Blue mold caused by *Penicillium oxalicum* on muskmelon (*Cucumis melo*) in Thailand

Chaninun Pornsuriya^{1,2} · Ithipon Chitphithak²

Received: 25 June 2018 / Accepted: 23 November 2018 / Published online: 28 November 2018
© Australasian Plant Pathology Society Inc. 2018

Abstract

Fruit rot infected with blue mold was found on muskmelon growing in a polytunnel located at Songkhla province southern Thailand in 2018. The blue mold fungus isolated from the infected fruit was identified as *Penicillium oxalicum* based on morphological characteristics and DNA sequences of the internal transcribed spacer (ITS) and the large subunit (*LSU*) regions of ribosomal DNA, and the calmodulin (*CaM*) gene region. Pathogenicity tests showed that *P. oxalicum* isolate was pathogenic to healthy muskmelon and Koch's postulates were satisfied by re-isolating the pathogen. This is a first report of muskmelon fruit rot caused by *P. oxalicum* in Thailand.

Keywords *Penicillium oxalicum* · Fruit rot · Muskmelon · Thailand

Cucumis melo var. *reticulatus*, belonging to the Cucurbitaceae family, is commonly called muskmelon or cantaloupe. The shape is round, with light green skin and a light brown reticulated rind, and with green or orange pulp. Recently, muskmelon has become popular among the consumers and economically important in Thailand. Consumers prefer this fruit for its texture, sweetness, aroma, and it is a rich source of phytonutrients such as ascorbic acid, carotene, folic acid and potassium (Menon and Ramana Rao 2012). Fungal diseases of cucurbits that reduce yield and quality at various fruit growing stages include Fusarium wilt caused by *Fusarium oxysporum* f. sp. *melonis* (Oumouloud et al. 2013), downy mildew caused by *Pseudoperonospora cubensis* (Savory et al. 2011), powdery mildew caused by *Golovinomyces cichoracearum* and *Podosphaera xanthii* (Aguilar et al. 2012), and gummy stem blight caused by *Stagonosporopsis cucurbitacearum* (syn. *Didymella bryoniae*) (Wolukau et al. 2007; Nuangmek et al. 2018). Furthermore, yellow leaf disease of muskmelon caused by *Tomato leaf curl virus* was reported in Thailand (Samretwanich et al. 2000).

In January 2018, both young and mature muskmelon fruit showing symptoms of blue mold were found in a polytunnel in Hatyai city, Songkhla, Thailand. After fruit set, the female flower still attached to the base of fruit was infected first, and the fungal mycelia then colonized the fruit tissues. The infected fruit showed water soaked spots at the bottom and the lesion later extended to the upper part of the fruit. Bluish green sporulation was abundantly visible on the lesion (Fig. 1a, b). The infected fruit rotted completely, causing severe yield losses. Rotted muskmelons were collected and fungal spores were directly plated on to water agar (WA) and incubated at room temperature (28–30 °C). A hyphal tip was transferred to potato dextrose agar (PDA) and incubated at room temperature for 7 days. A ten-fold serial dilution was done for single spore isolation. Since *Penicillium* spp. are not common pathogens on muskmelon, the objective was to identify the *Penicillium* sp. causing blue mold fruit rot of muskmelon, and to determine its pathogenicity by artificial inoculations made on detached healthy fruit.

Colonies on PDA were circular or elliptical, with even margins, attaining a diameter of 4.0 cm within 7 days at room temperature. Colony surfaces were flat and velvety in texture. Colony centers were blue-green fading to white towards the colony margins (Fig. 1c, d). Conidiophores were monoverticillate, or biverticillate and asymmetrical. Phialides were cylindrical (Fig. 1e, f). Conidia were consistently elliptical and smooth, measuring $2.7\text{--}3.9 \times 4.1\text{--}5.6 \mu\text{m}$ as observed with the light microscope (Fig. 1g). Based on these morphological features, the isolates from muskmelon were identified as *Penicillium*

✉ Chaninun Pornsuriya
chaninun.p@psu.ac.th

¹ Pest Management Biotechnology and Plant Physiology Laboratory, Prince of Songkla University, Hatyai, Songkhla 90110, Thailand

² Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University, Hatyai, Songkhla 90110, Thailand

Fig. 1 Blue mold rot caused by *Penicillium oxalicum* on muskmelon: **a** water soaked lesion on the young infected fruit, **b** sporulating lesion on mature fruit. Pathogen images: 7 day-old culture on PDA at 28–30 °C (**c**, top view; **d**, bottom view), **e–f** conidiophores, and **g** conidia in chain formation. The scale bars represent 20 μ m

oxalicum, in the *Penicillium* section *Lanata Divaricata*. A culture of the fungal pathogen was deposited in the Culture Collection of Pest Management Department, Faculty of Natural Resources, Prince of Songkla University (PSU), Thailand, with accession number PSU-PM-CM01.

