First report of *Xanthomonas prunicola* causing bacterial leaf streaks on wheat in Argentina

J. A. Martino[1-2*], F. D. Fernández[1-2*], E. A. Pozzi[3], E. J. Alberione[3], C. T. Bainotti[3], N. Márquez[1-2], P. A. Tolocka[1-2], N. Salines[3], D. T. Gomez[3], G. M. Donaire[3], L. R. Conci[1-2], V. M. Alemandri[1-2†].

*These authors contributed equally

1- Instituto de Patología Vegetal (IPAVE), Centro de Investigaciones Agropecuarias (CIAP), Instituto Nacional de Tecnología Agropecuaria (INTA), C.P. X5020ICA, Córdoba, Argentina.
2- UFYMA (Unidad de Fitopatología y Modelización Agrícola) (INTA-CONICET), C.P. X5020ICA, Córdoba, Argentina.
3- INTA EEA Marcos Juárez, C.P. 2580, Marcos Juárez, Argentina.

Since 2018, bacterial-like symptoms, such as leaf streaks were observed on wheat plants (*Triticum aestivum* L.) in Córdoba province in Argentina, with 1 to 5% of disease incidence. Samples of wheat stem and spike collected in a trial of varieties for summer/autumn sowing in the experimental field of the INTA Marcos Juárez were disinfected, washed and macerated in mortars with sterile distilled water and extracts were streaked on Luria-Bertani (LB) agar. After 48 h incubation at 28 °C, circular, mucoid, convex, and cream colonies were observed and pure cultures were transferred to LB medium for further identification tests. Biochemical tests corroborated the detection of a Gram-negative bacillus. Conventional PCR was performed using DNA isolate from pure cultures and general primers for various species of genera *Xanthomonas* (Maes 1993) and *Pseudomonas* (Mulet et al. 2010). An isolate (Arg-1), with cream colored colonies was positive using general primers for *Xanthomonas sp* (amplified fragment of 444 bp). A bacterial suspension containing 10^8 CFU mL−1 grown for 48 h on LB medium at 28 °C was injected into three-week-old leaves of wheat plants to fulfill Koch's postulates. After 5 days, plants showed symptoms of chlorosis, streaks and then necrosis on the leaves. The bacteria were re-isolated from the inoculated plants, showing same symptoms observed in the original plants. Negative control plants, inoculated with sterile water remained without symptoms. The amplified 444 bp fragment described above was sequenced by the Sanger method (GenBank accession OM972662), as well as another 757 bp fragment amplified with universal primers that amplify the partial 16S rDNA gene (GenBank accession OM972661). Analyses of these sequences, as well as the protein profile of the isolate obtained by matrix assisted laser desorption/ionization time of-flight mass spectrometry (MALDI-TOF MS) Bruker Biotyper, allowed to identify only the genus *Xanthomonas*. With the purpose of determine the species status, the complete genome of isolate Arg-1 was sequenced using Oxford Nanopore Technologies (ONT). Total gDNA was isolate from pure cultures using a commercial kit (Wizard Genomic DNA Purification Kit, Promega). gDNA library was constructed using Ligation Sequencing Kit (SQK-LSK109) and sequenced using ONT platform on a MinION 1kb device. Raw basecalled sequences were filtered using Filtlong and assembled using Trycycler. The genome was assembled in a single contig comprising 5.410.641 bp with 4740 predicted CDSs and 63.9% GC content. Genome sequence was deposited in GenBank under accession number CP094827 and SRA data SRX14635308. Whole-genome Average Nucleotide Identity (ANI) analysis showed values of ~ 97% against the reference genomes of *Xanthomonas prunicola* (PHKX01.1, PHKV01.1 and PHKW01.1) and 100% in complete 16S rRNA gene sequences (1547 bp). These findings suggest that a new wheat pathogen within the genus *Xanthomonas* is present in Argentina, as well as was reported in Uruguay and USA (Clavijo et al. 2021). To our knowledge, this is the first report of *X. prunicola* affecting wheat in Argentina and the first complete genome registered for this specie. Accurate and specific diagnostics are required for the detection of *X. prunicola* in wheat crops to implement correct prevention and control strategies to this disease, avoiding the dissemination in lots where it has not yet been found.

References:

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