Diseases Caused by Bacteria and Phytoplasmas

First Report of *Pseudomonas oryzae* Causing Stem and Leaf Rot on Muskmelon in China

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Muskmelon (*Cucumis melo* L.) is an important economic crop in China, which is planted on more than 376, 000 ha with over 13 million metric tons of annual fruit production. In February 2020, a serious bacterial stem and leaf rot disease on muskmelon plants was observed in greenhouses in Ligu Town, Ledong County, Hainan Province, China (18.54° N, 108.87° E), with disease incidences being approximately 10 to 12%. Initially, soft rot symptoms appeared on petioles and stems, showing yellow bacterial ooze signs, which was different from the milky white ooze produced by *Erwinia tracheiphila* infection. The infected tissues of petioles, stems, and leaves eventually developed into browning and withering symptoms. To isolate and identify the causal agent, the lesion tissues were sterilized by immersion in 75% ethanol for 30 s, washed three times with sterile water, and then cut and soaked in 1 ml of distilled water for 10 min. The suspension was serially diluted and spread on Luria-Bertani agar (LB) medium. After incubation at 28°C for 24 to 36 h, the resulting bacterial colonies were tiny and were streaked on LB plate for further culture. After purification, the colonies were yellow, circular, and smooth-margined, and two independent representative isolates (CM-11 and CM-12) were used for further validation experiments. The electron microscope analysis showed that the pathogen was rod-shaped, with a length of $1.34 \pm 0.22 \mu\text{m}$ and a width of $0.54 \pm 0.06 \mu\text{m}$ ($n = 50$), and it had a single terminal flagellum. The gram staining of the two isolates was negative. Moreover, the tested strains were positive for catalase but negative for oxidase and were able to utilize D-glucose, L-arabinose, and D-mannitol.

Morphological, physiological, and biochemical characteristics of both isolates were consistent with those of *Pseudomonas* spp. To verify the species identity of the bacterial pathogens, genomic DNA of isolates CM-11 and CM-12 was extracted, and several conserved genes were amplified and sequenced, including the 16S rRNA gene with primers 27F/1492R (GenBank MW187499 and MW187500), *rpoB* gene with primers V4/LAPS27 (MW201910 and MW446819), and *gyrB* gene with primers gyrBBAUP2/APrU (MW187501 and MW187502) (Mulet et al. 2010). In the BLAST analysis, the 16S rRNA sequences showed a 99% similarity to that of *Pseudomonas oryzae* strains TH19 (LC026009) and AA21 (MG571765). The *rpoB* and *gyrB* sequences showed high similarity (>98%) to *P. oryzae* strains FDAARGOS_657. The phylogenetic tree analysis of *rpoB* and *gyrB* genes further verified that the two isolates CM-11 and CM-12 were most closely related to *P. oryzae* species. Consequently, the two pathogenic isolates CM-11 and CM-12 were identified as *P. oryzae*. Both strains of CM-11 and CM-12 were tested to accomplish Koch's postulates. Young branches of muskmelons (cultivar Yugu, 10 days after pollination) were chosen as the material for inoculation. Ten healthy detached branches were placed in 15-ml tubes by submerging the cutting wound in 5 ml of the bacterial suspension (10^8 CFU/ml). Ten additional branches were implemented with sterilized distilled water as a negative control. The inoculated branches were placed in a plastic box containing moistened paper at 28°C. Rotting symptoms appeared within 5 days after infection, whereas the control samples remained healthy. Bacteria were reisolated from diseased tissues, and the 16S rRNA gene sequences of the isolates showed the same as those from the original pathogen. Panicle blight and grain discoloration disease caused by *P. oryzae* on rice has been described in China (Hou et al. 2020). It was also recently found that *P. oryzae* caused center blackening disease on muskmelon fruit in Korea (Choi et al. 2019). This study indicated that it was a causative agent of stem and leaf rot disease during the field growth period. To the best of our knowledge, this is the first report of *P. oryzae* causing muskmelon stem rot in China.

References:

- Choi, O., et al. 2019. Int. J. Food Microbiol. 291:1
- Hou, Y., et al. 2020. Plant Dis. 104:3055.
- Mulet, M., et al. 2010. Environ. Microbiol. 12:1513.

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