First Report of *Enterobacter cloacae* Causing Bulb Decay on Garlic in China

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Enterobacter cloacae is a symbiotic bacterium, which is one of the species in intestinal microbiota in many humans and animals (Fanaro et al. 2003). In some cases, it causes harmful diseases in humans (Mezzatesta et al. 2012). More and more studies showed that *E. cloacae* caused disease on plants, such as macadamia (Nishijima et al. 2007), ginger (Nishijima et al. 2004), mulberry (Zhu et al. 2010), onion (Zaid et al. 2011), chili pepper (García-González et al. 2018), and rice (Cao et al. 2020). Garlic (*Allium sativum* L.) is one of crops with economic importance in the world. It is also widely grown in China. During 2018 to 2020, naturally infected garlic bulbs from garlic fields in Kaifeng of Henan Province (34.55° N; 114.78° E) showed dry brown discoloration and rot symptoms. The diseased garlic seriously affected its edible value. Voucher specimens collected in June 2019 were deposited in the Plant Disease Laboratory of Tianjin Agricultural University under accession number PATAU190620. To identify the causal agent of this disease, the bulb tissues of infected garlic were surface disinfested in 0.6% sodium hypochlorite, dipped in 75% ethanol, and then dipped in sterile distilled water. These bulbs were plated on LB medium and incubated at 37°C. A number of white colonies grew on the medium after plating for 16 h. All colonies were

round, white, opaque, smooth, and gram-negative, which is a typical characteristic of *Enterobacter*. To confirm the initial identification of the isolated bacterium, the fragments of 16S rRNA gene and *gyrA* gene of six colonies were amplified, respectively. The PCR products were purified and sequenced. All 16S rRNA and *gyrA* sequences were identical to each other. The sequences of 16S rRNA gene and *gyrA* gene were deposited in GenBank with accession numbers MW730711 and MW768876, respectively. BLAST searches were conducted using the sequences of 16S rRNA and *gyrA*. The results showed 99.72 and 96.91% identity with the corresponding sequences of *E. cloacae* strain CBG15936 (CP046116.1), respectively. Phylogenetic trees were performed using the neighbor-joining method of MEGA X based on the sequences of 16S rRNA gene and *gyrA* gene. Phylogenetic trees indicated that isolates are most likely *E. cloacae*. Pathogenicity tests were performed by puncturing garlic bulbs with a hypodermic needle, followed by dipping in bacterial suspension with the concentration of 2×10^8 CFU/ml for 5 min. As a control, the garlic bulbs were treated with sterile water. The inoculated and control bulbs were incubated at 30°C. Seven days after inoculation, brown discoloration and rot developed on all inoculated garlic bulbs. No symptoms were observed in the control group. The symptoms were similar to those observed on the original diseased garlic bulbs. The inoculated and control garlic bulbs were 10 replicates in each independent biological experiment. The pathogenicity tests were conducted three times with similar results. The bacteria were reisolated from symptomatic diseased garlic and confirmed as *E. cloacae* by morphological and sequence analyses as above. The reisolated bacteria were identified by biochemical and physiological characteristics using API 20E strips. The results of the identification were identical to those of the edible ginger strains and the chili pepper strains. As far as we know, this is the first report of *Enterobacter* bulb decay caused by *E. cloacae*. The results are of great significance not only for the management of garlic bulbs during postharvest handling and storage but also for the further research of opportunistic human pathogens *E. cloacae*.

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