First report of pineapple root rot caused by *Pythium graminicola* in Thailand

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Isolates of *Pythium graminicola* Subramaniam were isolated from soil samples taken from the rhizosphere of pineapple cv. Pattavia (*Ananas comosus* Merr) showing symptom of root rot from pineapple plantations in Thailand. They were characterized by filamentous, inflated sporangia and plerotic oospores with usually 1-7 antheridia. The ITS region of the ribosomal nuclear DNA was amplified and sequenced for identification and building a phylogenetic tree to confirm the species. *P. graminicola* isolate T25 was proved to be pathogenic isolate of pineapple root rot. This is the first report of *P. graminicola* causing pineapple root rot in Thailand.

Key words: *Pythium graminicola*, pineapple root rot, ITS region, Phylogenetic tree, Pathogenicity

Introduction

Pineapple (*Ananas comosus* Merr.) is one of the most important economic plants in Thailand. It is grown mainly for fresh, canned fruits and juice, and is the only source of bromelain, an enzyme used in pharmaceuticals. Thailand is one of the ten leading exporters of processed pineapples. In 2006, Thailand exported fresh fruits and processed pineapple to Europe, America and Japan over 800 thousand tons, the export value was about 20 billion baht (National Food Institute. 2007). The growth area of pineapple in Thailand has been expanded because the increased worldwide demand for pineapple products has greatly stimulated plantings. Root rot symptoms are commonly found in the fields and become major losses in pineapple plantations. Root rot of pineapple in Thailand has been firstly reported by Leelasetthakul (1972) which caused by *Phytophthora parasitica* and also reported by other researchers

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(Kueprakone *et al*.,1977; Silayoy, 1987; Department of Agriculture, 2008). But in 2008, Department of Agriculture, Ministry of Agriculture and cooperatives, Thailand reported that pineapple root rot caused by *Pythium* spp. but it was not identified into species level. However, *Pythium graminicola* was reported to be pathogenic to pineapple root rot in Hawaii (van der Plaats-Niterink, 1981) and *Pythium arrhenomanas* has been reported for the most common species that infected to pineapple (Rohrbach and Apt, 1993).

The genus *Pythium* belongs to Oomycetes which was discovered by Pringsheim in 1858 (Martin, 1991). It is often known as a pathogen causing root rot and damping-off disease. The taxonomy of the genus *Pythium* is mainly based on the morphological descriptions and the keys provided by Middleton (1943), Waterhouse (1968) and van der Plaats-Niterink (1981). However, morphological observations are now being supplemented with molecular techniques such as PCR and sequencing. The internal transcribed region (ITS) of the ribosomal nuclear DNA and the nucleotide sequence of this region has become a useful tool in fungal taxonomy and it is currently used to identify different species of *Pythium* (Singh *et al*., 2003; Paul, 2001; Nechwatal *et al*., 2005).

The objective of this study was to identify and confirm *Pythium graminicola* causing root rot of pineapple cv. Pattavia based on morphology and molecular phylogeny.

Materials and Methods

Isolation and morphological study

Soil samples were collected from the rhizosphere of pineapple plants showing typical symptoms of root rot during the rainy season. The samples were kept in individual clean plastic bags, brought to the laboratory at Department of Plant Pest Management Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand for isolation to pure culture.

Isolation method was modified from Nechwatal *et al*. (2005)**.** Soil samples were mixed with sterile water (1/4 w/v) and baited with leaflets of pineapples (1x1 cm) floated on the surface of the water. After 2-3 days incubation at room temperature, discoloured baits were blotted on sterile paper towel to remove excess water and plated onto water agar (WA). Two days later, hyphal tips growing from the baits were transferred to Potato dextrose agar (PDA) and isolated to pure culture. Cultures were stored on PDA slant with sterile paraffin oil for further identification and maintenance.

