RESOURCE ANNOUNCEMENT

Complete Genome Sequence Resources of Six Strains of the Most Virulent Pathovars of *Xanthomonas arboricola* Using Long- and Short-Read Sequencing Approaches

e-Xtra*

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Announcement

Species *Xanthomonas arboricola* includes pathovars that cause serious diseases in *Prunus*, *Juglans*, and *Corylus* spp. (*X. arboricola* pvs. *pruni*, *juglandis*, and *corylina*, respectively).

Herein, we present the complete genome sequences of six strains of *X. arboricola*, two from each of the above-mentioned pathovars, five isolated in Spain and one from the International Center for Microbial Resources French Collection of Plant-associated Bacteria (CIRM-CFBP). Complete genome sequences were obtained by PacBio long-read sequencing technology and only assembly, followed by polishing with Illumina short reads that allowed an enhanced quality of the results. These complete and closed genomes will be used in future studies to elucidate host range and virulence mechanisms of these important xanthomonads.

X. arboricola is a Gram-negative, plant-associated bacterial species. Three *X. arboricola* pathovars are responsible for harmful diseases that cause significant economic losses: *X. arboricola* pv. *pruni*, *X. arboricola* pv. *juglandis*, and *X. arboricola* pv. *corylina*.

X. arboricola pv. *pruni* causes bacterial spot of *Prunus* spp., affecting *Prunus dulcis*, *P. salicina*, *P. persica*, *P. domestica*, *P. armeniaca, P. avium*, *P. cerasus*, and ornamental trees such as *P. laurocerasus* (Bergsma-Vlami et al. 2012; EFSA Panel on Plant Health 2014). This disease was first described in Michigan (United States) (Smith 1903) but today it is distributed worldwide (EPPO 2021a). Disease symptoms are spots and shot-holes on leaves, necrotic and sunken lesions on the fruit, twig and trunk cankers, and, in the most severe cases and only in some hosts, defoliation and fruit dropping (EPPO 2021b).

X. arboricola pv. *juglandis* was first described at the beginning of the 20th century (Pierce 1901). It causes walnut bacterial blight (WBB), the most well-known disease of walnut (*Juglans* spp.), which is characterized by necrotic spots on leaves and fruit. This bacterium has also been associated with brown apical necrosis (BAN), which is recognized by apical necrotic

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lesions near the blossom end, and with vertical oozing canker (VOC), which is characterized by vertical cankers with brown exudates that occur on the trunk and branches of walnut trees (Hajri et al. 2010; Kałużna et al. 2021). *X. arboricola* pv. *juglandis* has been detected in all major walnut-growing areas, including Australia, New Zealand, Canada, the United States, some Asian countries, and Europe (EPPO 2021a). Among European countries, the disease causes the most severe losses in France, Greece, Italy, Spain, and Turkey (Giraud and Prunet 2007; Kałużna et al. 2014). Losses due to WBB have been reported above 50% on untreated trees in northern California (United States) (Buchner et al. 2010); meanwhile, in 2013 and 2014, a BAN outbreak produced yield losses of nearly 50% in Turkey (Ustun et al. 2016).

X. arboricola pv. *corylina* causes bacterial blight in hazelnut (*Corylus* spp.). The disease was first reported in Oregon (United States) (Barss 1913) but today it is distributed worldwide, being present in countries such as Canada, Chile, Iran, Australia, New Zealand, and the United States and in many areas of Europe (EPPO 2021a). The symptoms of bacterial blight include spots on leaves, fruits, and twigs; trunk cankers; and defoliation. Losses due to this pathogen have not been accurately estimated but are considered high at the global level (Kałużna et al. 2021; Lamichhane and Varvaro 2014).

In this work, the complete genome sequences of two strains of each of the above-described pathovars were determined. The strains were *X. arboricola* pv. *pruni* IVIA 2626.1 and CITA 33, *X. arboricola* pv. *juglandis* IVIA 1317 and IVIA 2499, and *X. arboricola* pv. *corylina* CFBP 1846 and IVIA 3978. Pathogenicity of *X. arboricola* pv. *pruni* strains was confirmed in previous works by inoculation in several hosts (Garita-Cambronero et al. 2016a); meanwhile, pathogenicity of *X. arboricola* pvs. *juglandis* and *corylina* was verified in this work by leaf infiltration in their respective hosts to ensure the information provided by their original bacterial collections. Walnut and hazelnut leaves were infiltrated with *X. arboricola* pv. *juglandis* and *X. arboricola* pv. *corylina* suspensions, respectively (OD_{600} = 1), and symptoms were observed after 7 days postinoculation. Moreover, to fulfill Koch's postulates, *X. arboricola* pvs. *juglandis* and *corylina* strains were reisolated from inoculated leaves resulting in typical yellow colonies on the plates, which were identified as the bacterial species inoculated (Supplementary Fig. S1).

