

First report of *Alternaria alternata* causing leaf blight on *Actaea dahurica* in China

H. F. Sun^{1*}, H. Y. Yang¹, X. J. Mu², H. Wang¹, N. Li¹, Y. Yan¹, M. Y. Wei¹

*Corresponding Author: Haifeng Sun 359896987@qq.com

1. College of Pharmacy, Heilongjiang University of Chinese Medicine, Heilongjiang, 15000, China.
2. Sanofi Beijing Pharmaceutical Co., Ltd, Guangdong, 510000, China.

In July 2019, leaf blight on *Actaea dahurica*, a plant with high value in Chinese traditional medicine, was discovered in a 2 ha planting area in Heilongjiang Province (129.6°E, 44.6°N), China. Disease incidence was 90% in the field. Symptoms consisted of irregular black spots with gray margins on both sides of the leaf, often at the leaf margin, mostly on the older leaves. To isolate the pathogen, ten diseased leaves were randomly collected, surface disinfested, and 5 x 5 mm segments were removed from the margin of the lesions. Leaf segments were placed onto potato dextrose agar (PDA) and incubated at 25 °C for 7 days. Ten pure cultures with the same morphological characteristics were obtained from three leaves showing typical symptoms. Cultures on PDA initially had a cottony mycelium, white-gray to gray. After two to three weeks of growth, mycelium color changed from gray to black. Conidiophores were clustered, dark at the base, tapering to the apex, born from simple sublates, unbranched, with 1 to 5 septa, and $70.4\text{-}530.3 \times 5\text{-}7.5 \mu\text{m}$ in size. Conidia were $12.5\text{-}82.5 \times 5.2\text{-}20.3 \mu\text{m}$, usually in chains, had 2 to 8 transverse septa, 0 to 4 longitudinal or oblique septa, and a smooth brown surface. Simple, pale, vimineous or verrucous beaks developed from the apical cells with 0 to 4 septa. The morphological characteristics were consistent with *Alternaria* species (Simmons, 2007). To fulfill Koch's postulates, pathogenicity tests were carried out on three-month-old *A. dahurica* plants. A spore suspension was prepared from PDA cultures of isolates

SM0101 and SM0102 and adjusted to 10^5 spores/mL using a hemocytometer. Each leaf was sprayed with 2 mL of the spore suspension, then incubated at 25 °C for 7 days. The same number of healthy *A. dahurica* plants were sprayed with sterile water as a control. After 7 days, small brown necrotic spots appeared on inoculated plants, but the control group showed no symptoms. A fungus with the same characteristics as that used for inoculation was re-isolated from the lesions. This experiment was replicated three times, and the results of each experiment were consistent. Genomic DNA was extracted from isolates SM0101 and SM0102 and used for PCR amplification of the rDNA internal transcribed spacer regions (ITS), RNA polymerase II gene (*RPB2*) and *Alternaria* allergen a 1 (*Alt a 1*) gene sequences using the primer pairs ITS1/ITS4 (White et al. 1990), *RPB2*-5F2/*RPB2*-7CR (Khodaei and Arzanlou, 2013) and *Alt*-for/*Alt*-rev (Hong et al. 2005), respectively. The ITS (OL703042, OL616086), *RPB2* (OL703043, OL898416), and *Alt a 1* sequences (OL616087, OL898415) were deposited in GenBank. The sequences obtained in this study had the highest match to corresponding sequences of *Alternaria alternata* CBS 916.96 (AF347031, KC584375, AY563301). For isolate SM0101 the matches were ITS (461/461 bp), *RPB2* (897/985 bp), and *Alt a 1* (488/488 bp). For isolate SM0202 the matches were ITS (457/457 bp), *RPB2* (893/985 bp), and *Alt a 1* (484/484 bp). A phylogenetic analysis was performed using MEGA7 software. The alignment included sequences from 16 ex-type *Alternaria* species and the two isolates causing leaf blight on *A. dahurica*. Branch supports were calculated with 1,000 bootstrap replicates, and phylogenetic inference was performed using the maximum likelihood estimation. The fungus isolated from *A. dahurica* clustered with *A. alternata*. This is the first report of *A. alternata* on *A. dahurica* in the world. This report will help to identify the disease symptoms in the field and provides a basis for research into the occurrence, distribution, and control of leaf blight on *A. dahurica*.

References

- Hong, S. G. et al. 2005. Fungal Genet. Biol. 42:119.
- Khodaei, S., and Arzanlou, M. 2013. Arch. Phytopathol. Plant Prot. 46:2224.

Simmons, E. G. 2007. *Alternaria: An Identification Manual*. CBS Fungal Biodiversity Center, Utrecht, the Netherlands.

White, T. J., et al. 1990. Page 315 in: *PCR Protocols: A Guide to Methods and Applications*. Academic Press, San Diego, CA.

