

First Report of Root Rot Caused by *Plectosphaerella cucumerina* on Cabbage in China

Pan-Liang Li[§], A-Li Chai[§], Yan-Xia Shi, Xue-Wen Xie and Bao-Ju Li*

Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, China

Abstract Severe root rot was observed in fields of cabbages (*Brassica oleracea* L.) in 2015 in China. Cardinal symptoms of this disease included root rot and wilting leaves. A fungus was isolated from diseased tissues consistently. Based on the morphological features and molecular analysis of the ITS-5.8S rDNA and D1/D2 domain of the 28S rRNA gene, it was identified as *Plectosphaerella cucumerina*. This is the first report of *P. cucumerina* causing cabbage root rot in China and the world.

Keywords Cabbage, D1/D2, ITS, Pathogenicity, *Plectosphaerella cucumerina*, Root rot

Cabbage, *Brassica oleracea* L., belonging to the Brassicaceae family, is one of the most economically important vegetable crops in the world. China is the country with rich species of Brassicaceae all over the world and with a history of more than 500 years for cabbage cultivation. The world's total production of cabbage and other cruciferous vegetables was 70.1 million tons in 2012. China is the largest country in cabbage production, with 32.8 million tons produced, which is 47% of the world's output [1]. Furthermore, cabbages have medicinal value in traditional Chinese medicine. It can improve glucose metabolism and prevent cancer. The cabbages are well known in Korea and China for their great nutritional value and medicinal applications. Several fungi have been known as pathogens of cabbages worldwide. These include *Alternaria* leaf spot (*Alternaria alternata*, *A. brassicae*, and *A. brassicicola*) [2], anthracnose (*Colletotrichum capsici*) [3], *Fusarium* wilt (*Fusarium oxysporum*) [2, 4] and so on. However, cabbage root rot was also an important infection limiting factor of the productivity of the plants. It

would be worth taking notice of the pathogen causing cabbage root rot.

In October 2015, root rot symptoms were observed on cabbage plants in commercial fields in Lanzhou City (103°40' E, 36°03' N), Gansu Province, China. The disease incidence on cabbage plants varied from 30% to 55% in those fields, with 40% to 60% yield loss. The infected cabbages initially showed leaf blade wilting in the afternoon every day (Fig. 1A). And it would be normal in the morning and evening. As the disease progressed, leaf stalks and roots of the infected plants were rotten, and the whole plants died (Fig. 1B).

Diseased leaf stalks and roots were collected from the infected cabbages in several fields. The typical samples were cut into 2–3 mm pieces and disinfested in 1% sodium hypochlorite (NaOCl) for 2 min, then rinsed with sterilized sterile water for 3 times and placed on potato dextrose agar (PDA) after drying. All the Petri dishes were incubated at 25°C for a 12 hr light-dark cycle for 5 days. To obtain the pure culture, each colony was purified by using a single-spore technique and transferred to a fresh PDA with the following incubation at 25°C. Specimens of cabbages were deposited in the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences for future reference. A total of 20 isolates were obtained from the diseased samples, and all the colonies were morphologically the same. One isolate (GL11101616) was selected for further morphological and molecular identification. Colonies on PDA at 25°C for 10 days were buff to salmon pink, slimy, aerial mycelium sparse, 7.5 cm in diameter (Fig. 1C). Aerial mycelium was sparse, hyaline, septate, branched, and sometimes forming hyphal coils (Fig. 1D). Conidia were hyaline, smooth, thin-walled, elliptical to ovoid, monospore, 0–1 septate, guttulate, measured 3.8–9.1 × 2.5–4.5 μm (Fig. 1E) and were aggregated in slimy heads. Conidiogenous

Mycobiology 2017 June, 45(2): 110-113
<https://doi.org/10.5941/MYCO.2017.45.2.110>
pISSN 1229-8093 • eISSN 2092-9323
© The Korean Society of Mycology

***Corresponding author**

E-mail: libaojuivf@163.com

[§]These authors contributed equally to this work.

