Diseases Caused by Bacteria and Phytoplasmas

First Report of Bud Soft Rot on *Agave angustifolia* Caused by *Pantoea dispersa* in México

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Plant Dis. 105:3286, 2021; published online as <https://doi.org/10.1094/PDIS-02-21-0316-PDN>. Accepted for publication 7 May 2021.

The agave (*Agave* spp.) is an important crop in México, with 120,897 ha grown mainly for alcoholic beverage production (SIAP 2019). In September 2020, in the municipality of Huitzuco de los Figueroa (18.328692°N; 99.3998°W), Guerrero State, México, a serious disease was observed affecting *Agave angustifolia*. Disease incidence was 8% of 150 plants sampled over an approximate area of 2.5 ha. Initial symptoms of soft rot of the bud developed and produced an abundant exudate that appeared from the apical part to the base of the plant. In severe infections, the plants showed total maceration of the bud, and consequently death of the plants was observed. Symptomatic plant tissue was superficially disinfected with 1% NaOCl for 30 s and rinsed in sterile water three times. The disinfected tissues were macerated and with a loop spread in nutrient agar. The plates were incubated at 28°C for 2 days. Yellowish bacterial colonies were isolated, and eight colonies were selected for characterization. The bacterial strains were gram-negative and rod-shaped, negative for fluorescent pigment tests and Kovacs' oxidase. Two isolates designated AGA1 and AGA2 were identified by PCR amplification and sequencing of the partial 16S rRNA gene with the primer 27F/1492R (Lane 1991), and partial *fusA*, *rpoB*, and *gyrB* genes (Delétoile

et al. 2009). Sequences were deposited in GenBank, with the accession numbers for 16S rRNA, AGA1 as MW548406 and AGA2 as MW548407; for specific genes *fusA* (AGA1 = MW558445, AGA2 = MW558446), *rpoB* (AGA1 = MW558447, AGA2 = MW558448), and *gyrB* (AGA1 = MW558449, AGA2 = MW558450), and they were compared with the sequences available in GenBank using BLASTn. 16S rRNA gene sequences for AGA1 and AGA2 aligned with *Pantoea dispersa* (MT921704.1, 99.9% identity). Housekeeping genes also aligned 99 to 100% to *P. dispersa* (*fusA* = 100%, CP045216.1; *rpoB* = 99.8% MH015167.1 and *gyrB* = 99%, MK928270.1). Phylogenetic analysis of concatenated genes showed that strains AGA1 and AGA2 cluster with *P. dispersa*. To confirm pathogenicity, eight plants of 6-month-old *A. angustifolia* were inoculated with strain AGA1 using sterile toothpicks dipped in 10⁸ CFU/ml bacterial suspension. The toothpicks were inserted in the middle part of the bud. Four plants were inoculated with sterile water as control. The plants were covered with plastic bags and housed in a greenhouse (average temperature and relative humidity of 25°C and 85%, respectively). Pathogenicity tests were repeated two times. After 7 days, all inoculated plants developed symptoms similar to those observed in the field. Control plants did not show symptoms. From the plants that showed symptoms, the pathogen was reisolated again and was identified by morphological and molecular characterization, following the method previously described, fulfilling Koch's postulates. In México, *Erwinia cacticida* and *Pantoea ananatis* has been previously reported on *A. tequilana* that as causing soft rot and red leaf ring, respectively (Fucikovsky and Aranda 2006; Jiménez-Hidalgo et al. 2004). To our knowledge, this is the first report of *P. dispersa* causing bud soft rot on *A. angustifolia* in México. More studies on monitoring and control strategies of bud soft rot on *A. angustifolia* are required.

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The author(s) declare no conflict of interest.

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Keywords: prokaryotes, field crops, etiology

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