Diseases Caused by Bacteria and Phytoplasmas

First Report of *Pseudomonas oryzae* Causing Rice Panicle Blight and Grain Discoloration in China

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Pseudomonas oryzae has been reported as an opportunistic human pathogenic bacterium since the 1970s (Keikha et al. 2019); whether it is pathogenic to plants, particularly rice, remains unclear. In 2018, severe panicle blight (disease incidence ~15%, cultivar Tianyouhuazhan) was observed in rice fields in Fuyang, Hangzhou, China. Affected grains initially exhibited light-brown spots and later developed to wholly dark-brown on the glumes, which is different from the reddish-brown grains produced by *Burkholderia glumae* infection. These discolored grains were partially filled or aborted, causing panicles to be blighted. Blighted panicles were collected for pathogen isolation. Infected and discolored grains were sterilized by immersion in 70% ethanol for 30 to 60 s, rinsed three times with distilled water, and then cut, crushed, and soaked in 1 ml of distilled water for 10 to 30 min. The suspension was streaked onto nutrient agar (NA) medium and incubated for 16 to 24 h at 37°C. Circular, smooth-margined, yellow colonies were selected for further purification on NA containing ampicillin (50 µg/ml). The cells of obtained colonies were all gram-negative and rod-shaped, were able to utilize L-arabinose, D-glucose, D-mannose, D-mannitol, malate, potassium gluconate, and trisodium citrate, were positive for oxidase, and were negative for catalase. These morphological, physiological, and biochemical data indicate that the

characteristics of these isolates are similar to those of *Pseudomonas* genus. To determine bacterial species, the isolated strain H31 was used in the following molecular and pathogenicity assays. For molecular identification, 16S rRNA gene was amplified with common primers 27F (5'-AGAGTTT GATCATGGCTCAG-3') and 1492R (5'-AAGGAGGTGATCCAGCCG-3') (Hou et al. 2020). The amplified fragment was sequenced (GenBank MN565981), and the sequence shared 99% similarity with *Pseudomonas oryzae* strains H72 (GenBank KX390639.1) and LMG7040 (GenBank NR_117269.1) by NCBI BLAST analysis. In addition, a 837-bp fragment (GenBank MT185597) from *rpoD* gene and a 948-bp fragment (GenBank MT199661) from *gyrB* gene were amplified and sequenced using corresponding primers (rpoDP70F, 5'-ACGACTGACCCGGTACG CATGTAYATGMGNGARATGGNACNGT-3'; rpoDP70R, 5'-ATA GAAATAACCAGACGTAAGTTNGCYTCNACCATYTCYTTYTT-3'; gyrBPF, 5'-CAGGAAACAGCTATGACCAYGSNGGNGNAARTTYRA-3'; and gyrBPR, 5'-TGTAACACGACGGCCAGTGCNGGRTCYTTYT CYTGRC-3') (Yamamoto et al. 2000). Both fragments showed the highest homology to *P. oryzae* strain FDAARGOS_657 (GenBank CP044074.1) with a sequence similarity of 97 and 94%, respectively. Based on the above results, the isolated bacterium was identified as *P. oryzae*. For the pathogenicity test, bacterial suspension at 10⁸ CFU/ml was injected into the panicle bud at the booting stage of rice (cv. Tianyouhuazhan). Water was used as a control. Every nine Tianyouhuazhan plants were grown in a plastic box (50 × 20 × 16 cm), and three boxes were set as a replication. Plants were grown outdoors at 30 to 37°C with relative humidity over 75%. The heading rice showed light-brown spots on the grains at 7 to 10 days after inoculation; 2 to 3 weeks later, the whole grains were dark-brown, aborted, or partially filled, with similar symptoms to those observed in the field. In contrast, control plants remained healthy and symptomless. The same *P. oryzae* was reisolated in the inoculated rice plants. This is the first report of human pathogen *P. oryzae* causing rice panicle blight and grain discoloration in China.

References:

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