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Bacterial shoot blight caused by *Pseudomonas cerasi*, a new pathogen of pear tree

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Abstract

In 2018, bacterial shoot blight was observed on Asian pear trees (*Pyrus pyrifolia*) in an orchard in Jinju, South Korea. The young pear shoots were infected and showed shoot blight symptoms: the shoot tips shriveled and turned tan in color, and this spread from the shoot tip down the stem and into the leaves. The causal pathogen was isolated from the diseased lesions and identified as *Pseudomonas cerasi* based on API tests and multilocus sequence analysis (MLSA) using the 16S rRNA, *gyrB*, *rpoB*, and *rpoD* gene sequences. The symptoms after artificial inoculation were identical to natural field symptoms, whereas negative control plants were asymptomatic. Bacteria were re-isolated from the artificially induced lesions on blossoms and shoots, and their identity was confirmed by *rpoD* sequencing. This is the first report of bacterial shoot blight on pear tree caused by *Pseudomonas cerasi* Griffin in the world.

Keywords Bacterial shoot blight · Pear · *Pseudomonas cerasi* · Multilocus sequence analysis

The consumption of pears in Korea is decreasing as the number of single-member households increases. Moreover, many young Koreans only consume pears on traditional Korean holidays, such as Korean Thanksgiving and Lunar New Year's Day (USDA GAIN report 2018; www.gain.fas.usda.gov). The patterns of pear consumption in Korea are changing with pears now used more as a food sweetener than as a raw material, and as a health supplement in the form of hot pressed juice. Korea produces about 265,757 metric tons of pears per year on 10,861 ha. In the past decade, the area of planted pears has steadily decreased by about 41% (Korean Statistical Information Service 2018; www.kosis.kr.eng). Nevertheless, pears are still a popular fruit in Korea.

In 2018, bacterial shoot blight was observed on Asian pear trees (*Pyrus pyrifolia*; cv. Singo) in an orchard in Jinju, South Korea. It occurred in pear trees planted in orchards and along roads and 5% of the branches per infected tree were diseased. Young infected pear shoots exhibited shoot blight symptoms: the shoot tips shriveled and became tan colored, and this spread from the shoot tip down the stem and into the leaves (Fig. 1).

Pear shoots with blight were collected from an orchard in Jinju, South Korea. Bacteria were isolated from diseased lesions according to the protocol set out by Choi et al. (2017). Briefly, small pieces (3 × 3 mm) taken from the diseased shoot tissues were disinfested by placing them in 1% sodium hypochlorite solution for 30 s, washing twice in sterile distilled water, and transferring to 1.5-ml microtubes containing 0.5 ml sterile distilled water. The tissues were macerated with a sterile tip to make a suspension. A 100-μl aliquot of the suspension was spread on 1/10 tryptic soy broth agar (TSBA) and incubated at 28 °C for 2 days. Gram-negative, non-spore forming, rod-shaped pseudomonads were consistently recovered from diseased twigs exhibiting blight symptoms. Two bacterial colonies identical in appearance were selected for testing hypersensitive reactions on tobacco leaf. The two isolates (MHGNU B107a–b) exhibited opaque, circular, raised colonies with entire margins on TSBA, and produced a hypersensitive reaction on a tobacco leaf. A representative isolate MHGNU B107a was deposited in the Korean Agricultural Culture Collection (KACC 21449).

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Fig. 1 Bacterial shoot blight caused by *Pseudomonas cerasi* on pear. **a** Bacterial shoot blight; **b** Enlarged image

The two isolates, MHGNU B107a–b, were subjected to pathogenicity tests on pear flowers and young shoots (cv. Singo) of trees growing in pots. A bacterial suspension (10^8 CFU/ml) was sprayed on the flowers on a twig and infiltrated into another three young shoots. The inoculated pear flowers and shoots were covered with transparent plastic bags for 2 days. After removing the plastic bags, the pear plants were placed in a plant growth chamber at 25 °C and 90% humidity with a 12-h light (150 μmoles/m^2/s). Sterile distilled water was used as a negative control. The two isolates, MHGNU B107a–b, produced blossom and shoot blight symptoms that were identical to the symptoms observed in the field (Fig. 2a), and there was no difference between the isolates. The negative control inoculated with water showed no symptoms (Fig. 2b). Bacteria were re-isolated from the inoculated lesions and confirmed by *rpoD* gene sequencing to satisfy Koch's postulates.