Received March 4, 2017

Revised March 10, 2017

Accepted May 3, 2017

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

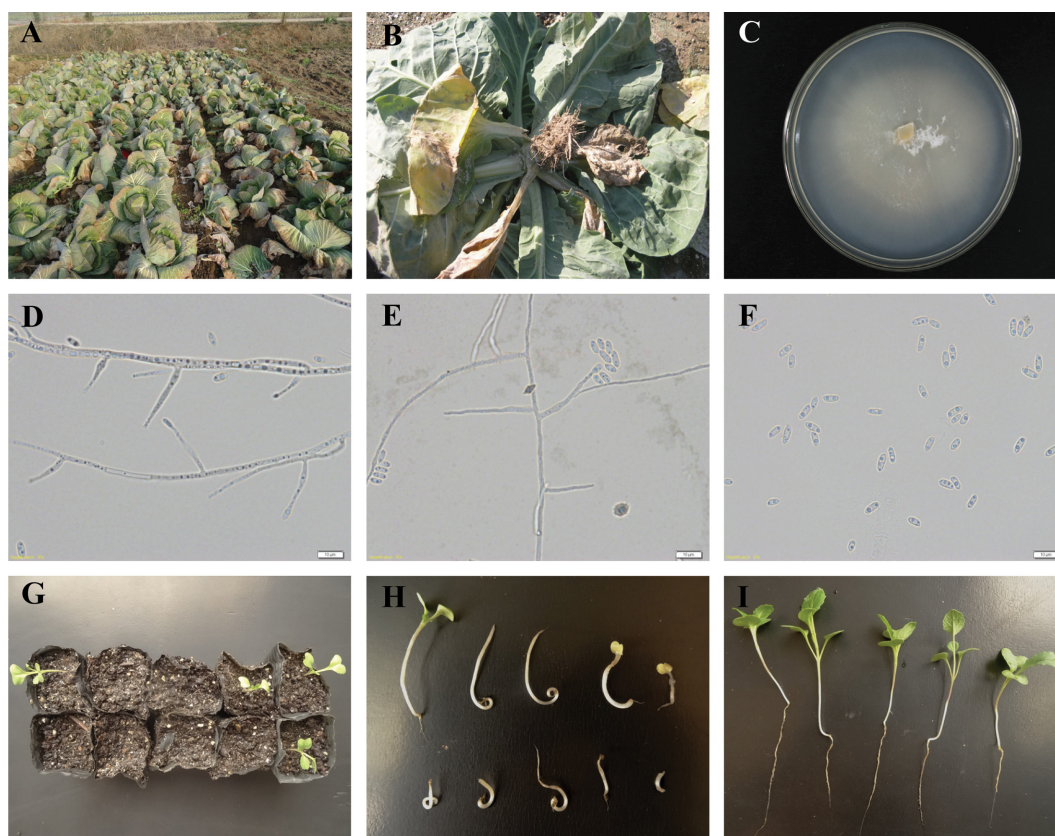


Fig. 1. Disease symptoms and morphological features of *Plectosphaerella cucumerina*. A, Root rot of cabbage plants infected by *P. cucumerina* in the field; B, Roots of cabbages turned brown and rotted, yellow lesions appeared on the leaves, and later leaf stalks decayed; C, Mycelial colony of *P. cucumerina* on potato dextrose agar at 25°C for 14 days; D, Aerial mycelium and conidiogenous cells; E, Conidiogenous cells; F, Conidia; G–I, Symptoms observed in pathogenicity tests; G, Roots of inoculated seedlings decayed and vanished, and finally the plants wilted; H, Brown lesions appeared on roots; I, No symptom was observed on the control (scale bars: D–F = 10 µm).

cells were monopodialic, hyaline, solitary, straight or crooked and 1-septate occasionally, measuring $9.9\text{--}36.9 \times 1.4\text{--}3.5$ µm (Fig. 1E). Chlamydospores were absent. Based on morphological and culture characteristics, the fungus was identified as *Plectosphaerella cucumerina* (Lindf.) W. Gams [5] (anamorph: *Plectosporium tabacinum* (J. F. H. Beyma) M. E. Palm, W. Gams & Nirenberg) [6].