The assessment of growth rates for the isolates of *Pythium*.spp. were grown on PDA, V8A and Potato-Carrot agar (PCA) (van der Plaats-Niterink, 1981) in 90 mm Petri dishes, and incubated at room temperature (28-30°C). Hyphal growth was recorded every 24 hr for 3 days. Colony morphology was observed after incubation for 7 days at room temperature. Investigation on sporangial development was made on agar discs which cut from the edge of actively growing colony on PDA, and floated in sterile distilled water for 24 hr at room temperature. Oogonia, antheridia and oospore characteristics were determined after 3 days of incubation at room temperature on V8A and PCA. At least 30 mature oogonia/oospores were chosen at random for recording at 400 magnification with the light microscope. The morphological identification was based on the work of Waterhouse (1968) and van der Plaats-Niterink (1981).

Sequence analysis

Sequence analysis of the ITS regions of the rDNA repeats were performed to determine the phylogenetic relationship.

For DNA extraction, 33 isolates of *P. graminicola* were grown in 50 ml test tubes containing 20 ml PDB (Potato dextrose broth) at 28°C on an arbital shaker (180 rpm) for 3-10 days. The mycelium was harvested by filtration. Excess water was removed from mycelium by pressing in a paper towel. The DNA was extracted by following the protocols of Lee and Taylor (1990) with some modifications. A mycelial mat was placed in a prechilled mortar, frozen with liquid nitrogen, and ground to fine powder. Mycelial powder was suspended in 600 µl CTAB buffer (cetyltri-methyl-ammonium bromide), vortex and incubated at 65°C for 30 min, and 600 µl CIA (chloroform: isoamyl alcohol, 24:1 (v/v)) was added. The solution was incubated for 25 min on a shaking platform and centrifuged at 7,000 rpm for 5 min at 4°C. The aqueous phase (top) was transferred to a new microcentrifuge tube and repeat CIA extraction. After the second CIA was washed 300 µl isopropanal was added and mixed by inverting the tube several times. The tube was stored at room temperature, and then was centrifuged at 10,000 rpm for 10 min, the supernatant was decanted and drained on a paper towel for 30 min, the pellet was resuspended with 50 ml sterile ddH_2O.

ITS rDNA

Polymerase chain reaction (PCR) amplification of ITS1, 5.8S and ITS2 regions was performed with universal primers ITS1(TCC GTA GGT GAA

CCT GCG G) and ITS4 (TCC TCC GCT TAT TGA TAT GC) (White *et al*., 1990). The reaction was carried out in 50 µl volumes containing 1 µl genomic DNA, 3 µl $MgCl_2$, 1 µl dNTPs, o.4 µl of each primer and 1 µl *Taq* DNA polymerase in 5 µl PCR buffer. Amplifications were done with the following temperature cycling parameters: denaturation at 94°C for 3 min for first cycle and 1 min each for subsequent cycles, annealing for 30 sec at 55°C, and elongation for 1 min at 72°C. To assess the efficiency of the amplification, 5 µl PCR products were electrophoresed in a 1% agarose gel in 1xTAE buffer. The remaining volumes of the PCR amplicons were purified for DNA sequencing. Purified template DNA was sequenced by Shanghai Sangon Biological Engineering Technology & Services Co.,Ltd (Shanghai, P.R China).

In order to determine the phylogenetic relationship, sequence analysis of the ITS regions of the rDNA repeats were performed and data compared to related species retreived from GenBank (Table 1.). Isolates of *Pythium volutum* were used as outgroup. All sequence editing and aligning were carried out using BioEdit, version 7.0.2 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Sequencing data were analysed and neighbour-joining phylogenetic analyses conducted using ClustalW, version 1.83. Phylogenetic analysis was performed using PAUP* 4.0b8 (Swofford, 2001). Trees were drawn using Treeview.

Pathogenicity **test**

All isolates of *P. graminicola* were tested for pathogenicity by Koch's Postulate on detached leaves and roots of pineapple cv. Pattavia.

