Diseases Caused by Bacteria and Phytoplasmas

First Report of Onion (*Allium cepa*) Bulb Rot Caused by *Pantoea agglomerans* in China

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Funding: Funding was provided by National Natural Science Foundation of China (NFS No. 21967015); Gansu Province science and technology key research and development project (20YF3FA038); Gansu Jiayuguan city science and technology major project (20-04). Plant Dis. 106:1057, 2022; published online as <https://doi.org/10.1094/PDIS-07-21-1393-PDN>. Accepted for publication 14 September 2021.

Onion (*Allium cepa* L.) is one of the most cultivated vegetables throughout the world. With an average annual production quantity of 18 million kilograms over a recent 21 years, China is the world's biggest onion producer (Hanci 2018). Among them, onion is mainly cultivated in the provinces of Gansu, Shandong, Yunnan, Jiangsu, Zhejiang, and Henan. A survey in Gansu Province in the last several years showed that the incidence of onion bulb rot was 30 to 80%. In April 2020, bulbs appeared water-soaked, and then rot symptoms were observed during storage in Lanzhou City, Gansu Province, China. The initial symptoms of bulb rot disease were yellowish brown color and production of an abundant exudate in the inner bulb scales when cut. Gradually, symptomatic bulbs became soft, watery, and decayed. In severe infections, the onions showed total rot of the bulb. Therefore, we sampled some diseased onions and isolated pathogenic bacteria from the junction of lesions along with healthy parts on Luria-Bertani (LB) medium. Three representative single colonies were obtained on LB medium, and the culture characteristics were raised elevation, mucoid texture, round, and smooth with entire margin; the brown at the beginning and turned yellow later; and scanning electron microscopy observations showed that these isolates were short rod-shaped. The physiological and biochemical determination revealed that the isolates were positive for yellow pigment, Voges-Proskauer test, growth at 37°C, nitrate reduction, catalase, glucose, sucrose, D (–)-salicin, starch hydrolysis, motility, and pellicle. They were negative for indole production, methyl red, lactose, gelatin liquefaction, glycerol, and gram staining (Gavini et al. 1989; Nabrdalik et al. 2018). Based on these morphological, physiological, and biochemical characteristics, three isolates were initially

identified as *Pantoea agglomerans* (Guo et al. 2020). A representative isolate, L1, was selected to extract DNA, and the conserved sequences of the pathogen gene were sequenced according to 23S ribosomal RNA (23S rRNA), DNA gyrase subunit B (*gyrB*), and elongation factor G (*fusA*) (She et al. 2021) housekeeping gene. The sequence alignment of the 23S rRNA gene (*P. agglomerans*, MZ314289, 930 bp) showed that the homology between the strain L1 and *P. agglomerans* (CP016889) with similarity of 99.54%, and based on the sequence alignment of *gyrB* (*P. agglomerans*, MZ337547, 1,189 bp) and *fusA* (*P. agglomerans*, MZ350961, 1,037 bp) genes, the similarity with *P. agglomerans* (FJ617386 and MG845872) was 100%. A phylogenetic analysis based on the 23S, *gyrB*, and *fusA* housekeeping gene sequences was performed by using the neighbor-joining methods in MEGA 7.0 under the p-distance (Kumar et al. 2016), which included *P. agglomerans* strains AR1a, TH81, L15, and ASB05, *P. eucalypti* strain LMG 24197, *P. dispersa* strains BJQ0007 and DSM 32899, *P. ananatis* strains LMG 20103 and AJ13355, and *P. vagans* strains C9-1, LMG24199, and PV989. The phylogenetic distribution generated five primary phylogroups, and strain L1 formed a clade with the other four *P. agglomerans* strains. Thus, the strain L1 was identified as *P. agglomerans*. To satisfy Koch's postulates, 10 onions were divided into two groups, five in each group, and needle puncture wounded on the surface of each onion. In the experimental group, 400-μl bacterial suspensions were injected with a sterile syringe, and the other five onions were injected with the same amount of sterile distilled water as the negative control. Inoculated onions were incubated in the greenhouse incubator (28°C, humidity >80%). After 4 days of incubation, all onions inoculated with strain L1 appeared water-soaked and displayed rot symptoms, and no symptoms were observed in the negative control. Subsequently, pathogens were reisolated from inoculated bulbs and identified as *P. agglomerans* according to molecular identification described above. To the best of our knowledge, this is the first report of bulb rot disease of stored onion caused by *P. agglomerans* in China.

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The author(s) declare no conflict of interest.

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Keywords: vegetables, yield loss and economic impacts, onion, *Pantoea agglomerans*, bulb rot

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