 days inoculation. *P. palmivora* isolate CHP25-S08 showed the significantly largest diameter of brown lesions (mean of 26.94 mm) on durian leaf. In this study, the *P. cucurbitacearum* isolate CHP22-S05, CHP14-N11 and CHP14-S11 gave large diameters ranged from 18.24 to 22.15 mm. In addition, both isolates of *P. splendens* (CHP17-N05 and CHP18-N03) also maked brown lesions ranged from 16.04 to 17.40 mm in diameter. However, there were no significant differences in diameter of lesions in the groups of control, *P. deliense* (CHP25-S10) and *Mortierella* (CHP25-S01, CHP06-S03 and CHP33-S06). The pathogenicity are shown in Figure 3 and Table 4.

Table 4. Lesion diameters on detached leaves of Montong durian cultivar inoculated with ten isolates after 4 days of inoculation

Isolates	Species	Lesion diameters (mm)*
Control	-	4.56 ± 0.08^{e}
CHP25-S08	*Phytophthora palmivora*	26.94 ± 2.16^{a}
CHP22-S05	*Pythium cucurbitacearum*	22.15 ± 0.22^{b}
CHP14-N11	*Pythium cucurbitacearum*	20.21 ± 1.00^{bc}
CHP14-S11	*Pythium cucurbitacearum*	18.24 ± 0.11^{cd}
CHP17-N05	*Pythium splendens*	17.40 ± 0.49^{d}
CHP18-N03	*Pythium splendens*	16.04 ± 0.32^{d}
CHP25-S10	*Pythium deliense*	4.86 ± 0.29^{e}
CHP25-S01	*Mortierella chlamydospora*	4.76 ± 0.04^{e}
CHP06-S03	*Mortierella capitata*	4.95 ± 0.24^{e}
CHP33-S06	*Mortierella* sp.	4.63 ± 0.17^{e}

*Values expressed are mean ± SE

a-c means with the different letters in the same column were significant by ANOVA at $p<0.05$

