Diseases Caused by Bacteria and Phytoplasmas

First Report of *Pantoea dispersa* Causing Brown Blotch Disease in *Flammulina filiformis* in China

Haijing Hu,¹ Miao Xu,¹ Haiyan Song,² Zhijun Zhai,¹ Minghui Chen,¹ Jianping Zhou,¹ Dianming Hu,^{1,†} and Yang Gao^{1,†}

¹Bioengineering and Technological Research Centre for Edible and Medicinal Fungi, Jiangxi Agricultural University, Nanchang, Jiangxi 330045, P.R. China

²Key Laboratory of Crop Physiology, Ecology and Genetic Breeding (Jiangxi Agricultural University), Ministry of Education of the P.R. China, Nanchang, Jiangxi 330045, P.R. China

Funding: Funding was provided by the National Natural Science Foundation of China (32070023), the Key Projects of Youth Fund of Jiangxi Province (20192ACBL21017), and Natural Science Foundation of Education Department of Jiangxi Province (GJJ190168, GJJ190228, GJJ200465). Plant Dis. 106:1056, 2022; published online as <https://doi.org/10.1094/PDIS-06-21-1270-PDN>. Accepted for publication 2 September 2021.

Flammulina filiformis (previously known as *F. velutipes*) is one of the most frequently cultivated and consumed edible mushrooms in China. In October 2020, brown blotch disease was observed on the pileus of *F. filiformis* at a mushroom factory in Ganzhou (25.74°N; 114.95°E), Jiangxi, China, with a disease incidence of ~6%. Symptoms initially appeared as small, irregular spots on the infected pileus, with color ranging from pale yellow to light brown. Such spots were enlarged and pitted at high relative humidity within several days, and finally caused malformation of the caps and yield reduction. To isolate the causal agent, the blotches on *F. filiformis* caps were homogenized and diluted with sterilized distilled water, and the resulting suspension (100 µl) was spread onto LB agar plates. After incubation at 28°C for 48 h, three colonial types were obtained: (i) yellow, convex, and smooth colonies; (ii) light yellowish, irregular, and rough colonies; and (iii) milky white, glistening, and smooth colonies. The first colonial type was predominant. A single colony of each type was randomly selected and streaked on fresh LB agar plates to obtain pure cultures (PF1, PF2, and PF3, respectively). To test pathogenicity of the three isolates, young *F. filiformis* fruiting bodies (8- or 10-day-old primordium) grown in culture bottles were inoculated by spraying a bacterial suspension (10⁸ CFU/ml, 3 ml per bottle on average), and cultivated in a mushroom house at 15 ± 2°C and 95% relative humidity. The fruiting bodies sprayed with sterilized distilled water served as

controls. Pathogenicity tests were repeated three times, and at least five culture bottles were included in each experiment. Among the three types of bacteria, only isolate PF1 induced symptoms similar to the original disease. Brown spots were observed on treated pileus 10 days after inoculation, and a fresh weight reduction of 30.9% per culture bottle was observed. In contrast, fruiting bodies treated with water remained asymptomatic. The same yellow colonies were also reisolated from the infected pileus and identified by subsequent methods. Isolate PF1 was gram negative, motile, and short rods. Biochemical analysis showed that the strains belonged to genus *Pantoea* (positive for citric acid, inositol, mannitol, methyl red test, and Voges-Proskauer test but negative for lysine, ornithine, phenylalanine, H₂S, urease, D-melibiose, sorbitol, adonitol, and raffinose). Further PCR amplification and sequencing of four genes, 16S rRNA gene with primer 27F/1492R, *fusA* gene with primer fusA3/fusA4, *gyrB* gene with primer gyrBf1/gyrBr1, and *rpoB* genes with primer Vic3/Vic2 (Delétoile et al. 2009; Palemon et al. 2021), were performed to identify the species. A BLASTn showed 100, 100, 99.51, and 99.26% homology, respectively, with those of *Pantoea dispersa* (MT072166, CP045216, CP076369, MH015168). The four gene sequences were deposited in GenBank (accession nos. MZ373179, MZ393661, MZ393662, MZ393663). A phylogenetic analysis based on the four concatenated genes also showed that the isolate PF1 well clustered with the type strain of *P. dispersa*. This species has been reported to cause leaf blight in rice (Toh et al. 2019), soft rot in *Agave angustifolia* (Palemon et al. 2021), and bulb decay in onion (Chang et al. 2018). To the best of our knowledge, this is the first report of *P. dispersa* causing brown blotch diseases on cultivated *F. filiformis*, which was previously known to be caused by *Pseudomonas tolaasii* (Lee et al. 2002). Our results also indicate *P. dispersa* could induce malformation of pileus and lead to a severe yield loss if not controlled effectively. Therefore, it should be considered in future disease management of *F. filiformis* cultivation.

References:

- Chang, C. P., et al. 2018. Australas. Plant Pathol. 47:609.
- Delétoile, A., et al. 2009. J. Clin. Microbiol. 47:300.
- Lee, H. I., et al. 2002. Lett. Appl. Microbiol. 35:276.
- Palemon, F., et al. 2021. Plant Dis. 105:3286.
- Toh, W. K., et al. 2019. Plant Dis. 103:1764.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: winter mushroom, pathogen identification, prokaryotes, *Pantoea dispersa*

[†]Indicates the corresponding authors.

D. Hu; hudianming1@163.com, and Y. Gao; gaoyang@jxau.edu.cn