Pathogenicity tests on detached leaves

Pathogenicity test was carried out as described by Soytong *et al*. (2005) with some modifications. Six-month-old pot-grown pineapple was used for the assessment of the pathogenicity. Each isolate, pineapple leaves of approximately the same age (the same position on the plant) were collected, clipped on base and apex (length *ca* 10 cm), and surface-sterilized by soaking in 10% ethanol for 3 min and then each pineapple leaf was wounded with needle-pricking method (0.5 cm in length and 1 min in depth from the surface). The wounded leaves were inoculated with a plug (5 mm diameter) of tested fungal isolate which taken from the margin of an actively growing colony on PDA. The control was treated with a plug of PDA. The inoculated leaves were kept in sealed plastic boxes containing moist paper towels at room temperature (28-30°C). The lesion diameter was recorded and confirmed by re-isolation after 5 days of incubation. The experiments were designed as completely

randomized design (CRD) with four replicates and data was subjected to statistical analysis, and the variance of lesion diameter was computed, then treatment means were compared using the Duncan's Multiple Range Test (DMRT) at P=0.01.

Table 1. *Pythium* sequences from GenBank used in this study.

Pythium* species	**Host/substrate**	**Locality**	**ITS GenBank accession No.**
P. aphanidermatum	-	-	AY598622
P. aristosporum	*Triticum aestivum*	Canada	AY598627
P. aristosporum	-	-	AB160843
P. aristosporum	-	-	AB095042
P. arrhenomanes	-	-	AF330183
P. arrhenomanes	-	-	AF330181
P. arrhenomanes	-	-	AF330182
P. arrhenomanes	-	-	AJ233444
P. arrhenomanes	*Zea mays*	USA	AY858635
P. arrhenomanes	-	-	AB095039
P. arrhenomanes	-	-	AF330180
P. arrhenomanes	-	-	AF330179
P. arrhenomanes	-	-	AJ233439
P. arrhenomanes	-	-	AF330174
P. arrhenomanes	-	-	AF330178
P. graminicola	-	-	AF330173
P. graminicola	*Saccharum officinarum*	Jamaica	AY598625
P. graminicola	-	-	AY243091
P. graminicola	-	-	AF330165
P. graminicola	-	-	AF330165
P. phragmitis	*Phragmites australis*	Germay	AY594259
P. torulosum	-	-	AB095046
P. torulosum	-	-	AB160846
P. torulosum	-	-	AF330194
P. vanterpoolii	*Triticum sativum*	UK	AY598685
P. vanterpoolii	-	-	AB095043
P. vanterpoolii	-	-	AJ233461
P. volutum	-	-	AJ233464
P. volutum	*Triticum* sp. and *Hordeum* sp.	Janpan	AY598686

* ITS sequences of *Pythium* species were retrieved from GenBank

Pathogenicity tests on pineapple roots

The pathogenicity test was done by using completely randomized design (CRD) with four replicates. The experiment was carried out using the young suckers of pineapple cv.Pattavia. All isolates of *P. graminicola* were grown on

PDA in 9-cm-diameter Petri dishes. The inoculum was prepared by the method of Shang *et al*. (1999) with modifications. Seven-to ten-day-old culture was flooded with 20 ml sterile distilled water for 48 hrs and a flame sterilized glass spreader was used to rub the colony surface to dislodge the sporangia into sterile distilled water. Sporangial suspension was incubated at 20°C for 1 hr to allow the sporangia to release their zoospores. Zoospores suspension was adjusted to $1x10^6$ zoospores/ml (Chern *et al*., 1998). Pineapple suckers were stripped off the lower leaves and placed in 8 cm diameter of clear glass containing 100 ml distilled water. After 2 weeks, the plants were removed from plastic cup, and 10 roots end per plant were cut (3 mm) before inoculation. The root system of each tested plant was placed in sterile distilled water inoculated with 100 ml zoospore suspension ($1x10^6$ zoospores/ml). Control plant was placed with 100 ml sterile distilled water without inoculum. All tested plants were maintained indoor near a sunny window until root rot occurs approximately 1 week, and then removed from plastic cup. Disease severity index (DSI) was recorded by modified following scale of Ahmed *et al.* (1999) as follows: 1 = no root rot, 2= 1-25% root rot, 3=26-50% root rot, 4=51-75% root rot and 5= >76-100% root rot.

Results and Discussion

Isolation and morphological study

The 33 isolates of *P. graminicola* were isolated from rhizosphere soils of pineapple cv. Pattavia which causing root rot. All isolates were studied for their growth rate and morphology for identification.