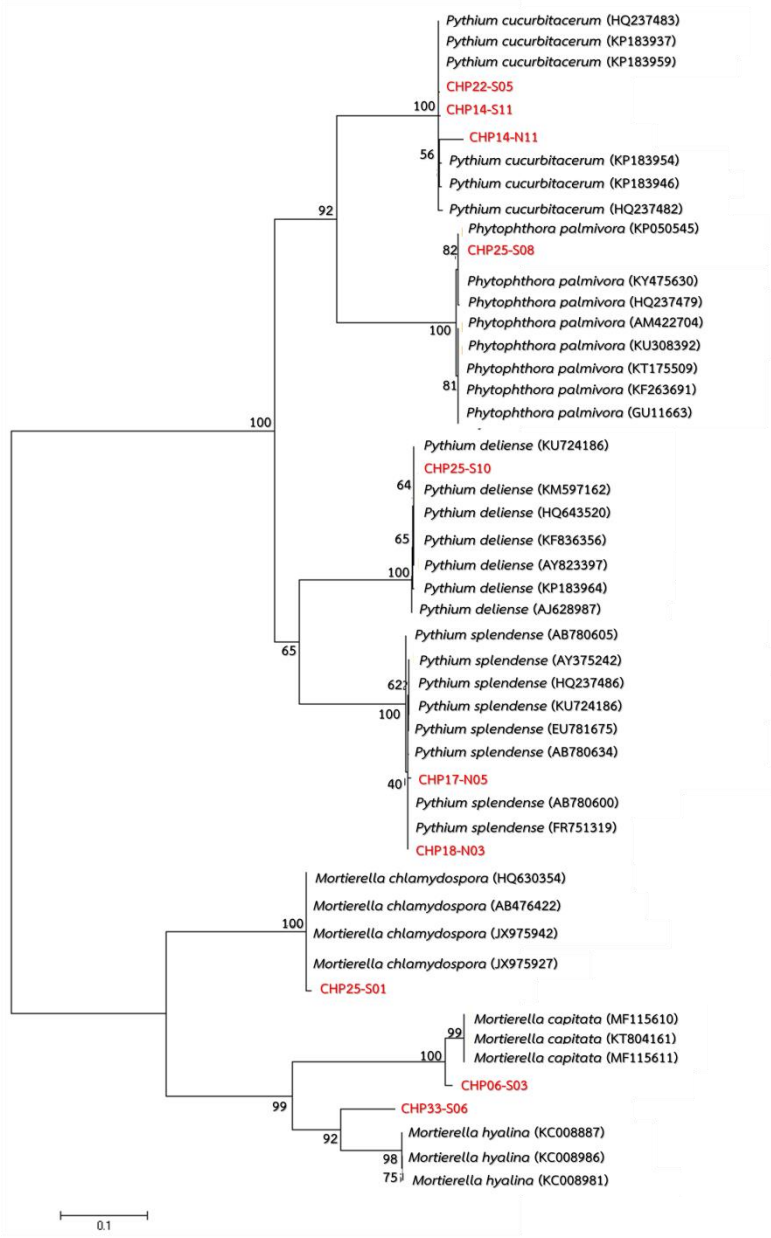

Figure 2. Phylogenetic tree obtained from the analyses of ITS-nrDNA sequence data, representing the identification of *Pythium*, *Phytophthora* and *Mortierella* and the numbers above the nodes are the percentage of the trees from bootstrap analysis

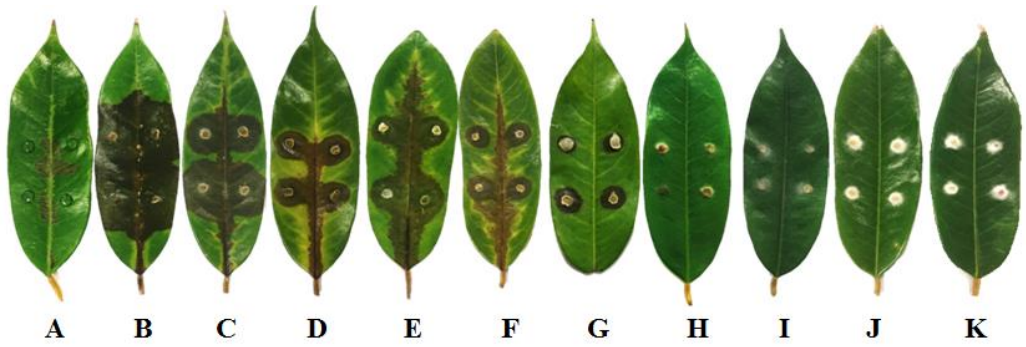

Figure 3. Pathogenic and non-pathogenic results of detached leaf assays in leaves of Monthong durian inoculated with isolates of control (A), CHP25-S08 (B), CHP22-S05 (C), CHP14-N11 (D), CHP14-S11 (E), CHP17-N05 (F), CHP18-N03 (G), CHP25-S10 (H), CHP25-S01 (I), CHP06-S03 (J) and CHP33-S06 (K) after 4 days of inoculation

Discussion

The genus *Phytophthora* and *Pythium* were classified in Pythiaceae and Peronosporales. Both genera are very similar in morphology (Uzuhashi *et al*., 2010; Schroeder *et al*., 2013). For *Phytophthora palmivora* the sporangium is thin-walled, limoniform shaped with an apical papilla, zoospores which packed in the sporangium are released directly through an opening at apical papilla. For genus *Pythium*, the sporangium have globose shaped without papilla and sporangium produce zoospores in a vesicle. Zoospores in the sporangium are released when the vesicle break (Burgess *et al*., 2008). However, some isolates are not found stage of zoospores release. *Phytophthora palmivora* and *Pythium* sp. have various shape and size of sporangium (Santoso *et al*., 2015). Including, the colony morphology of some *Pythium* sp. isolates are similar appearance to *Mortierella* sp. in culture that some *Mortierella* sp. produce zygospores that look like *Pythium* oospores (Domsch *et al*., 2007). However, colonies of *Mortierella* are fast growing, producing a concentric pattern and having smaller spore than *Pythium* sp. An accurate morphological identification is essential but difficult to identify or impossible to do. So, molecular identification provides a rapid and reliable. Based on morphological and ITS-nrDNA sequences, ten isolates were identified as *P. palmivora, P. cucurbitacearum, P. splendens, P. deliense, M. chlamydospora, M. capitata* and *Mortierella* sp. Because of CHP33-S06 shown to be less closely related to *M. hyalina* that it is not enough to identify as this species only based on ITS sequences of nrDNA.