To confirm the species identification, the isolate was cultured on PDA at room temperature for 3 days for DNA extraction. Fungal genomic DNA was extracted from mycelia by the mini preparation method (Saitoh et al. 2006). The internal transcribed spacer (ITS1–5.8S–ITS2), and parts of the large subunit (*LSU*) and the calmodulin (*CaM*) gene were amplified using

BIO-RAD T100™ Thermal Cycler (BioRad, Hercules, CA, USA). Amplification of the gene region used the primer pairs ITS1/ITS4 (White et al. 1990) for ITS, LROR/LR5 (Vilgalys and Hester 1990) for *LSU*, and CMD5/CMD6 (Hong et al. 2006) for *CaM*. The PCR was performed in 25 μ l of reaction mixture containing 10 pmol of each primer, 2 $\times$ Dreamtaq Green PCR Master Mix (Thermo Scientific) and 50 ng of DNA template. An initial denaturation step for 3 min at 95 °C was followed by 35 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 50 °C, and extension for 1 min at 72 °C, with a final extension step of 10 min at 72 °C for all

Fig. 2 Maximum likelihood (ML) phylogenetic tree of *Penicillium oxalicum* including related species from GenBank, based on rDNA ITS sequences. *Penicillium glabrum* is used as the outgroup. ML bootstrap support $\geq 80\%$

primer pairs. The PCR products were visualised by agarose gel electrophoresis. The ITS, *LSU* and *CaM* gene regions were sequenced at Macrogen Korea (Seoul). Using BLAST analyses, the sequences obtained were compared with known sequences available in Genbank (The National Center of Biological Information). The BLAST search revealed that the sequences sampled from the isolate PSU-PM-CM01 had a 100% match with *P. oxalicum* WJF44 (KU877612, rice rhizosphere soil, China) for the ITS regions, and 99% with *P. oxalicum* TJM-F1 (MH169231, unknown source, China) and *P. oxalicum* HP7-1 (KP284553, soil, China) for *LSU* and *CaM* gene regions, respectively. These sequences have been deposited in GenBank with accession numbers LC386215,

LC386316 and LC386216 for ITS, *LSU* and *CaM*, respectively. For phylogenetic analyses, nucleotide sequences of the ITS regions were aligned with sequences of ex-type cultures from closely related taxa (*Penicillium* section *Lanata Divaricata*) (Visagie et al. 2015) using MUSCLE of the MEGA v. 7.0 (Kumar et al. 2016) and manual adjustments were applied when necessary. The alignments were submitted to TreeBase (submission ID <http://purl.org/phylo/treebase/phyloids/study/TB2:S22860>). Further, the data were analysed using the maximum likelihood (ML) with partial deletion of gaps and 1000 bootstrap replicates. According to the phylogenetic relationships, our *P. oxalicum* isolate (PSU-PM-CM01) from *C. melo* var. *reticulatus* was clearly confirmed as *P. oxalicum*

closely related to *P. oxalicum* CBS 219.30 (ex-type) *P. oxalicum* CBS 173.81 and *P. oxalicum* CV 822 (Fig. 2).

For pathogenicity tests, detached healthy muskmelon fruit (10-day-old) were surface sterilized by immersing the fruit in 70% ethanol for 3 min and then rinsing two times in sterile distilled water and drying with sterilized tissue paper. The flowering end of the fruit were sprayed with a spore suspension of *P. oxalicum* (1×10^6 conidia/ml) prepared from 10-day-old colonies of the isolates. The control treatment was sprayed with sterilized distilled water. Four replicate fruit were tested per treatment and the experiment was carried out two times. All fruit were placed in moist plastic box and then kept in a polytunnel (30–32 °C, 60% relative humidity) for 2 weeks. Water-soaked lesions appeared on fruit 7 days after inoculation, and these then rotted within 14 days. The fungal pathogen was re-isolated and its morphology matched *P. oxalicum*.

