First Report of Oat Halo Blight Caused by *Pseudomonas coronafaciens* in South Korea

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In May 2019, halo blight symptoms were observed on leaves of oats (*Avena sativa* L. ‘Joyang’) at the tillering stage. Plants were cultivated in experimental fields in Wanju County (Lat. 35.842006°, Long. 127.049588°), located in the southern region of South Korea. Symptom consisted of water-soaked spots, which progressed into blotches or streaks with brown oval to spindle-shaped centers surrounded by yellow halos (Fig. 1S). These symptoms were similar to those reported for oats infected by *Pseudomonas coronafaciens* in other parts of the world (Haber and Harden, 1992; Harder and Harris, 1973; Wilkie, 1972). To isolate the causal agent of the symptoms, 1 to 3 cm-long pieces of symptomatic leaves from 10 different oat plants were surface sterilized with 70% ethanol for 30 s and macerated in sterile distilled water. The macerates were streaked onto nutrient agar plates, and grown at 28°C for 48 h. In total, four pure colonies were selected and were cream-colored, wet, and shiny, with convex surfaces. The isolated colonies did not produce fluorescent siderophores when cultured on King’s medium B. We selected two representative colonies (SP-1 and X-1) for further characterization. The 16S rRNA, *gyrB*, and *rpoD* loci were amplified using the 27F/1492R primers (Lane, 1991), *gyrB*+271ps/*gyrB*-1022ps, and for *rpoD*+147p and *rpoD*-1222ps, respectively (Sarkar and Guttman, 2004). The sequences were deposited in GenBank with the following accession numbers: MN134544 (SP-1) and MN134571 (X-1) for the 16S rRNA gene, MN599033 (SP-1) and
MN599034 (X-1) for the gyrB gene, and MN599035 (SP-1) and MN599036 (X-1) for the rpoD gene. Analyses of these sequences indicated that the two isolates belong to the same clade of pathotypes as P. coronafaciens (Fig. 2S). These pathotype formed a clade distinct from that of other P. syringae pathovars, as described recently by Dutta et al. (2018). For confirmation, PCR analysis of two different specific primer sets was performed. The 498 bp products from the SP-1 and X-1 were amplified using a specific primer pair for P. coronafaciens, Pc-12-F and Pc-12-R (An et al., 2015), and 600 bp products with primers, P1 and P2, to detect the sequence within the plasmid pCOR1 harboring the genes for toxin production (Dutta et al., 2018). Consequently, the bacteria isolated from the lesions on the oat plants were P. coronafaciens strains. To fulfill Koch’s postulates, pathogenicity tests were conducted in which isolates SP-1 and X-1 were inoculated onto surface-sterilized leaves of 2-week-old, disease-free, greenhouse-grown ‘Joyang’ oat plants. Leaves were inoculated using a 1-ml needle syringe with 400 µl of a bacterial suspension (10^8 colony forming units (CFU)/ml) or 400 µl sterile water (for the control group). The plants were incubated for 1 week at 23°C in 100% relative humidity under a 10 h photoperiod. After 5 days, the inoculated leaves began to exhibit typical brown necrotic spots surrounded by yellow halos. Bacteria were isolated from these diseased leaves and the 16S rRNA gene sequences of the isolates were the same as those from the blighted field-grown leaves. The pathogenicity test was repeated twice. These findings indicated that P. coronafaciens strains accounted for the symptoms observed in field grown oats. To the best of our knowledge, this is the first report of oat halo blight disease caused by P. coronafaciens in South Korea. The pathogen has the potential to impair plant health and result in economic losses, as reported for other global locations (Elliott 1920; Harder and Harris 1973; Wilkie, 1972).
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Fig. S1. Symptoms of oat halo blight. A. Symptom of halo blight on leaves of field-grown oats, cultivar "Joyang". (B) Symptoms of halo blight on “Joyang” oat plants inoculated with two Korean isolates, Pseudomonas coronafaciens (X-1 and SP-1) under laboratory conditions. Leaves injected with sterile water were negative controls (Con).
Fig. 2S. Phylogenetic analysis of partial gyrB (A) and rpoD (B) genes of the oat halo blight isolates with other Pseudomonas species and pathovars. The alignment was generated using Clustal W. The evolutionary tree was inferred using the Neighbor-joining method. Phylogenetic analyses were conducted using MEGA7 software. Bootstrap values are shown as notes based on 10,000 replications. The accession numbers of the bacterial strains are listed, and the bacterial strain with “*” represents a pathotype strain.