Draft genomes of both *X. arboricola* pv. *pruni* strains (IVIA 2626.1 and CITA 33) were already available (Garita-Cambronero et al. 2014, 2016b); moreover, the complete genome of another *X. arboricola* pv. *pruni* strain has been recently published (Back et al. 2020). The complete sequence of genomes from two *X. arboricola* pv. *juglandis* strains (417 and CPBF 427) were published (Pereira et al. 2015; Teixeira et al. 2021); meanwhile, other *X. arboricola* pv. *juglandis* complete genomes were recently submitted to GenBank under the accession numbers NZ_LR824643.1, NZ_HG999365.1, and NZ_HG999362.1. Some complete genomes of *X. arboricola* pv. *corylina* strains were also recently reported: the one of *X. arboricola* pv. *corylina* A7 (Núñez et al. 2022) and those with GenBank accession numbers HG992341.1, HG992338.1 (with its plasmid XAC301_p24; accession number HG992339.1), and HG992342.1 (with its plasmid CFBP6600_p24; accession number HG992343.1). Most of these genome sequences were obtained using just a single sequencing platform and only some of them combining two and by a hybrid assembly approach.

In recent years, approaches combining sequencing platforms have been popularized because they may result in high-quality sequences and closed bacterial genomes (Arias-Giraldo et al. 2020; Teixeira et al. 2020; Zhang et al. 2021). The genomes presented in this work were obtained using Pacific Biosciences' long-read technology, combined with the Illumina platform to get high-quality de novo complete genomes. Although PacBio offers long reads that diminish the risk of misassembles due to repeat elements, it has also been demonstrated to show relatively high sequence error rates (Dominguez Del Angel et al. 2018). These errors can be corrected using Illumina short but accurate reads (Dominguez Del Angel et al. 2018). Therefore, combined approaches allowed us to obtain complete circularized assemblies of the genomes and high-quality sequences, which is crucial to understand minimal genomic differences among closely related genomes such as those of the different *X. arboricola* pathovars, and to elucidate every genomic element on them. The assembly of bacterial genomes with data from two different sequencing platforms may be performed by hybrid assembly or by long-read-only assembly followed by polishing with Illumina short reads (Zhang et al. 2021). The last option was chosen because it has been identified as an accurate technology for bacterial genomes (Zhang et al. 2021).

In order to obtain bacterial DNAs, *X. arboricola* strains were grown 48 h in Luria-Bertani broth and incubated overnight at 27°C on a rotatory shaker. Genomic DNA extraction was performed using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions, with minor variations. Briefly, each centrifugation was sped up to 1,000 rpm, the elution from the columns was performed three times instead of twice, and sterilized bidestilled water at 70°C was used. Concentration and quality of the DNA were measured in a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, U.S.A.).

Sequencing, raw data filtering, and assembly were performed by Macrogen (Seoul, South Korea). In brief, PacBio long reads were preprocessed and assembled de novo with HGAP3 (GUI.HGAP v3.0), run with the default options. After assembly, Illumina reads were applied for error correction using Pilon v1.21 (Walker et al. 2014), as shown in Supplementary Table S1. After this, the corresponding consensus sequence was obtained.

Regarding *X. arboricola* pv. *pruni* IVIA 2626.1, HGAP3 was only able to assemble the chromosome sequence but lacked plasmid pXap41, which was previously identified in this strain by our group (Garita-Cambronero et al. 2016b). For this reason, de novo assembly of the plasmid was performed. Illumina and PacBio reads mapping the pXap41 plasmid (NCBI accession number FR875157.1) (Pothier et al. 2011) were extracted using mira 4.9.6 (Chevreux et al. 1999) and minimap2 2.18-r1015 (Li 2018), respectively. After this, a hybrid assembly of the plasmid was achieved with the Unicycler v0.4.8 pipeline (Wick et al. 2017), and its depth was calculated by aligning short and long reads against the assembly with Bowtie 2 version 2.4.4 (Langmead and Salzberg 2012) and minimap2 2.18-r1015, and using SAMtools 1.12 (command: samtools coverage) (Danecek et al. 2021) to determine the mean coverage. Just in case more misassemblies had occurred, *X. arboricola* pv. *juglandis* and *X. arboricola* pv. *corylina* Illumina reads were run under the plasmidSPAdes pipeline (SPAdes genome assembler v3.15.2) (Antipov et al. 2016; Prjibelski et al. 2020) to assemble putative plasmids; however, no new plasmids were assembled.

