

## First Report of soybean (*Glycine max* L.) disease caused by *Pseudomonas aeruginosa* in Cuba

**O. Plasencia-Márquez, M. Corzo, Y. Martínez-Zubiaur**, Phytopathology Group, National Center for Animal and Plant Health (CENSA), Cuba; **D. Rivero**, UCTB “Los Palacios”, Instituto Nacional de Ciencias Agrícolas (INCA), Cuba; **G. Devescovi, V. Venturi**, Bacteriology Group, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy.

Soybean (*Glycine max* L.) has become one of the most widely consumed foods; however, diseases caused by microorganisms can affect yields and seed quality. In March 2015, during routine survey in a soybean growing area in Pinar del Río province, Cuba, disease symptoms were observed in some leaves. These included water-soaked necrotic spots surrounded by chlorotic halos, especially on the margins of the leaves. To identify the possible pathogens involved, leaves were disinfected with tap water, 70% ethanol and were rinsed with sterile distilled water. Small segments from diseased tissue were macerated in sterile 0.85% NaCl solution, decimal dilutions were performed and 20  $\mu$ L aliquots were streaked onto King's B medium (KB). After 24 hours of incubation at 28 °C, a fluorescent pseudomonad was isolated. Colonies were round, smooth and produced yellowish-green diffusible pigments on KB. Physiological and morphological characteristics were determined using standard microbiological techniques (Schaad *et al.*, 2001). The isolate was oxidase and arginine dihydrolase positive, reduced nitrates to dinitrogen, hydrolyzed gelatine and was levan and potato rot negative. Using API 20NE gallery (bioMérieux), the strain was identified as *Pseudomonas aeruginosa*. It was further confirmed by amplifying and sequencing the 16SrRNA, *gyrB*, *rpoD* and *rpoB* genes. The sequences were deposited in GenBank, under accession numbers KY652227, KY652228, KY652229 and KY652230, respectively. BLAST analyses were performed using the NCBI server against the whole nucleotide collection and in all cases, sequences had 99% identity to that *P. aeruginosa* strains PA-3 (KT266561.1), ATCC 27853 (CP015117.1), PA7790 (CP014999.1) and PcyII-10 (LT673656.1), respectively. Strain pathogenicity was evaluated in 15-days soybean plants by spraying a  $10^8$  CFU\*mL<sup>-1</sup> bacterial suspension on the leaves. Control plants were sprayed with 0.85% NaCl solution. The plants were maintained at 28°C in a moist chamber at 100 % relative humidity for 48 hours before being moved to a greenhouse. Water-soaked necrotic spots with intense chlorotic halos appeared after 72 hours and after five days, the entire plant turned yellow and wilted. No symptoms were observed in negative control plants. The bacterium was re-isolated from symptomatic plants and had the same cultural, physiological and biochemical characteristics to that inoculated, fulfilling Koch's postulates. *P. aeruginosa* is a well-known human

opportunistic pathogen, widely distributed in nature, usually as a harmless saprophyte. It has also been reported as a pathogen in *Arabidopsis thaliana*, sweet basil (*Ocimum basilicum*) and lettuce (*Lactuca sativa*) (Starkey *et al.* 2009; Mondal *et al.*, 2012; Gao *et al.*, 2014). To our knowledge, this is the first report of *P. aeruginosa* causing leaf infection in soybean.

## References

1. Gao J *et al.* Plant Dis. 98(11):1577, 2014.
2. Mondal KK *et al.* Plant Dis. 96(1):141.2, 2012.
3. Schaad NW *et al.* Laboratory Guide for the Identification of Plant Pathogenic Bacteria, 3rd edition. APS Press, St. Paul, MN, 2001.
4. Starkey M *et al.* Nat. Protoc. 4(2):117-24, 2009.