To determine pathogenicity, isolate GL11101616 was used to inoculate healthy cabbages (cv. Zhonggan No. 21). The conidia suspension was prepared from 7-day-old cultures grown in potato dextrose broth on a shaker (120 rpm) at 25°C. Briefly, 20 cabbage seeds were pre-germinated on a piece of moistened filter paper in Petri dishes for 3 days and then inoculated by soaking cabbage embryonic roots (approximately 5 mm) into conidial suspensions (1×10^6 conidia/mL) for 10 min. Meanwhile, another embryonic roots of healthy cabbage seedlings were similarly dipped into sterilized water served as controls. All treated seedlings were planted in 5.0 cm diameter plastic pots containing pasteurized soil matrix. Then the plants were placed in a greenhouse at 15°C (night)/25°C (day) with natural daylight conditions. Ten days after inoculation, brown lesions appeared

on roots in all inoculated cabbages, and finally, the plants wilted. Some cotyledons and roots of cabbages decayed and vanished (Fig. 1G and 1H). The disease incidence in inoculated plants was 100%. No symptoms were observed on the control plants (Fig. 1I). *P. cucumerina* was successfully re-isolated from inoculated cabbages and was identical with the original isolates GL11101616. Thus, Koch's postulates were accomplished and it showed that *P. cucumerina* was the causal agent of the disease. The experiment was repeated twice and the results were similar.

To confirm the identification based on morphological characteristics, genomic DNA was extracted from fungal mycelium using the plant Genomic DNA Kit (Isolate Plant DNA Minikit, Tiangen, China). The internal transcribed spacer (ITS)-5.8S rDNA and D1/D2 domain of the 28S rRNA gene were amplified using primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3')/ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') [7] and NL-1 (5'-GCA TAT CAA TAA GCG GAG GAA AAG-3')/NL-4 (5'-GGT CCG TGT TTC AAG ACG G-3') [8], respectively. The PCR procedure for amplification of the ITS-5.8S rDNA region was referenced to White *et al.* [7]. For the D1/D2 domain of the 28S rRNA

gene, PCR was performed with thermocycling profile as Atkins *et al.*s [9]. PCR products were sequenced at Biomad in Beijing, China (BigDye Terminator Ready Reaction Mix v3.1; Applied Biosystems, Foster City, CA, USA). The resulting sequences were blasted in the GenBank database of National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov/BLAST/>). The obtained two sequences and downloaded relevant sequences of *Plectosphaerella* sp. were edited and aligned using MEGA ver. 5.1 software package [10]. Phylogenetic analysis based on ITS-5.8S rDNA and D1/D2 domain of the 28S rRNA gene sequences were conducted using the neighbour-joining method with a bootstrap of 1,000 replicates [11]. The sequence of *Verticillium*

dahliae was used as an out-group. BLAST analysis confirmed the identity of the fungus, showing 100% similarity to the ITS-5.8S rDNA sequence of *P. cucumerina* (KT699136), and 100% identity with the D1/D2 domain of the 28S rRNA sequence of *P. cucumerina* (JQ999955). The sequences of ITS-5.8S rDNA (562 bp) and D1/D2 domain of the 28S rRNA genes (608 bp) of the isolate GL11101616 have been submitted to NCBI database (GenBank accession Nos. KT596812 and KT596813, respectively). Phylogenetic inference based on the ITS gene region can make a good distinction between *P. cucumerina* among the other *Plectosphaerella* spp. [12]. In the phylogenetic tree, the representative isolate was highly clustered within a clade comprising reference isolates