With this, the growth rate and morphology of 10 selected isolates of *P. graminicola* were reported in this paper. They were recorded morphological characteristics by comparison to the work of van der Plaats-Niterink (1981) that is presented in Table 2. In general, colonies appeared like rosetted on PDA, radiated pattern on PCA and V8A. Appressoria commonly produced and sporangia are not observed on solid agar, but readily produced in water, showing toruliod sporangia (Fig. 1). Oogonia produced abundantly in single culture, strictly globose, smooth-walled, terminally and intercalary borne. Antheridia are usually monoclinous, often also diclinous, usually crook-necked, 1-7 per oogonia. Oospores are single, plerotic, completely filling the oogonia (Fig. 2).

Our research finding has been found that the pineapple root rot is caused by *P. graminicola* which van der Plaats-Niterink (1981) reported as the same species. But it is contradicted to previously reports of Leelasetthakul (1972), Kueprakone *et al*. (1977), Silayoy (1987) and Department of Agriculture (2008) who stated that pineapple root rot in Thailand caused by *P. parasitica*

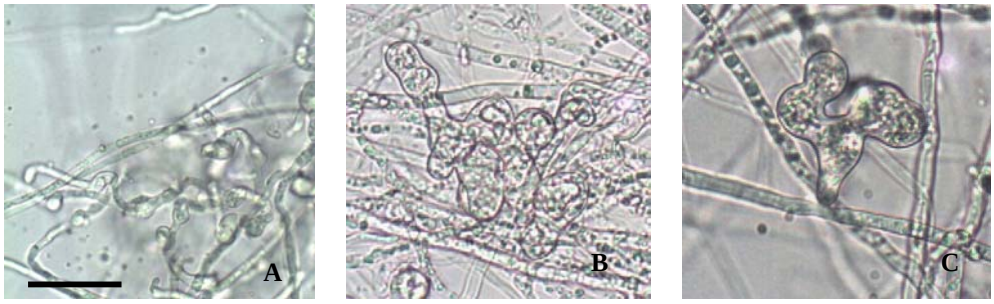

Fig 1. Appressoria and sporangia of *Pythium graminicola*.
A = Appressoria, B and C = toruloid sporangia, Bar = 30 µm.

