The Korean Journal of Mycology www.kjmycology.or.kr

RESEARCH NOTE

Occurrence of Tan-brown Leaf Spot Caused by *Pilidium concavum* on *Fragaria ananassa* in Korea

Mi-Jeong Park[1]*, Chang-Gi Back[1], Jong-Han Park[1], Kyung-Sook Han[2]

[1]Horticultural and Herbal Crop Environment Division, National Institute of Horticultural and Herbal Science, Wanju 55365, Korea
[2]Urban Agricultural Research Division, National Institute of Horticultural and Herbal Science, Wanju 55365, Korea

*Corresponding author: mijpark@korea.kr

Abstract

In 2015, a new leaf spot disease was observed on strawberry seedlings in Wanju, Korea. Tanned brown spots appeared on the leaves of the infected plants, and often coalesced to form larger necrotic areas, resulting in the death of foliage. An isolate was obtained in pure culture. On the basis of morphological characteristics and molecular analysis of internal transcribed spacer rDNA sequence, the causal agent was identified as *Pilidium concavum*. Pathogenicity tests revealed the isolate was pathogenic to the leaves and fruits of strawberry. To the best of our knowledge, this is the first report of *P. concavum* causing tan-brown leaf spot on strawberry in Korea.

Keywords: *Fragaria ananassa*, *Pilidium concavum*, Strawberry, Tan-brown leaf spot

Kor. J. Mycol. 2017 December, 45(4): 377-380
https://doi.org/10.4489/KJM.20170044

pISSN : 0253-651X
eISSN : 2383-5249

Received: 30 October, 2017
Revised: 2 November, 2017
Accepted: 3 November, 2017

Strawberry (*Fragaria ananassa* Duch.) is one of the most economically important fruits in Korea. In November 2015, a leaf spot symptom was found on strawberry seedlings in a farm located in Wanju, Korea. Tanned brown spots appeared on the leaves of strawberry seedlings left out in the field after planting (Fig. 1A, 1B). The severely affected leaves withered and finally died. Approximately 1%~3% of seedlings showed these symptoms.

A fungal isolate was obtained from the diseased leaves using the single hyphal tip method. One pure culture (Fig. 2I) was deposited in the Korean Agricultural Culture Collection under the accession number KACC48095. A colony of the fungus on potato dextrose agar (PDA) attained 90 mm diameter at 23°C (optimal temperature) in three weeks, and was beige to buff, slimy, concentric, and consisting of sparse aerial mycelia, with an entire margin. Morphological characteristics were observed and measured from the fungal structures that were produced on fruits inoculated with the isolate. Morphologically, conidiomata were disk-shaped, flattened, brown to dark brown, sometimes blackish, and 0.2~1 mm in size (Fig. 2A~2E). Conidiophores were hyaline, unbranched or branched, and

up to 62.5~87.5 μm in length and 1.8~2.0 μm in width (Fig. 2F, 2G). Conidia were hyaline, aseptate, allantoid, and 5~9 μm in length and 1.7~2.5 μm in width (Fig. 2H). The morphological features of the causal fungus were consistent with the description of *Pilidium concavum* (Desm.) Hönh [1].

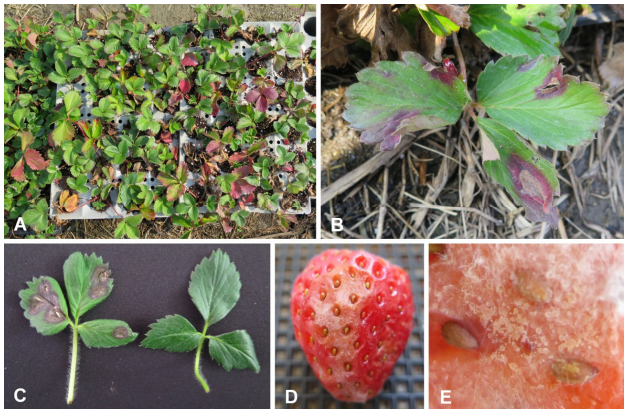

Fig. 1. Symptoms associated with *Pilidium concavum* on strawberry. A, Diseased seedlings thrown out on one side of the field; B, Close-up of leaf spot lesions on strawberry leaf; C, Similar symptoms (left) appearing on strawberry leaf after seven days of inoculation, compared with the untreated control (right); D, Symptoms occurring on strawberry fruit; E, Immature conidiomata formed on lesions of strawberry fruit.