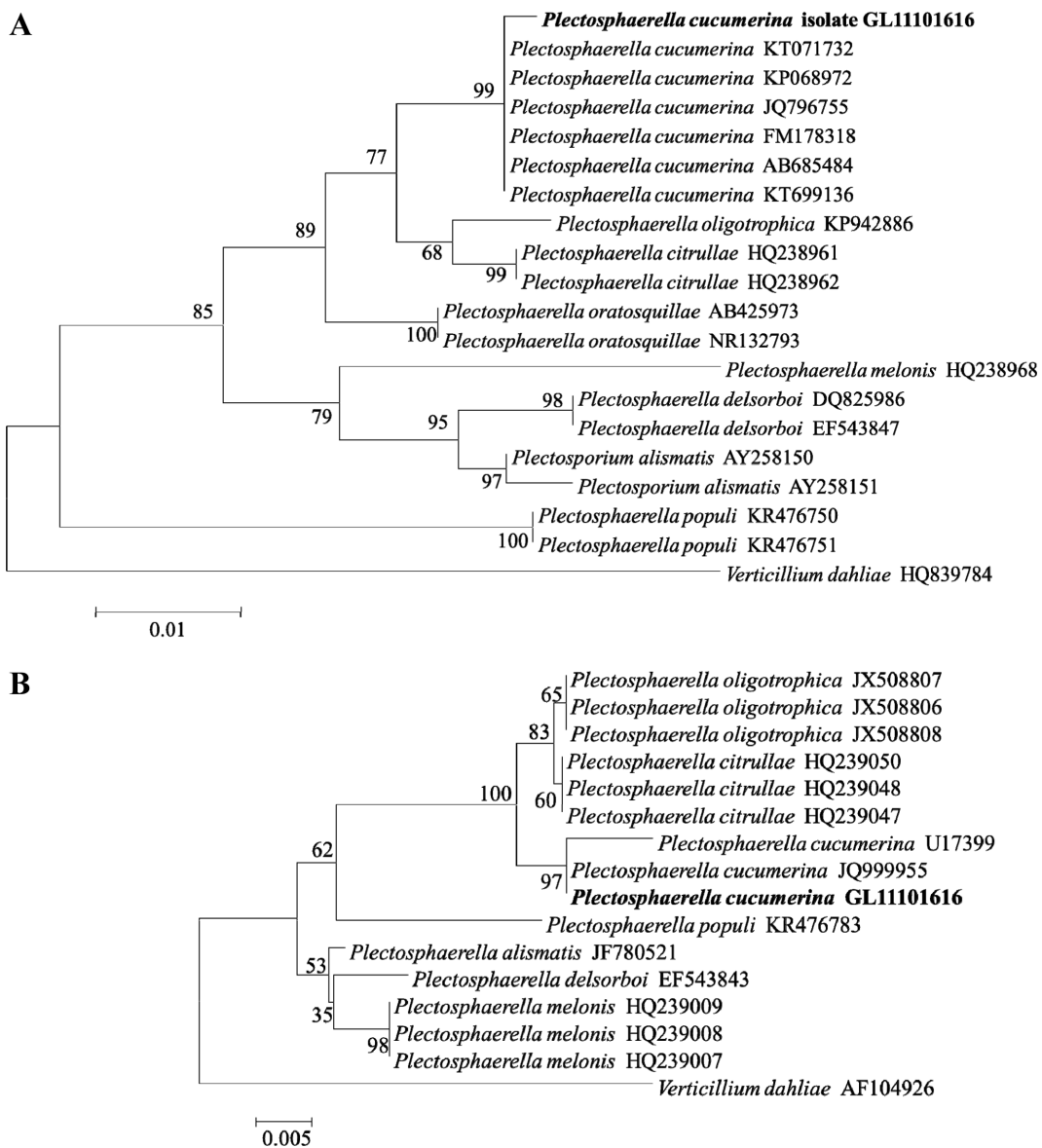


Fig. 2. Phylogenetic tree constructed with the ITS-5.8S rDNA (A) and D1/D2 domain of the 28S ribosomal DNA gene (B) sequences of *Plectosphaerella cucumerina* isolates and related species, including one isolate newly obtained from this study (in bold), and other isolates retrieved from GenBank. Bootstrap values resulting from 1,000 replicates are shown at the branch points. *Verticillium dahliae* served as the out-group. The fungal strains identified in this study are shown in boldface.

of *P. cucumerina* (Fig. 2).