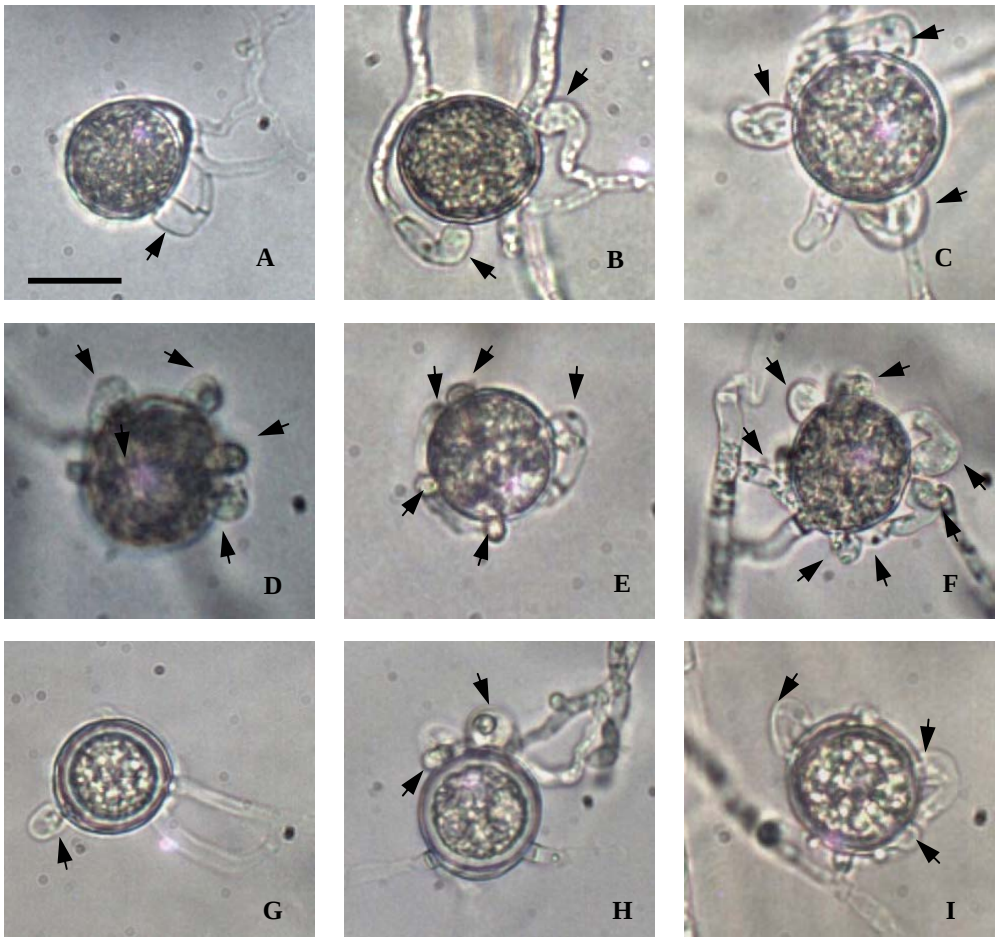

Fig. 2. Oogonia, oospores and antheridia (arrow heads) of *Pythium graminicola*.
A = Oogonia with single, monoclinous antheridia, B-F = Oogonia with two mono- or diclinous antheridia.
G-I = Oospores with one or more antheridium,. Bar=20 µm.

according to morphological studies. With this, the causing agents of pineapple root rot may possible caused by both species, *P. parasitica* and *P. graminicola*, which may depends on different in ecological diversity.

Sequence analysis

Thirty-three isolates of *Pythium* spp. were identified by morphological characters as *P. graminicola*. The isolates were studied to confirm morphological identification by using ITS sequences with the length of the complete ITS1, 5.8S and ITS2. The BLAST searches which indicated the species of *P. graminicola* that can be seen in Fig. 3. It is clearly demonstrated to identify and confirm to be *P. graminicola* as a valid identification.

Although, *P. graminicola* has been difficulty considered to separate from *P. arrhenomanas* in the past due to overlapping of morphological characters, but it could be clearly distinguished from this species by molecular evidence (Chen and Hoy, 1993). As our result, *P. graminicola* from the related species, *P. arrhenomanas*, *P. aristosporum*, *P. arrhenomanes*, *P. vanterpoolii* and *P. torulosum* which showing in Fig. 3. *Pythium* spp. isolate T3, T25 and T27 were grouped in the species of *P. graminicola*.

Therefore, morphological observation and molecular analysis should be used for identify different species of *Pythium.* This agrees with Singh *et al*.(2003), Paul (2001), Lévesque and De Cock, (2004) and Nechwatal *et al*.(2005) who have used morphology and molecular analysis for identify the different species of *Pythium*.

Pathogenicity test

Ten isolates of *P. graminicola* were proved to be pathogenic to pineapple root rot. Lesion lengths on leaves after 5 days inoculation of *P. graminicola* isolate T3, T5, T7, T9, T11, T12, T18, T25, T26 and T27 were 1.00, 0.79, 0.93, 0.98, 0.83, 1.02, 0.94, 1.40, 0.81 and 0.98, respectively. The Disease severity index on root test were 4, 2, 3, 3, 2, 4, 5, 2, 4 and 1, respectively. The isolate of *P. graminicola*, T25 was high significantly different at P=0.01 and lesion was larger than the other isolates on detached leaves. Moreover, the tested roots were faster infection in the inoculated plants. Therefore, *P. graminicola*, T25 was the most aggressive isolate both on detached leaf and root tests which disease severity index showed over 76-100% root rot (Table 3.and Fig. 4).