Plant disease caused by *P. oxalicum* has been reported on many host plants: for example, stem and fruit rot of cucumber (*Cucumis sativa*) in Canada (Jarvis et al. 1990) and tomato stem and fruit rot in Mexico (Picos-Munoz et al. 2011). Blue mold fruit rot caused by *P. oxalicum* on melon has been reported in Korea (Kwon et al. 2002) but the variety was different (*C. melo* cv. *gayabaegja*). There have been no reports of *P. oxalicum* causing blue mold fruit rot on muskmelon in Thailand or anywhere else, so this is the first such report. Consequently, further research is required to find efficacious methods to manage the disease.

Acknowledgements The authors would like to thank the Prince of Songkla University for funding and facilities, and Mr. Diregrit Plyduang for providing a photograph of blue mold rot on maturing muskmelon fruit. The copy-editing service of RDO/PSU and the helpful comments of Assoc. Prof. Dr. Seppo Kärtilä are gratefully acknowledged.

References

- Aguiar BM, Vida JB, Tessmann DJ, de Oliveira RR, Aguiar RL, Alves TCA (2012) Fungal species that cause powdery mildew in greenhouse-grown cucumber and melon in Paraná state, Brazil. *Acta Scientiarum Agronomy* 34:247–252

- Hong SB, Cho HS, Shin HD, Frisvad JC, Samson RA (2006) Novel *Neosartorya* species isolated from soil in Korea. *Int J Syst Evol Microbiol* 56:477–486
- Jarvis WR, Barrie SD, Traquair JA, Stoessl A (1990) Morphological and chemical studies of *Penicillium oxalicum*, newly identified as a pathogen on greenhouse cucumbers. *Can J Bot* 68:21–25
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874
- Kwon JH, Kang SW, Kim JS, Park CS (2002) Blue mold on melon (*Cucumis melo*) caused by *Penicillium oxalicum*. *Research in Plant Disease* 8:220–223
- Menon SV, Ramana Rao TV (2012) Nutritional quality of muskmelon fruit as revealed by its biochemical properties during different rates of ripening. *Int Food Res J* 19:1621–1628
- Nuangmek W, Aiduang W, Suwannarach N, Kumla J, Lumyong S (2018) First report of gummy stem blight caused by *Stagonosporopsis cucurbitacearum* on cantaloupe in Thailand. *Can J Plant Pathol* 40:306–311
- Oumouloud A, El-Otmami M, Chikh-Rouhou H, Claver AG, Torres RG, Perl-Treves R, Alvarez JM (2013) Breeding melon for resistance to fusarium wilt: recent developments. *Euphytica* 192:155–169
- Picos-Munoz PA, Garcia-Estrada RS, Carrillo-Fasio JA, Leon-Felix J, Allende-Molar R (2011) First report of blue mold caused by *Penicillium oxalicum* in tomato (*Solanum lycopersicum*) in Mexico. *Plant Dis* 95:1195
- Saitoh KI, Togashi K, Arie T (2006) A simple method for a mini-preparation of fungal DNA. *J Gen Plant Pathol* 72:348–350
- Samretwanich K, Chiemsombat P, Kittipakorn K, Ikegami M (2000) Yellow leaf disease of muskmelon from Thailand caused by *Tomato leaf curl virus*. *Plant Dis* 84:707
- Savory EA, Granke LL, Quesada-Ocampo LM, Varbanova M, Hausbeck MK, Day B (2011) The cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. *Mol Plant Pathol* 12:217–226
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically ribosomal DNA from several *Cryptococcus* species. *J Bacteriol* 172:4238–4246
- Visagie CM, Houbraken J, Seifert KA, Samson RA, Jacobs K (2015) Four new *Penicillium* species isolated from the fynbos biome in South Africa, including a multigene phylogeny of section *Lanata-Divariata*. *Mycol Prog* 14:96
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetic. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a guide to methods and applications*. Academic, San Diego, pp 315–322
- Wolukau JN, Zhou XH, Li Y, Zhang YB, Chen JF (2007) Resistance to gummy stem blight in melon (*Cucumis melo* L.) germplasm and inheritance of resistance from plant introductions 157076, 420145, and 323498. *HortScience* 42:215–221