P. cucumerina is a very common fungus on decayed plant material or in the soil [13]. *P. cucumerina* has been reported on many crops, such as potato, tomato, muskmelon, tobacco, soybean, pepper, and other important economic crops [13]. However, the pathogenicity of the fungus is rarely realized in agricultural production in many countries. In Korea, it was only reported as the pathogen of the wild arrowhead (*Sagittaria trifolia* L.) in 2004 [14]. In China, *P. cucumerina* was first reported on tomato in 2014 [15]. And then it was reported as a pathogen of sunflower [16] and alfalfa [17] in 2015. What's more, this is first observed that *P. cucumerina* as a pathogen of cruciferous vegetables. In fact, the host range of *P. cucumerina* should be accomplished in most species of cruciferous vegetables. The reason is that cabbages and other Brassicas are often routinely cultivated in neighboring fields in China. This practice may become one potential reason for the spread of *P. cucumerina*. Our results emphasize the importance of this disease management because of cabbages' economic value. To the best of our knowledge, this is the first report of *P. cucumerina* causing root rot on cabbage in China and the world.

ACKNOWLEDGEMENTS

This work was supported by National Key Research & Development (R&D) Plan (2016YFD0201002), Modern Agro-industry Technology Research System in China (CARS-25), the Agricultural Science and Technology Innovation Program of the Chinese Academy of Agricultural Sciences (CAAS-ASTIP-IVFCAAS) and funded by Key Laboratory of Biology and Genetic Improvement of Horticultural Crops, Ministry of Agriculture, China.

REFERENCES

1. FAO Statistics Database [Internet]. Rome: Food and Agriculture Organization of the United Nations; 2015 [cited 2015 Feb 2]. Available from: <http://www.fao.org/faostat/en/#data/QC>.
2. French AM. California Plant Disease Host Index. Sacramento (CA): California Department of Food and Agriculture; 1989.
3. Williams TH, Liu PS. A host list of plant diseases in Sabah, Malaysia. Phytopathological Papers No. 19. Kew: Commonwealth Mycological Institute; 1976.
4. Tai FL. Sylloge fungorum sinicorum. Peking: Science Press; 1979.
5. Carlucci A, Raimondo ML, Santos J, Phillips AJ. *Plectosphaerella* species associated with root and collar rots of horticultural crops in southern Italy. *Persoonia* 2012;28:34-48.
6. Palm ME, Gams W, Nirenberg HI. *Plectosporium*, a new genus for *Fusarium tabacinum*, the anamorph of *Plectosphaerella cucumerina*. *Mycologia* 1995;87:397-406.
7. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal 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 (CA): Academic Press; 1990. p. 315-22.
8. O'Donnell K. Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). *Curr Genet* 1992; 22:213-20.
9. Atkins SD, Clark IM, Sosnowska D, Hirsch PR, Kerry BR. Detection and quantification of *Plectosphaerella cucumerina*, a potential biological control agent of potato cyst nematodes, by using conventional PCR, real-time PCR, selective media, and baiting. *Appl Environ Microbiol* 2003;69:4788-93.
10. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;28:2731-9.
11. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406-25.
12. Crous PW, Wingfield MJ, Guarro J, Hernández-Restrepo M, Sutton DA, Acharya K, Barber PA, Boekhout T, Dimitrov RA, Dueñas M, et al. Fungal planet description sheets: 320-370. *Persoonia* 2015;34:167-266.
13. Abad P, Pérez A, Marqués MC, Vicente MJ, Bruton BD, García-Jiménez J. Assessment of vegetative compatibility of *Acremonium cucurbitacearum* and *Plectosphaerella cucumerina* isolates from diseased melon plants. *Bull OEPP* 2000;30:199-204.
14. Cho WD, Shin HD. List of plant diseases in Korea. 4th ed. Suwon: Korean Society of Plant Pathology; 2004.
15. Xu J, Xu XD, Cao YY, Zhang WM. First report of greenhouse tomato wilt caused by *Plectosphaerella cucumerina* in China. *Plant Dis* 2014;98:158.
16. Zhang YY, Li M, Liang Y, Zhou HY, Zhao J. First report of sunflower wilt caused by *Plectosphaerella cucumerina* in China. *Plant Dis* 2015;99:1646.
17. Wen Z, Duan T, Christensen MJ, Nan Z. *Microdochium tabacinum*, confirmed as a pathogen of alfalfa in Gansu Province, China. *Plant Dis* 2015;99:87-92.