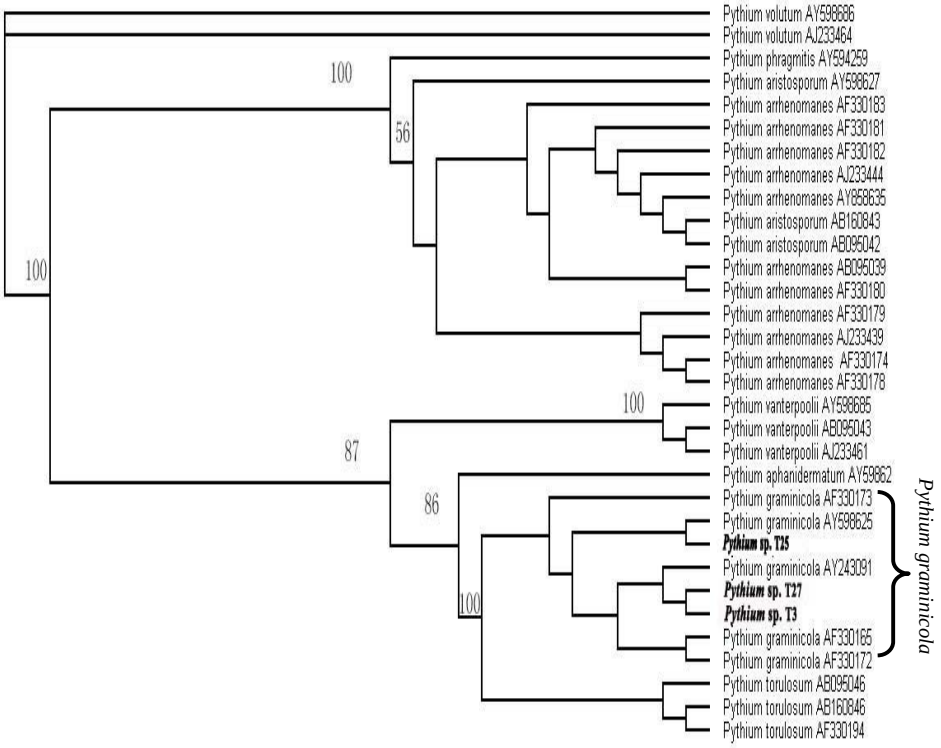

Fig. 3. Phylogenetic tree of *Pythium* species including the related species from GenBank that constructed after distance-based analysis of ITS1, 5.8S and ITS2 regions of rDNA. Numbers at the branches indicate the percentage of bootstrap values after 1,000 replications (values below 50% not shown). *Pythium volutum* was used as an outgroup.

Table 3. Virulence of *Pythium graminicola* isolates causing pineapple root rot.

Isolates	lesion length (cm)[1/]	disease severity index (DSI)[2/]
P. graminicola T3	1.00^{bc}	4
P. graminicola T5	0.79^{d}	2
P. graminicola T7	0.93^{c}	3
P. graminicola T9	0.98^{bc}	3
P. graminicola T11	0.83^{d}	2
P. graminicola T12	1.02^{b}	4
P. graminicola T18	0.94^{bc}	3
P. graminicola T25	1.40^{a}	5
P. graminicola T26	0.81^{d}	2
P. graminicola T27	0.98^{bc}	4
control	0^{e}	1

[1/] Average of four replications. Means of the lesion length of leaves followed by a common letter were not significantly different (P=0.01) by DMRT.

[2/] disease severity index of pineapple root rot, 1 = no root rot, 2= 1-25% root rot, 3=26-50% root rot, 4=51-75% root rot and 5= >76-100% root rot(modified from Ahmed *et al.*,1999).

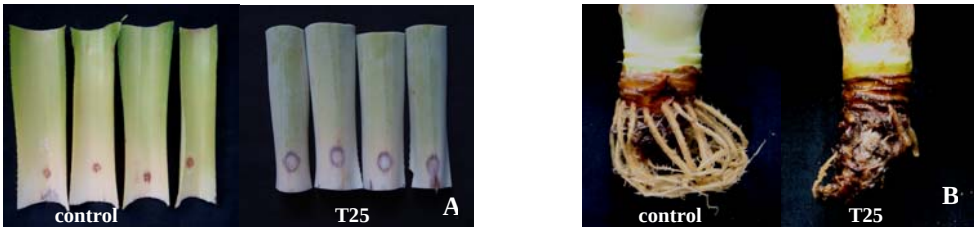

Fig. 4. Pathogenicity test on detached leaves and roots of *Pythium graminicola* T25.
A = The lesion on detached leaves after 5 days of inoculation.
B = Root rot after 1 week of inoculation.