Root and stem rot disease in durian are almost related to *Phytophthora palmivora* as the responsible pathogen (Lim and Chan, 1986; Cooke *et al*., 2009; Abad and Cruz, 2012). Nowadays, *P. cinnamomi*, *P. cucurbitacearum*, *P. vexans* and *P. deliense* are also found associated with durian tree-decline (Lin and Sangchote, 2003; Vawdrey *et al*., 2005; Santoso *et al*., 2015). *P. splendens* were successful in produced lesions on leaves of durian. So, this present study demonstrated the first publication for the association of *P. splendens* pathogen in durian. However, *P. deliense* and genus *Mortierella* are not associated with leaf rot disease in durian which genus *Mortierella* has not been reported for plant pathogen. So, those isolates should be testing on the another commercial durian cultivars in future.

Conclusion

Direct soil plating and soil baiting techniques were isolated and identified for pathogenic fungi from soil that were collected from durian orchards in Chumphon province. Due to insufficient morphological identification, it is easy to misidentify and difficult to key to the species level of *Phytophthora* and *Pythium*. The molecular identification are essential to verify the species level identification which can provide rapid and reliable. Based on morphological and ITS-nrDNA sequences, the ten isolates were identified as *Phytophthora palmivora*, *Pythium cucurbitacearum*, *P. deliense*, *P. splendens*, *Mortierella chlamydospora*, *M. capitata* and *Mortierella* sp. Pathogenicity test by detached leaf method, the high virulence of leaf rot disease of durian are caused by *P. palmivora* and *P. cucurbitacearum*. In this study, *P. splendens* was successful in produced lesions on leaves of Monthong durian. So, this is the first publication of *P. splendens* as pathogen of durian. However, *P. deliense* and genus *Mortierella* are not associated with leaf rot disease of durian.

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References

Abad, R. G. and Cruz, K. J. T. (2012). Incidence of *Phytophthora* fruit rot on four durian cultivars in Davao city, Philippines. Banwa 9:1-9.

Bais, K. (2016). Why Thailand is the leading exporter of durian, mangosteen and other tropical fruits. Utar Agriculture Science Journal 2:5-15.

Burgess, L. W., Knight, T. E., Tesoriero, L. and Phan, H. T. (2008). Diagnostic manual for plant diseases in Vietnam. Vietnam: Goanna Print Pty Ltd.

Chomchalow, N., Somsri, S., and Songkhla, P. N. (2008). Marketing and export of major tropical fruits from Thailand. Assumption University Journal of Technology 11:133-143.

Cooke, D. E. L., Drenth, A., Duncan, J. M., Wagels, G. and Brasier, C. M. (2000). A molecular phylogeny of *Phytophthora* and related oomycetes. Fungal Genetics and Biology 30:17-32.

Cooke, T., Persley, D. and House, S. (2009). Diseases of fruit crops in Australia. Australia: CSIRO Publishing.

Drenth, A. and Sendall, B. (2001). Practical guide to detection and identification of *Phytophthora*. Brisbane: CRC for Tropical Plant Protection.

Domsch, K. H., Gams, W. and Anderson, T. H. (2007). Compendium of soil fungi. Berchtesgaden, IHW-Verlag.

Doyle, J. J. and Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. Focus 12:13-15.

Erwin, D. C., Garcia, S. E. and Tsao, P. H. (1983). *Phytophthora*: Its biology, taxonomy, and pathology. United States: The American Phytopathlogical Society.