Genome sequences were annotated with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) version 5.2 (Haft et al. 2018; Li et al. 2021; Tatusova et al. 2016).

Using the approach mentioned above, complete assemblies of the genomes of *X. arboricola* pv. *pruni* strains IVIA 2626.1 and CITA 33, *X. arboricola* pv. *juglandis* strains IVIA 1317 and IVIA 2499, and *X. arboricola* pv. *corylina* strains CFBP 1846 and IVIA 3978 were obtained. The resulting *X. arboricola* pv. *pruni* IVIA 2626.1 genome comprised two circular contigs, a 5.11-Mb chromosome and a 41.10-kb plasmid, while CITA 33 was composed of a 5.09-Mb chromosome and 41.11-kb plasmid (Table 1). These complete genomes constituted an improvement compared with the available draft genomes (Garita-Cambronero et al. 2014, 2016b) because the draft genome of *X. arboricola* pv. *pruni* IVIA 2626.1 consisted of 214 contigs (92-fold coverage) and that of *X. arboricola* pv. *pruni* CITA 33 consisted of 501 contigs (95-fold-coverage). The resulting *X. arboricola* pv. *juglandis* IVIA 1317 and IVIA 2499 genomes were composed of one contig, corresponding to a chromosome of 5.27 Mb in both cases (Table 1). *X. arboricola* pv. *corylina* CFBP 1846 had only one circular chromosome of 5.22 Mb, while IVIA 3978 presented two circular contigs, a chromosome of 5.06 Mb and a putative plasmid of 18.38 kb (Table 1). According to the PLSDB v. 2020_11_19 tool (Galata et al. 2019) (search strategy: mash screen, winner-takes all), this plasmid is slightly similar to *X. fragariae* strain PD885 plasmid pPD885-27 (NZ_LT853884.1) and *X. hortorum* pv. *gardneri* strain CFBP 8129 plasmid CFBP8129_p26 (NZ_LR828256.1).

Moreover, in order to assess the quality and completeness of the genome assemblies, the benchmarking universal single-copy ortholog (BUSCO) software v5.2.2 (Manni et al. 2021) was used with the bacteria and the *Xanthomonadales* lineages (bacteria_odb10 and xanthomonadales_odb10, respectively). All of the chromosomes exhibited 100% complete (C) and single-copy BUSCOs (S) compared with bacteria_odb10. Using the lineage xanthomonadales_odb10, which contains 1,152 BUSCOs, a percentage of complete BUSCOs ≥99.7% was observed in all of the strains (Table 1).

The average nucleotide identity was calculated using BLASTN+ with pyani 0.2.11 (Pritchard et al. 2016). This analysis allowed a precise taxonomic identification of the strains and construction of a percentage identity tree where genomes from the strains grouped with their corresponding pathovars (Fig. 1). In the case of *X. arboricola* pv. *juglandis*, strains IVIA 1317 and IVIA 2499 were grouped with WBB and VOC strains identified in previous works (Cesbron et al. 2015; Pereira et al. 2015; Teixeira et al. 2021) but separated from CPBF 1494 and CPBF 765, isolated from pecan (*Carya illinoinensis*), which clustered into another branch of the

Table 1. Relevant features of the complete genome assemblies of *Xanthomonas arboricola* pv. *pruni* strains IVIA 2626.1 and CITA 33 (and comparison with the available draft genomes), *X. arboricola* pv. *juglandis* strains IVIA 1317 and IVIA 2499, and *X. arboricola* pv. *corylina* strains CFBP 1846 and IVIA 3978