Conclusion

P. graminicola was firstly proved to be pathogenic to pineapple cv. Pattavia causing root rot in Thailand. It was confirmed by morphology under microscopic observation and molecular phylogeny as a valid confirmation.

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References

Ahmed, A.S., Perez-Sanchez, C., Egea, C. and Candela, M.E. (1999). Evaluation of *Trichoderma harzianum* for controlling root rot caused by *Phytophthora capsici* in pepper plants. Plant Pathology. 48(1): 58-65.

Chen, W. and Hoy, J.W. (1993). Molecular and morphological comparison of *Pythium arrhenomanes* and *P. graminicola*. Mycological Research 97: 1371-1378.

Chern, L.L., Ann, P.J. and Young, H.R. (1998). Root and foot rot of loquat in Taiwan Caused by *Phytophthora*. Plant Disease. 82(6): 651-656.

Department of Agriculture. (2008). [Online]. Available : http://prachuap.doae.go.th/pineappleforprachuap/lok&malang.files/lok&malang.htm.

Kueprakone, U., Kamhangridthirong, T. and Arthan, T. (1977). Morphology and Taxonomy of *Phytophthora* spp. isolated from economic plant. Bangkok : Department of Agriculture.

Lee, S.B. and Taylor, J.W. (1990). Isolation of DNA from fungal mycelium and single spores. 284-287. in Innis, M.A. Gelfand, D.H., Sninsky, J.J and White, T.J. editors. PCR Protocols: A Guide to methods and applications. San Diego: Academic Press.

Leelasetthakul, K. (1972). Pineapple disease. Bangkok : Office of Agriculture Thailand.

Lévesque, C.A. and de Cock, A.W.A.M. (2004). Molecular phylogeny and taxonomy of the genus *Pythium*. Mycol. Res. 108(12): 1363-1383.

Martin, F.N. (1991). *Pythium*. Plant Pathology Department University of Florida, Gainesville, pp. 12.

Middleton, J. T. (1943). The taxonomy host range and geographical distribution of the genus *Pyhium*, Mem. Torrey Bot. Club. 1-171,

National Food Institute. (2007). Pineapple. [Online]. Available: http://www.nif.or.th/ stat/ pineapple.asp. (25/12/2007).

Nechwatal, J., Wielgoss, A. and Mendgen, K. (2005). *Pythium phragmitis* sp. nov., a new species close to *P. arrhenomanes* as a pathogen of common reed (*Phragmites australis*). Mycol. Res. 109(12): 1337-1346.

Paul, B. (2001). ITS region of the rDNA *of Pythium proliferatum*, a new species: its taxonomy and its comparison with related species, FEMS Microbiology Letters. 206, 191-196.

Rohrbach, G.K and Apt, J.W. (1993). [Online]. Available: Common name of plant disease. http://www.apsnet.org/online/common/names/pineappl.asp. (6/12/2007).

Shang, H., Chen, J., Handelsman, J. and Goodman, R.M. (1999). Behavior of *Pythium torulosum* zoospores during their interaction with tobacco roots and *Bacillus cereus*. Current Microbiology. 38(4): 199-204.

Silayoy, E. 1987. Disease of herb and fruit, and protection. Bangkok : Brachachonjumgud.

Singh, K. K., Mathew, R. Masih, E. I. and Paul, B. M. (2003). ITS region of the rDNA of *Pythium rhizosaccharum* sp. Nov. isolated from sugarcane roots: taxonomy and comparison with related species, FEMS Microbiology Letters. 221, 233-236.

Soytong, K., Pongnak, W. and Kasiolarn, H. (2005). Biological control of Thielaviopsis bud rot of Hyophorbe lagenicaulis in the field. Journal of Agricultural Technology. 1(2): 235-245.