Grunwald, N. J., Martin, F. N., Larsen, M. M., Sullivan, C. M., Press, C. M., Coffey, M. D., Hansen, E. M. and Parke, J. L. (2011). Phytophthora-ID.org: A sequence-based *Phytophthora* identification tool. Plant Disease 95:337-342.

Janick, J. and Paull, R. E. (2008). The encyclopedia of fruit and nuts. United Kingdom: Cambridge University Press.

Jeffers, S. N. and Aldwinckle, H. S. (1987). Enhancing detection of *Phytophthora cactorum* in naturally infested soil. Phytopathology 77:1475-1482.

Lim, T. K. and Chan, L. G. (1986). Fruit rot of durian caused by *Phytophthora palmivora*. Pertanika 9:269-276.

Lin, T. K. and Sangchote, S. (2003). Diseases of tropical fruit crops. Florida: Tropical Research and Education Center Homestead.

Martin, F. N., Abad, Z. G., Balci, Y. and Ivos, K. (2012). Identification and detection of *Phytophthora*: reviewing our progress, identifying our needs. Plant disease 96:1080-1103.

Oszako, T., Sikora, K., Belbahri, L. and Nowakowska, J. A. (2016). Molecular detection of oomycetes species in water courses. Folia Forestalia Polonica, Series A–Forestry 58:246-251.

Olson, J. D., Damicone, J. P. and Kahn, B. A. (2016). Identification and characterization of isolate of *Pythium* and *Phytophthora* spp. from snap beans with cottony leak. Plant Disease 100:1446-1453.

Parichatnon, S., Maichum, K. and Peng, K. C. (2017). Application of the malmquist productivity index on measurement of productivity trend of durian production in Thailand. International Journal of Business Marketing and Management 2:1-9.

Robideau, G. P., De Cock, A. W., Coffey, M. D., Voglmayr, H., Brouwer, H., Bala, K., Chitty, D. W., Desaulniers, N., Eggertson, Q. A., Gachon, C. M. M., Hu, C. H., Kupper, F. C., Rintoul, T. L., Sarhan, E., Verstappen, E. C. P., Zhang, Y., Bonants, P. J. M., Ristaino, J. B. and Levesque, C. A. (2011). DNA barcoding of Oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Molecular Ecology Resources 11:1002-1011.

Santoso, P. S., Aryantha, I. N. P., Pancoro, A. and Suhandono, S. (2015). Identification of *Pythium* and *Phytophthora* associated with durian (*Durio* sp.) in Indonesia: their molecular and morphological characteristics and distribution. Asian Journal of Plant Pathology 9:59-71.

Schroeder, K. L., Martin, F. N., De Cock, A.W. A. M., Levesque, C. A., Spies, C. F. J., Okubara, P. A. and Paulitz, T. C. (2013). Molecular detection and quantification of *Pythium* species: evolving taxonomy, new tools, and challenges. Plant Disease 97:4-20.

Singh, V., Pande, P. C. and Jain D. K. (2008). Text book of botany diversity of microbes and cryptogams. 4. New Delhi: Rastogi Publications.

Spies, C. F. J., Mazzola, M. and McLeod, A. (2011). Characterisation and detection of *Pythium* and *Phytophthora* species associated with grapevines in South Africa. European Journal of Plant Pathology 131:103-119.

Uzuhashi, S., Tojo, M. and Kakishima, M. (2010). Phylogeny of the genus *Pythium* and description of new genera. Mycoscience 51:337-365.

Vawdrey, L. L., Langdon, P. and Martin T. (2005). Incidence and pathogenicity of *Phytophthora palmivora* and *Pythium vexans* associated with durian decline in far northern Queensland. Australasian Plant Pathology 34:127-128.

Vawdrey, L. L., Martin, T. M. and De Faveri, J. (2005). A detached leaf bioassay to screen durian cultivars for susceptibility to *Phytophthora palmivora*. Australasian Plant Pathology 34:251-253.

White, T. J., Bruns, T., Lee, S. and Taylor, J. (1990). PCR protocols: a guide to methods and applications. San Diego: Academic Press.

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