	Strain, reference[a]										
	IVIA 2626.1			CITA 33			IVIA 1317	IVIA 2499	CFBP 1846	IVIA 3978	
Variables	G 2016b	This article		G 2014	This article		This article	This article	This article	This article	
Sequencing technology[b]	Ion	Illum Nova, PacBio		Ion	Illum Nova, PacBio		Illum Nova, PacBio	Illum Nova, PacBio	Illum Nova, PacBio	Illum Nova, PacBio	
Genomic feature	Draft genome	Chromosome	Plasmid	Draft genome	Chromosome	Plasmid	Chromosome	Chromosome	Chromosome	Chromosome	Plasmid
Number of contigs	214	1	1	501	1	1	1	1	1	1	1
Length (bp)	5,027,671	5,106,361	41,102	5,104,864	5,087,099	41,110	5,271,558	5,272,621	5,223,792	5,062,837	18,383
GC (%)	65.40	65.38	62.31	65.43	65.40	62.31	65.42	65.42	65.43	65.51	60.23
Coding sequence	4,720	4,265	46	4,348	4,241	46	4,380	4,385	4,371	4,212	24
Transfer RNA	47	53	0	50	53	0	53	53	54	53	0
Ribosomal RNA	3	6	0	3	6	0	6	6	6	6	0
Noncoding RNA	–	37	0	–	37	0	39	39	37	36	0
Transfer messenger RNA	–	1	0	–	1	0	1	1	1	1	0
Regulatory	–	6	0	–	6	0	6	6	6	6	0
Genes	4,770	4,362	46	4,401	4,338	46	4,479	4,484	4,469	4,308	24
Depth	92	121	1396.96	95	120	390	152	110	151	137	322
BUSCO (%)[c]	–	C:99.7 [S:99.7, D:0.0], F:0.3, M:0.0	–	–	C:99.9 [S:99.9, D:0.0], F:0.1, M:0.0	–	C:99.9 [S:99.8, D:0.1], F:0.0, M:0.1	C:99.9 [S:99.8, D:0.1], F:0.0, M:0.1	C:99.8 [S:99.8, D:0.0], F:0.1, M:0.1	C:99.7 [S:99.7, D:0.0], F:0.2, M:0.1	–
Xanthomonas arboricola pathovar	*pruni*	*pruni*	*pruni*	*pruni*	*pruni*	*pruni*	*juglandis*	*juglandis*	*corylina*	*corylina*	*corylina*
Isolation source	*Prunus salicina*	*P. salicina*	*P. salicina*	*P. dulcis*	*P. dulcis*	*P. dulcis*	*Juglans regia*	*J. regia*	*Corylus avellana*	*C. avellana*	*C. avellana*
GenBank accession number	LJGN00000000.1	CP076628	CP076627	JHUQ00000000.1	CP076701	CP076702	CP076725	CP076726	CP076619	CP076534	CP076535

[a] References: This article, G 2016b = Garita-Cambronero et al. 2016b, and G 2014 = Garita-Cambronero et al. 2014.
[b] Ion = Ion Torrent, Illum Nova = Illumina NovaSeq, and PacBio = PacBio RSII.
[c] Benchmarking universal single-copy ortholog (lineage dataset: xanthomonadales_odb10; *n:1152*).

tree. This last result may be explained because of the high diversity identified previously within the *X. arboricola* pv. *juglandis* group (Fernandes et al. 2021).

NCBI PGAP annotation resulted in 4,408 features (coding sequences and RNA sequences) for *X. arboricola* pv. *pruni* IVIA 2626.1 and 4,384 features for *X. arboricola* pv. *pruni* CITA 33; 4,479 features for *X. arboricola* pv. *juglandis* IVIA 1317 and 4,484 features for *X. arboricola* pv. *juglandis* IVIA 2499; and 4,469 for *X. arboricola* pv. *corylina* CFBP 1846 and 4,332 total features for *X. arboricola* pv. *corylina* IVIA 3978. Moreover, the annotations of the draft genomes of *X. arboricola* pv. *pruni* IVIA strains 2626.1 and CITA 33 were mapped against the annotations of the new complete genomes using Geneious Prime version 2021.2.2 (https://www.geneious.com), and a higher amount of complete genes was achieved: 0.90% of improved genes in the chromosome of *X. arboricola* pv. *pruni* strain IVIA 2626.1 versus IVIA 2626.1 draft; 0.71% of improved genes in the chromosome of strain CITA 33; and 1.22% of improved genes in the plasmid versus strain CITA 33 draft. Furthermore, errors such as duplicated genes were also corrected.

The information presented in this work will contribute to understanding the biology of these bacteria and to better explain infection mechanisms, particularly those specific processes involved in the specific host range of the most virulent pathovars of *X. arboricola*.

