



## High-Quality Draft Genome Sequence of *Xanthomonas* sp. Strain CPBF 424, a Walnut-Pathogenic Strain with Atypical Features

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**ABSTRACT** We report here the draft genome sequence of *Xanthomonas* sp. strain CPBF 424, isolated from a diseased walnut tree. Multilocus sequence analysis showed that this walnut-pathogenic isolate is located between the nonpathogenic *X. arboricola* and *X. prunicola* clusters. These features make this strain a promising reference to disclose new genetic determinants of pathogenesis.

The *Xanthomonas arboricola* species complex includes numerous phytopathogenic bacteria comprising different pathovars capable of infecting a wide range of plants (1–3) and causing severe disease symptoms and serious economic losses in important crops (4). Recently, particular attention has been given to *X. arboricola*-related strains shown to be phylogenetically distinct from pathogenic *X. arboricola* pathovar strains (5–8). *Xanthomonas* sp. strain CPBF 424 was isolated in April 2016 from asymptomatic dormant buds of a diseased walnut tree in Loures, Portugal, with common symptoms of walnut bacterial blight. Multilocus sequence analysis (MLSA) of the concatenated partial sequences of the *atpD* (750 bp), *dnaK* (759 bp), *efp* (339 bp), *fyuA* (684 bp), *glnA* (675 bp), *gyrB* (735 bp), and *rpoD* (586 bp) genes confirmed the strain's identity as a *Xanthomonas* sp., revealing that strain CPBF 424 is located between the nonpathogenic *X. arboricola* and *X. prunicola* clusters and diverges from *Xanthomonas arboricola* pv. juglandis strains, i.e., walnut-pathogenic bacteria, and from other *X. arboricola* pathovars (9). Pathogenicity tests on walnut plantlets further showed that CPBF 424 is pathogenic to walnut trees (10, 11), making this strain particularly appealing to provide new insights into xanthomonad pathoadaptations.

Here, we make available the whole-genome sequence of *Xanthomonas* sp. strain CPBF 424.

*Xanthomonas* sp. strain CPBF 424 was grown on bacterial culture medium M2 (yeast extract, 2 g liter<sup>-1</sup>; Bacto peptone, 5 g liter<sup>-1</sup>; NaCl, 5 g liter<sup>-1</sup>; KH<sub>2</sub>PO<sub>4</sub>, 0.45 g liter<sup>-1</sup>; Na<sub>2</sub>HPO<sub>4</sub> 12H<sub>2</sub>O, 2.39 g liter<sup>-1</sup>) at 28°C and 100 rpm for 48 h. DNA was extracted for sequencing using the E.Z.N.A. bacterial DNA purification kit (Omega Bio-tek, Norcross, GA). Genomic library preparation and genome sequencing were outsourced to GATC Biotech, AG (Konstanz, Germany) and conducted using an Illumina HiSeq platform with 2 × 150-bp paired-end reads, which resulted in 12,672,550 reads of raw sequence data with a sequencing coverage of 776×. *De novo* genome assembly was obtained with MIRA version 4.0 (12) using standard settings in accurate mode. This was followed by

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contig reassembly using SeqMan Pro from the Lasergene genomics package version 12.1.0 (DNASTar, Madison, WI) with Pro assembler parameters and read mapping using SeqMan NGen with standard settings to check for inconsistencies (overlapping contig extremities with no or low coverage with paired-read inconsistencies). A total of five irregularities were found on which contigs were broken open per the initial *de novo* assembly. Contigs were ordered using the Move Contigs function in Mauve 20150226 version 10 (13, 14) according to the genome of *X. arboricola* pv. *juglandis* CFBP 2528 (GenBank accession number [NZ\\_JZEF00000000](#)) (15). Automatic genome annotation was performed with a *Xanthomonas* genus database using the Prokka software tool version 1.12 (16).

The *Xanthomonas* sp. CPBF 424 genome had a total size of 4,896,146 bp and a G+C content of 65.89% represented by 10 contigs with an  $N_{50}$  value of 1,029,447 bp. The genome of CPBF 424 is estimated to be composed of 4,143 coding sequences (CDS), including 58 tRNAs and 4 rRNAs. Preliminary analysis with the EDGAR version 2.0 platform (17) allowed us to detect 3,502 coding sequences that are shared between CPBF 424 and *X. arboricola* pv. *juglandis* CFBP 2528, which was used as the reference genome.

The whole-genome sequence of strain CPBF 424 may contribute to elucidating new walnut pathoadaptations within the genus *Xanthomonas*.

**Data availability.** This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the BioProject accession number [PRJEB27248](#) (SRA accession number [ERR2767968](#)), and the sequence accession number is [UIHB00000000](#). The version described in this paper is version UIHB01000000.