To confirm the identity, the partial 16S rRNA region and *rpoD* gene were amplified and sequenced. PCR amplification was performed with primer pairs; 27mF (5′-AGAG TTTGATCMTGGCTCAG-3′) and 1492mR (5′-GGYTACCTTGTTACGACTT-3′) for the partial 16S rRNA region; PsEG30F (5′-ATYGAAATCGCCAARCG-3′) and PsEG790R (5′-CGGTTGATKTCCTTGA-3′) for the *rpoD* gene (Mulet et al. 2009); UP-1E (5'-CAGGAAAC AGCTATGACCAYGSNGGNGGNAARTTYRA-3′) and APrU (5′-TGTAAAACGACGGCCAGTGC NGGRTCYTTYTCYTGRCA-3′) for the *gyrB* gene (Ait Tayeb et al. 2005); and LAPSs (5′-TGGCCGAGAACCAG TTCCGCGT-3′) and LAPs27 (5′-CGGCTTCGTCCAGC TTGTTCAG-3′) for the *rpoB* gene (Hall et al. 2016). DNA extraction and PCR amplification were conducted as described previously (Choi et al. 2016). PCR was performed on a thermal cycler (T100; Bio–Rad, Hercules, CA, USA) using PCR premix (Bioneer, Daejeon, Korea), 1 μg of genomic DNA, and 1 mM of each primer: at 98 °C for 2 min, 30 cycles of 98 °C for 30 s, 55 °C for 30 s, and 70 °C for 1 min, and a final 4-min extension at 72 °C. The PCR products were examined by electrophoresis in 0.8% agarose gels, purified using Expin Gel SV (GeneAll Biotechnology, Seoul, Korea), and sequenced with the same primer pairs used for PCR amplification at Macrogen (Daejoen, South Korea). The GenBank accession numbers for the partial 16S rRNA, *rpoD*, *gyrB* and *rpoB* gene sequences of strain MHGNU B107a are: MK779199, MK779202, MN061942 and MN061943, respectively. The DNA sequences were analyzed using the BLAST program and compared with the NCBI/GenBank database (http://www.ncbi.nlm.nih.gov/blast/). Based on an analysis of 16S rDNA sequences the isolates shared the highest similarity (over 99.8%) with *Pseudomonas tremae* CFBP 6111(T) and *P. cerasi* 58(T).

Phylogenetic analysis using multilocus sequence analysis (MLSA) and comparison with sequences in the PADMB database (http://genome.ppws.vt.edu/cgi-bin/MLST/home.pl) was performed according to a previously reported method (Mulet et al. 2010). Phylogenetic analysis based on MLSA using the 16S rRNA, *gyrB*, *rpoB* and *rpoD* gene sequences was performed using the Neighbor-joining method and Tajima-Nei distance modeling in MEGA 7.0 (Kumar et al. 2016). The sequences of related pseudomonads were downloaded from GenBank. In the phylogeny based on MLSA using the 16S rRNA, *gyrB*, *rpoB* and *rpoD* gene sequences, MHGNU B107a and MHGNU B107b clustered in the same group as *P. cerasi* (Fig. 3).

Bacteria exhibiting virulence on pear plants were subjected to biochemical analysis using the Analytical Profile Index (API) system (BioMérieux, Marcy l'Étoile, France), according to the supplier's instructions. The API 20NE tests revealed that MHGNU B107a and MHGNU B107b utilized D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid, and trisodium citrate, but that both isolates did not utilize N-acetyl-glucosamine, D-maltose, adipic acid, or phenylacetic acid. Both isolates were positive for β-glucosidase and oxidase but were negative for the production of indole, arginine dihydrolase, urease, β-galactosidase, protease, and potato tube rot. Nitrate was not reduced to nitrite.

Fig. 2 Pathogenicity of *Pseudomonas cerasi* causing bacterial shoot blight on pear. **a** Blossom and shoot blight symptoms induced artificially by *P. cerasi* MHGNU B107a inoculation: blight of blossoms, petals, and shoots, and discoloration of the anther; **b** negative control with no symptoms

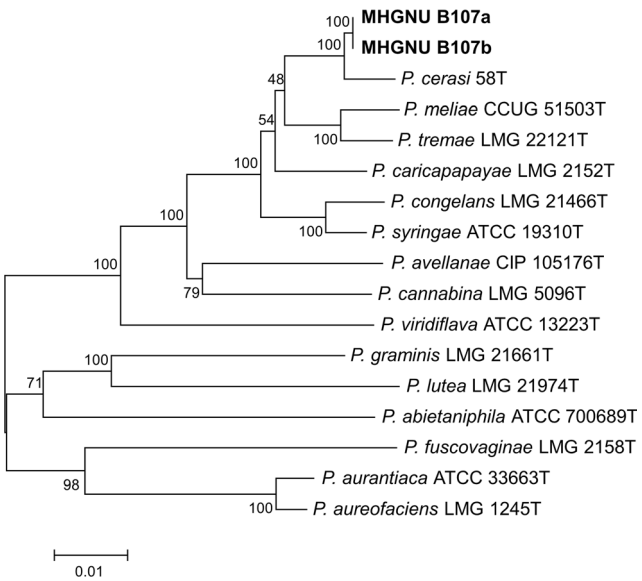

Fig. 3 Phylogenetic tree of *Pseudomonas* strains based on the phylogenetic analysis of four concatenated genes (16S rRNA, *gyrB*, *rpoB*, and *rpoD*). Distance matrices were calculated by the Jukes-Cantor method and are in the units of number of base substitutions per site. Dendrograms were generated by Neighbor-Joining. Numbers above the branches are the bootstrap values. Bars indicate the number of nucleotide substitutions per site. The isolates obtained in this study is in bold. Evolutionary analyses were conducted in MEGA7

Based on pathogenicity, biochemical tests and multilocus sequence analysis (MLSA) using the 16S rRNA, *gyrB*, *rpoB*, and *rpoD* gene sequences, the causal bacterium was identified as *Pseudomonas cerasi*. The disease caused by *P. cerasi* was first discovered in Polish cherry trees with flower dieback and necrotic spots on shoots, leaves and fruits (Kałużna et al. 2016). This is the first report of bacterial shoot blight on pear tree in the world. It is not known how this bacterial pathogen was introduced to the orchard Jinju. However, bacterial blight caused by *P. syringae* pv. *syringae* has been reported in Korea on apples (Seo et al. 1999; Cheon and Jeon 2015) and kiwifruit (Shin et al. 2004; Kim et al. 2017). The recent outbreak of this disease indicates that the bacterial species affects the fruiting of pear and poses a possible threat to pear cultivation and production in Korea.

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