Diseases Caused by Bacteria and Phytoplasmas

First Report of *Pantoea ananatis* Causing Leaf Spot Disease of Maize in Ecuador

A. Toaza,¹ R. B. Caiza,¹ A. D. Garrido,¹ C. R. Moreno,¹ J. Guevara,¹ H. Regalado,¹ F. J. Flores,^{2,3} L. A. Ramos,² and P. A. Garrido^{2,†}

¹ AGROCALIDAD - Agencia de Regulación y Control Fito y Zoonosanitario, Quito, Ecuador

² Universidad UTE, Centro de Investigación de Alimentos, CIAL, Facultad de Ciencias de la Ingeniería e Industrias, Quito, Ecuador

³ Departamento de Ciencias de la Vida, Carrera de Ingeniería en Biotecnología, Universidad de las Fuerzas Armadas ESPE, Sangolquí, Ecuador

Plant Dis. 105:3286, 2021; published online as <https://doi.org/10.1094/PDIS-02-21-0298-PDN>. Accepted for publication 6 May 2021.

Maize (*Zea mays*) is the second most cultivated grain crop in Ecuador, with growing significance as a source of fodder and food. During the rainy season (November and December) of 2018 and 2019, a disease of maize that was not previously observed in Ecuador was found at commercial fields of variety Misqui Sara, at four parishes of canton Quito (Tumbaco, Pifo, Puembo, and Checa), Pichincha Province. At tassel initiation, infected plants displayed symptoms of localized chlorotic streaks on leaves that expanded with time, and around a month later turned necrotic. Severely affected plants wilted and died. Symptoms appeared in lower leaves first and were later observed in upper leaves as the disease progressed. Disease incidence was between 20 and 30% in the affected plantations, with around 30% of infected plants wilting and dying, resulting in 20 to 25% yield losses. Upper leaves from 10 symptomatic plants, five from Puembo and five from Checa, were collected randomly. Two 0.5-cm² pieces of leaf from each plant were excised from the margins of the necrotic lesions, surface sterilized, and macerated in 9 ml of sterile peptone water. The 10⁻³ dilutions were plated onto nutrient agar and incubated at 28°C for 24 h. Yellow, mucoid colonies were isolated on nutrient agar. Three isolates from Puembo and two from Checa were selected for testing Koch's postulates

and further biochemical and molecular characterization. Isolates were gram-negative rods, oxidase negative, catalase, indol and citrate positive. Fragments of the 16S, gyrB, and rpoB loci were amplified and sequenced using the 27F/1492R (Lane 1991), UP-1/UP-2r (Yamamoto and Harayama 1995), and rpoBCM81-F/rpoBCM32b-R (Brady et al. 2008) primer pairs, respectively. All isolates presented identical sequences for the different loci, and therefore only sequences from isolate FP191505 were deposited in GenBank (accession nos. MW528428–MW528430). A search of homologous sequences using BLAST resulted in identities of 99.3, 99.7, and 100% for 16S, gyrB, and rpoB, respectively, with sequences from *Pantoea ananatis* type specimen LGM 2665 (Brady et al. 2008; Hauben et al. 1998; GenBank accession nos. NR_119362.1, EF988824.1, and EF988996.1), indicating that our isolates belong to this species. Pathogenicity tests were performed by syringe infiltration of bacterial suspensions. Each one of the five characterized *P. ananatis* isolates was inoculated in four leaves (500 µl of 1 × 10⁸ CFU ml⁻¹ per leaf) of three healthy maize plants. Negative control plants were infiltrated with sterile distilled water. Plants were incubated at 28 to 30°C and 60% relative humidity for 24 h. Later, plants were maintained in a greenhouse with 27°C day/21°C night temperatures and observed daily. After 6 weeks, all bacteria-inoculated plants developed symptoms of chlorosis and necrosis while the control plants were symptomless. Bacteria were reisolated from symptomatic leaves and identified as *P. ananatis* following the same methodologies used for the initial identification. To our knowledge, this is the first report of *P. ananatis* causing leaf spot of maize in Ecuador. The spread of the disease and the bacterium *Pantoea ananatis* can result in substantial economic losses.

References:

- Brady, C., et al. 2008. Syst. Appl. Microbiol. 31:447.
- Hauben, L., et al. 1998. Syst. Appl. Microbiol. 21:384.
- Lane, D. J. 1991. Page 115 in: Nucleic Acid Techniques in Bacterial Systematics. E. Stackebrandt and M. Goodfellow, eds. Wiley, London.
- Yamamoto, S., and Harayama, S. 1995. Appl. Environ. Microbiol. 61:1104.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: prokaryotes, cereals and grains, pathogen detection

[†]Indicates the corresponding author.
P. A. Garrido; patricia.garrido@ute.edu.ec