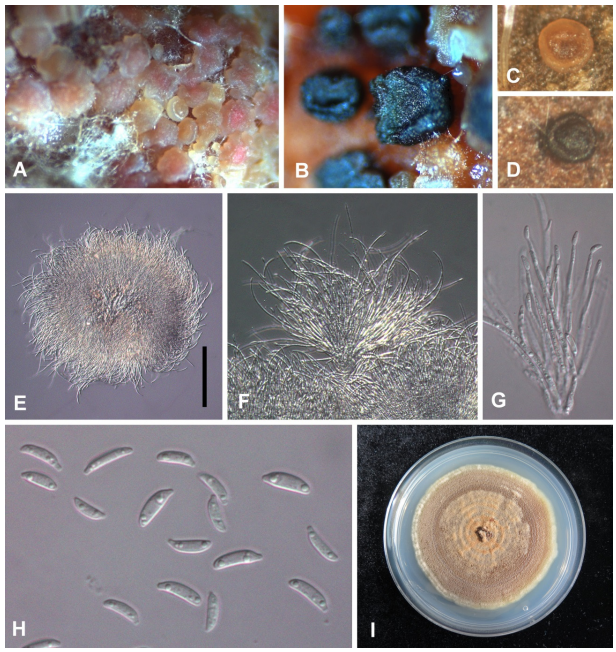

Fig. 2. Morphological and cultural features of *Pilidium concavum* causing tan-brown leaf spot on strawberry. A, Disk-shaped, flattened, brown to dark brown conidiomata; B, Blackish conidiomata; C, D, Close-up of conidiomata; E, Conidiomata as seen under a differential interference contrast (DIC) microscope; F, G, Conidiophores; H, Conidia; I, Colony grown on potato dextrose agar after incubation for 2 weeks (scale bars: E = 100 μm, F = 50 μm, G = 20 μm, H = 10 μm).

Genomic DNA was extracted from mycelia scraped from the surface of agar plates. The internal transcribed spacer (ITS) rDNA region was amplified using the primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) [2]. The PCR products were purified and directly sequenced by BIOFACT (Daejeon, Korea). The raw DNA sequence data for *P. concavum* was edited using SeqMan program (DNASTAR, Madison, WI, USA). The resulting sequence has been submitted to GenBank (accession number MG212519). Phylogenetic relationships between *P. concavum* and related species were inferred from ITS rDNA sequences, including five reference sequences of *Pilidium* spp. retrieved from GenBank. *Chaetomella zambiensis* (CPC 22465) was used as an outgroup. A phylogenetic tree was constructed by MEGA6 [3] using the neighbor-joining method with the Tajima-Nei model. A BLAST search of the resulting sequence showed 100% similarity with the sequences of *P. concavum* in GenBank. In the neighbor-joining tree (Fig. 3), the present isolate positioned into a clade comprising the *P. concavum* reference isolates. It was also confirmed that *P. concavum* is further related to *P. pseudoconcavum* [4], with a high value of 94%. The molecular phylogenetic analysis verified the identity of the present isolate.

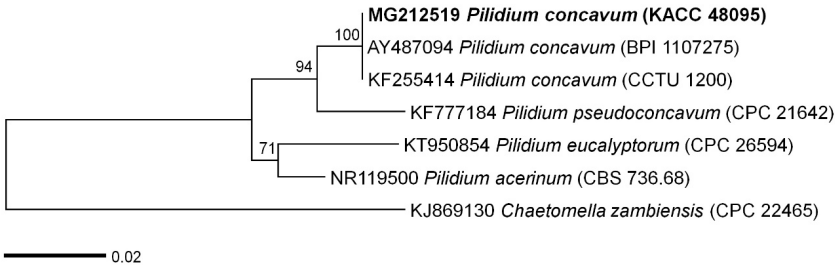

Fig. 3. Neighbor-joining tree based on the internal transcribed spacer rDNA sequence dataset, showing a phylogenetic affinity of the Korean isolate (in boldface) of *Pilidium concavum* with other *Pilidium* species. *Chaetomella zambiensis* was designated as the outgroup. Bootstrap values above 70% were shown at the nodes. The scale bar represents 0.02 nucleotide substitutions per site.