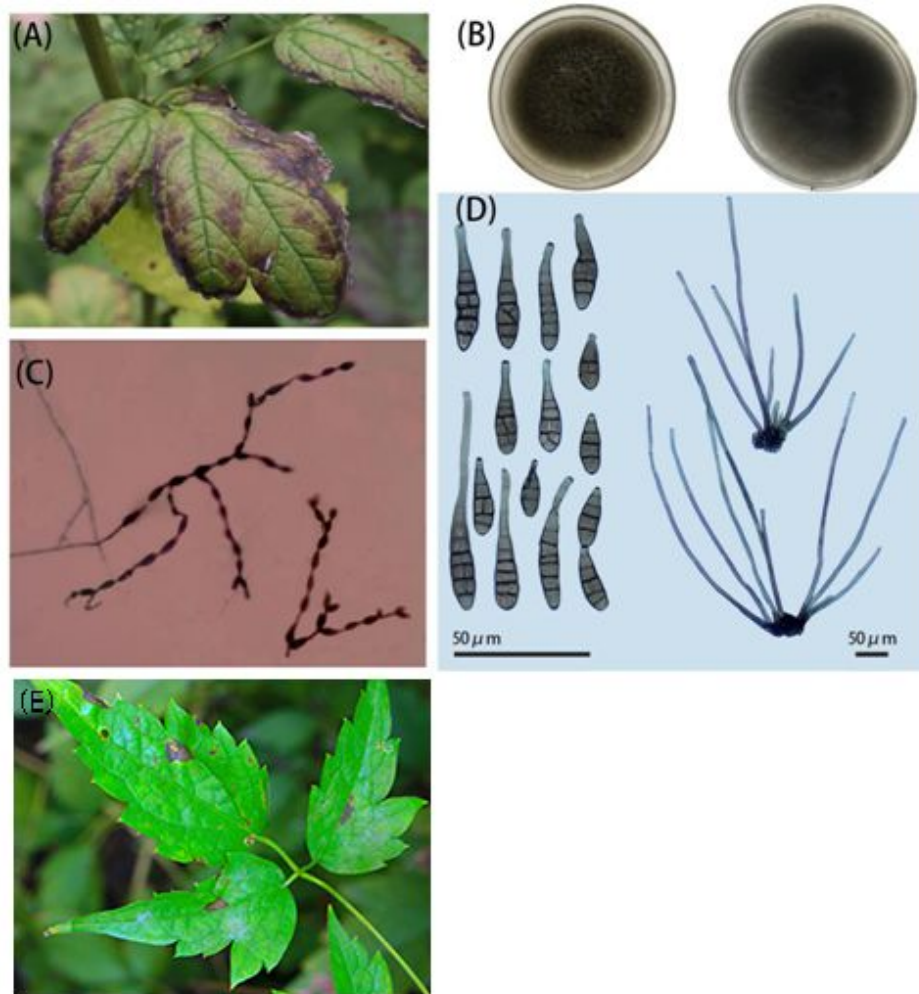


Figure 1. Fungal characteristics and symptoms of leaf spot caused by *A. alternata* on *A. dahurica*. (A) Symptoms in the field on the leaves of *A. dahurica* plants. (B) Fungal colony on PDA. (C) Sporulation pattern. (D) Conidiophores and conidia. (E) Symptoms on an inoculated leaf.

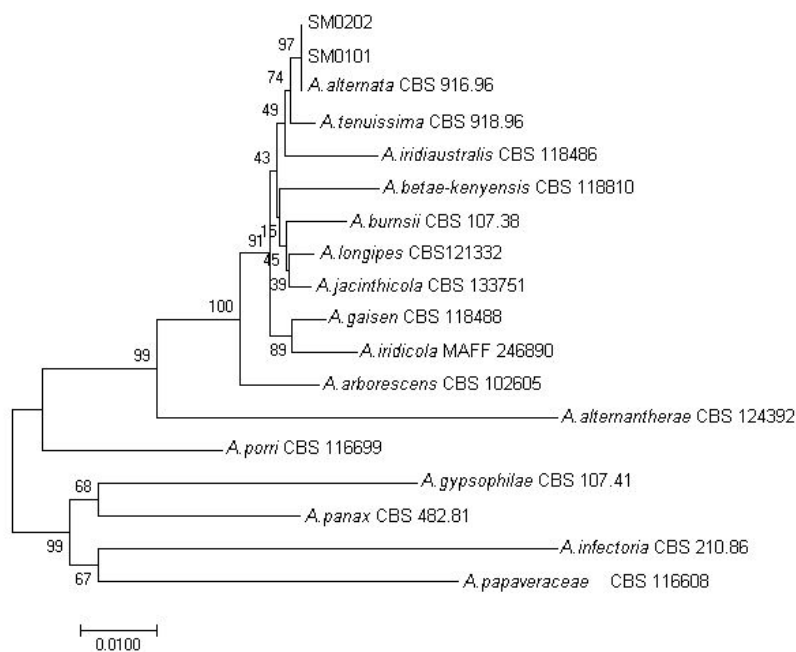


Figure 2. Phylogenetic tree constructed with sequences of ITS, *RPB2* and *Alt a 1* of isolates SM0101 and SM0202 obtained in this study and other species of *Alternaria* retrieved from GenBank. The tree was constructed using the maximum likelihood method from the alignment of ITS, *RPB2* and *Alt a 1* sequences using MEGA software (version 7).