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## REFERENCES

- Vauterin L, Hoste B, Kersters K, Swings J. 1995. Reclassification of *Xanthomonas*. *Int J Syst Bacteriol* 45:472–489. <https://doi.org/10.1099/00207713-45-3-472>.
- Janse J, Rossi M, Gorkink R, Derks J, Swings J, Janssens D, Scortichini M. 2001. Bacterial leaf blight of strawberry (*Fragaria* (x) *ananassa*) caused by a pathovar of *Xanthomonas arboricola*, not similar to *Xanthomonas fragariae* Kennedy & King. Description of the causal organism as *Xanthomonas arboricola* pv. *fragariae* (pv. nov., comb. nov.). *Plant Pathol* 50:653–665. <https://doi.org/10.1046/j.1365-3059.2001.00644.x>.
- Fischer-Le Saux M, Bonneau S, Essakhi S, Manceau C, Jacques MA. 2015. Aggressive emerging pathovars of *Xanthomonas arboricola* represent widespread epidemic clones distinct from poorly pathogenic strains, as revealed by multilocus sequence typing. *Appl Environ Microbiol* 81:4651–4668. <https://doi.org/10.1128/AEM.00050-15>.
- Lamichhane JR. 2014. *Xanthomonas arboricola* diseases of stone fruit, almond, and walnut trees: progress toward understanding and management. *Plant Dis* 98:1600–1610. <https://doi.org/10.1094/PDIS-08-14-0831-FE>.
- Essakhi S, Cesbron S, Fischer-Le Saux M, Bonneau S, Jacques MA, Manceau C. 2015. Phylogenetic and variable-number tandem-repeat analyses identify nonpathogenic *Xanthomonas arboricola* lineages lacking the canonical type III secretion system. *Appl Environ Microbiol* 81:5395–5410. <https://doi.org/10.1128/AEM.00835-15>.
- Jacques M-A, Arlat M, Boulanger A, Boureau T, Carrère S, Cesbron S, Chen NWG, Cociancich S, Darrasse A, Denancé N, Fischer-Le Saux M, Gagnevin L, Koebnik R, Lauber E, Noël LD, Pieretti I, Portier P, Pruvost O, Rieux A, Robène I, Royer M, Szurek B, Verdier V, Vernière C. 2016. Using ecology, physiology, and genomics to understand host specificity in *Xanthomonas*. *Annu Rev Phytopathol* 54:163–187. <https://doi.org/10.1146/annurev-phyto-080615-100147>.
- Garita-Cambronero J, Palacio-Bielsa A, López MM, Cubero J. 2017. Pan-genomic analysis permits differentiation of virulent and non-virulent strains of *Xanthomonas arboricola* that cohabit *Prunus* spp. and elucidate bacterial virulence factors. *Front Microbiol* 8:573. <https://doi.org/10.3389/fmicb.2017.00573>.
- López MM, Lopez-Soriano P, Garita-Cambronero J, Beltrán C, Taghouti G, Portier P, Cubero J, Fischer-Le Saux M, Marco-Noales E. 2018. *Xanthomonas prunicola* sp. nov., a novel pathogen that affects nectarine (*Prunus persica* var. *nectarina*) trees. *Int J Syst Evol Microbiol* 68:1857. <https://doi.org/10.1099/ijsem.0.002743>.
- Fernandes C, Pothier JF, Tavares F. 2018. Whole-genome sequencing of distinct *Xanthomonas arboricola* lineages isolated from a single walnut tree host, abstr poster 9, p 88. Abstr 6th *Xanthomonas* Genomics Conference and 2nd Annual EuroXanth Conf, Halle (Saale), Germany. <https://doi.org/10.13140/RG.2.2.13768.37128>.
- Albuquerque P, Fernandes C, Cruz L, Tavares F. 2017. Diversity of *Xanthomonas arboricola* pv. *juglandis* in Portugal evokes a cosmopolitan dispersion, abstr FEMS7-1173, P-487. Abstr 7th Congress of European

- Microbiologists. Federation of European Microbiological Societies, Valencia, Spain. <https://doi.org/10.13140/RG.2.2.16494.66884>.
11. Fernandes C, Albuquerque P, Cruz L, Tavares F. 2018. Genotyping and epidemiological metadata provides new insights into population structure of *Xanthomonas* isolated from walnut trees. bioRxiv <https://doi.org/10.1101/397703>.
  12. Chevreux B, Wetter T, Suhai S. 1999. Genome sequence assembly using trace signals and additional sequence information. *J Comput Sci Syst Biol* 99:45–46.
  13. Darling AC, Mau B, Blattner FR, Perna NT. 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome Res* 14:1394–1403. <https://doi.org/10.1101/gr.2289704>.
  14. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* 5:e11147. <https://doi.org/10.1371/journal.pone.0011147>.
  15. Cesbron S, Briand M, Essakhi S, Gironde S, Boureau T, Manceau C, Fischer-Le Saux M, Jacques MA. 2015. Comparative genomics of pathogenic and nonpathogenic strains of *Xanthomonas arboricola* unveils molecular and evolutionary events linked to pathoadaptation. *Front Plant Sci* 6:1126. <https://doi.org/10.3389/fpls.2015.01126>.
  16. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
  17. Blom J, Kreis J, Spänig S, Juhre T, Bertelli C, Ernst C, Goesmann A. 2016. EDGAR 2.0: an enhanced software platform for comparative gene content analyses. *Nucleic Acids Res* 44:W22–W28. <https://doi.org/10.1093/nar/gkw255>.