Data Availability

The complete chromosome sequence of *X. arboricola* pv. *pruni* strain IVIA 2626.1 has been deposited in GenBank under accession number CP076628, and the plasmid sequence under CP076627; the complete chromosome and plasmid sequences of *X. arboricola* pv. *pruni* strain CITA 33 have been deposited under accession numbers CP076701 and CP076702, respectively. The complete genome sequences of *X. arboricola* pv. *juglandis* strains IVIA 1317

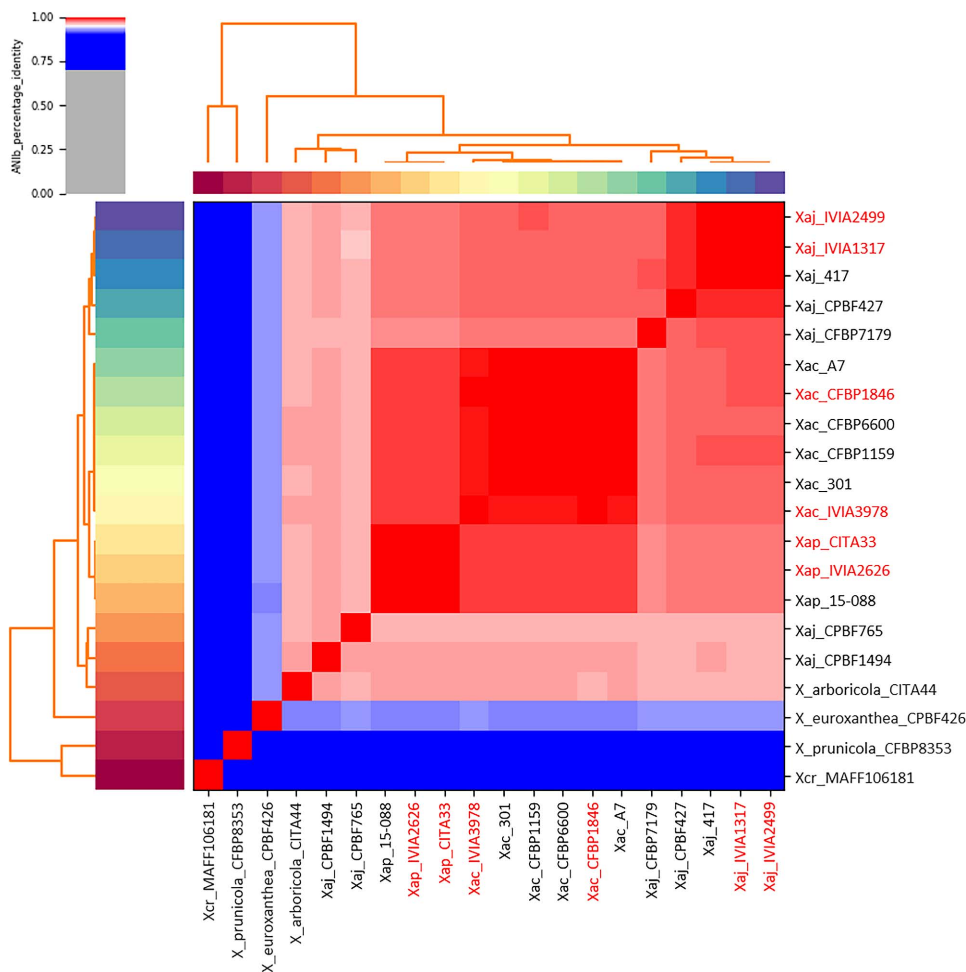

Fig. 1. Heatmap representing the percentage of identity (average nucleotide identity calculated using BLASTN+) between *Xanthomonas arboricola* pv. *pruni* (Xap) strains IVIA 2626.1 and CITA 33, *X. arboricola* pv. *juglandis* (Xaj) strains IVIA 1317 and IVIA 2499, *X. arboricola* pv. *corylina* (Xac) strains CFBP 1846 and IVIA 3978, and several *Xanthomonas* spp. strains (Supplementary Table S2). The shading scale represents higher to lower identity values.

and IVIA 2499 have been deposited in GenBank under accession numbers CP076725 and CP076726, respectively. The complete genome sequence of *X. arboricola* pv. *corylina* strain CFBP 1846 has been deposited in GenBank under accession number CP076619, and the complete chromosome and the putative plasmid sequences of *X. arboricola* pv. *corylina* strain IVIA 3978 have been deposited under accession numbers CP076534 and CP076535, respectively.

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