To fulfill Koch's postulates, the pathogenicity of the isolate was tested using leaves and fruits of strawberry. The inoculum was prepared by harvesting mycelial mats and conidia from seven-day-old cultures grown on PDA. Leaves of five plants, some wounded by a needle, and some left intact, were sprayed with hyphal and conidial suspensions. Five plants spray-inoculated with sterile water served as controls. The fungal suspensions were also sprayed on the surface of five non-wounded fruits. Five healthy fruits sprayed with sterile water served as controls. After inoculation, the plants were maintained in a growth chamber at 23°C for one week. Wounded leaves developed typical symptoms of tan-brown leaf spots after seven days of inoculation (Fig. 1C). On the surfaces of the inoculated fruits, conidiomata were abundantly produced (Fig. 1D, 1E). Control plants remained symptomless.

The fungus was consistently re-isolated from the lesions after inoculation, which proved that the isolate of *P. concavum* is pathogenic to strawberry.

Pilidium concavum exhibits a broad host spectrum that infects diverse crops and wild plants worldwide [5]. It has been reported to occur on the leaves and fruits of strawberry in Poland [6], Brazil [7], Belgium [8], China [9], the USA [10], and Iran [11]. However, there has been no previous record of strawberry associated with *P. concavum* in Korea. To our knowledge, this is the first report on tan-brown leaf spot pathogen on strawberry seedlings in Korea.

Acknowledgements

This work was carried out with the support of the "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01136802)" Rural Development Administration, Republic of Korea.

REFERENCES

1. Palm ME. Taxonomy and morphology of the synanamorphs *Pilidium concavum* and *Hainesia lythri* (Coelomycetes). Mycologia 1991;83:787-96.
2. White TJ, Bruns TD, Lee SB, Taylor JW. Amplification and direct sequencing of fungus ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. San Diego: Academic Press; 1990. p.315-22.
3. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 2013;30:2725-9.
4. Crous PW, Wingfield MJ, Guarro J, Cheewangkoon R, van der Bank M, Swart WJ, Stchigel AM, Cano-Lira JF, Roux J, Madrid H, et al. Fungal Planet description sheets: 154-213. Persoonia 2013;31:188-296.
5. Farr DF, Rossman AY. Fungal databases, U.S. National Fungus Collections, ARS, USDA [Internet]. Beltsville: Systematic Mycology and Microbiology Laboratory; 2016 [cited 2017 Oct 15]. Available from: http://nt.ars-grin.gov/fungaldatabases/.
6. Gołębniak B, Jarosz A. First report of tan-brown rot (*Hainesia lythri*) on strawberry fruits in Poland. Phytopathol Pol 2004;31:57-60.
7. Lopes UP, Zambolim L, Lopes UN, Pereira OL, Costa H. First report of *Pilidium concavum* causing tan-brown rot in strawberry fruits in Brazil. Plant Pathol 2010;59:1171-2.
8. Debode J, van Hemelrijck W, Heungens K, Maes M, Creemers P. First report of *Pilidium concavum* causing tan-brown rot on strawberry fruit in Belgium. Plant Dis 2011;95:1029.
9. Geng WL, Hu P, Ma Z, Zhao XY, Wei YM. First report of *Pilidium concavum* causing tan-brown leaf spot on strawberry in China. Plant Dis 2012;96:1377.
10. Fernández-Ortuño D, Bryson PK, Schnabel G. First report of *Pilidium concavum* causing tan-brown rot on strawberry nursery stock in South Carolina. Plant Dis 2014;98:1010.
11. Ayoubi N, Soleimani MJ, Zare R. *Pilidium concavum*, causing tan-brown rot on strawberry in Iran. J Plant Pathol 2016;98:667-9.