Swofford, D.L. (2001). PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b8. Sunderland: Sinauer Associates.

Waterhouse, G. M. (1968). The genus *Pythium Pringsheim*, Mycological paper, pp. 110. CMI,

White, T.J., Burns, T., Lee, S.B and Taylor, J.W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315-322 in Innis, M.A. Gelfand, D.H., Sninsky, J.J and White, T.J. PCR Protocols: A Guide to methods and applications. San Diego: Academic Press.

van der Plaats-Niterink, A. J. (1981). Monograph of the genus *Pythium*, Stud. Mycol. Baarn no. 21, 1-242.

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Table 2. Morphology and growth features of *Pythium graminicola* causing pineapple root rot.

Morphology	***P. graminicola*, reference data[1/]**	***Pythium graminicola*[2/]**									
		T3	**T5**	**T7**	**T9**	**T11**	**T12**	**T18**	**T25**	**T26**	**T27**
Hyphae wide (µm)	up to 6	3.94	3.33	4.48	3.30	3.28	3.27	3.48	4.10	3.88	4.37
Sporangium shape	toruloid	toruloid	toruloid	toruloid	toruloid	toruloid	toruloid	toruloid	toruloid	toruloid	toruloid
Oogonium diam (µm)	20-25 (av. 22.30)	22.70-30.40 (av. 25.73)	23.38-29.75 (av. 24.98)	21.68-31.15 (av. 25.85)	22.30-29.58 (av. 25.90)	21.68-29.28 (av. 24.18)	23.08-29.70 (av. 25.33)	23.28-29.75 (av. 25.13)	21.68-29.58 (av. 25.47)	23.73-30.88 (av. 26.83)	22.60-31.15 (av. 26.97)
Antheridium/oogonium	1-6	1-7	1-7	1-7	1-7	1-6	1-6	1-7	1-7	1-7	1-6
Monoclinous or diclinous antheridium	mono- or diclinous antheridium	mono- or diclinous antheridium	mono or diclinous antheridium	mono or diclinous antheridium	mono or diclinous antheridium	mono or diclinous antheridium	mono or diclinous antheridium	mono or diclinous antheridium	mono or diclinous antheridium	mono or diclinous antheridium	mono or diclinous antheridium
Oospore diam (µm)	-	15.2-25.1	15.3-24.4	16.6-25.5	16.1-24.7	17.1-24.4	16.6-25.5	15.5-23.9	14.6-24.0	16.3-23.1	14.6-24.8
Oospore wall thickness (µm)	up to 3	1.6-4.7	2.0-4.8	1.6-4.5	2.1-4.8	1.7-5.9	1.4-6.1	1.7-6.0	1.6-5.8	2.0-5.2	2.1-5.6
Aplerotic or plerotic oospore	plerotic oospore	plerotic oospore	plerotic oospore	plerotic oospore	plerotic oospore	plerotic oospore	plerotic oospore	plerotic oospore	plerotic oospore	plerotic oospore	plerotic oospore
growth rate on PDA (cm)	-	3.79	4.10	3.65	3.50	3.85	3.85	3.88	4.00	3.72	3.79
colony on PDA (cm)	-	rosette	rosette	rosette	rosette	rosette	rosette	rosette	rosette	rosette	rosette
growth rate on PCA (cm)	-	2.19	1.94	2.43	2.18	2.23	1.75	1.12	1.65	1.27	1.60
colony on PCA (cm)	radiate pattern	radiate pattern	radiate pattern	radiate pattern	radiate pattern	radiate pattern	radiate pattern	radiate pattern	radiate pattern	radiate pattern	radiate pattern
growth rate on V8A (cm)	-	3.37	3.80	2.93	3.65	4.05	3.85	3.75	3.59	3.74	3.64
colony on V8A (cm)	-	radiate pattern	radiate pattern	radiate pattern	radiate pattern	radiate pattern	radiate pattern	radiate pattern	radiate pattern	radiate pattern	radiate pattern

[1/]Data from van der Plaats-Niterink (1981).
[2/] Isolates of *Pythium graminicola* isolated from the rhizosphere of pineapple were examined with at least 30 organs.