RESOURCE ANNOUNCEMENT

Complete Genome and Plasmid Sequence Data of Three Strains of *Xanthomonas arboricola* pv. *corylina*, the Bacterium Responsible for Bacterial Blight of Hazelnut

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Abstract

Xanthomonas arboricola pv. *corylina* is the causal agent of bacterial blight of hazelnut. The bacterium has been listed as an A2 quarantine pathogen in Europe since 1978 and on the regulated non-quarantine pest list since 2019. Three isolates from various geographic regions and isolated at different times were sequenced using a hybrid approach with short- and long-read technologies to generate closed genome and plasmid sequences in order to better understand the biology of this pathogen.

Genome Announcement

Bacterial blight of hazelnut (*Corylus* spp.) was first reported in the early 20th century in Oregon (Barss 1913; Kałużna et al. 2021). The disease is caused by *Xanthomonas arboricola* pv. *corylina* and has since been reported in countries from all continents apart from Arctic and Antarctic (Kałużna et al. 2021). To limit the risk of introduction to other countries, especially via planting material, this Gram-negative bacterium was listed by the European Plant Protection Organization as an A2 quarantine pathogen in 1978 and as a regulated non-quarantine pest (Picard et al. 2018) since 2019 (European Union 2019).

The most important host for *X. arboricola* pv. *corylina* is *Corylus avellana* L. (the common hazel) but other plant species such as *Corylus pontica*, *Corylus maxima*, and *Corylus colurna* were also found to be susceptible, although considered as minor hosts (OEPP/EPPO 1986, 2004).

Here, we report the complete genome sequences of three strains of *X. arboricola* pv. *corylina* (Table 1). The strains were isolated between 1939 and 2007 from either *C. avellana* or *C. maxima* and from three different countries (Table 1). These complete genomes should contribute to unveiling the ecology, evolution, and virulence of this economically relevant bacterium for hazelnut cultivation.

The CFBP 1159[PT] and CFBP 6600 strains were initially obtained as freeze-dried cultures in glass ampoules from the international strain collection Collection Française de Bactéries Associées aux Plantes (CFBP) (Beaucouzé, France). Strains were revived, stored, and handled as described previously (Dia et al. 2020). Strain Xac301 was isolated in 2007 in Poland from symptomatic leaf spots of a hazelnut (Puławska et al. 2010). Characteristic mucoid, yellow colonies were obtained and a pure colony initially called RIPF X12 (=Xac301) was further grown on yeast extract nutrient agar (YNA) medium. This isolate was identified as *X. arboricola* pv. *corylina* based on cellular fatty acid content converted to methyl esters as well as in *gyrB*

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Keywords
bacteria, bacterial pathogens, bioinformatics, complete genome, genomics, hazelnut, microbe-genome sequencing, *Xanthomonas arboricola* pv. *corylina*

Table 1. Genome metrics and accession numbers of the newly sequenced *Xanthomonas arboricola* pv. *corylina* genomes

Variables[a]	Strain		
	CFBP 1159[PT]	CFBP 6600	Xac301
Origin (year)	United States (1939)	France (1977)	Poland (2007)
Host	*Corylus maxima*	*C. avellana*	*C. avellana*
Genome size (bp)	5,080,866	5,234,232	5,294,219
G+C content (%)	65.56	65.42	65.37
Total number of genes	4,279	4,499	4,461
Number of plasmids	0	1	1
Illumina data			
Total number of reads	1,511,890	1,952,794	2,516,854
Average read length (bp)	301	301	251
Average coverage (×)	77	100	110
Oxford Nanopore data			
Total number of reads	50,946	27,923	129,031
Read length N_{50} (bp)	32,779	38,810	22,574
Average coverage (×)	44	20	295
SRA accession number (MinION/MiSeq)	ERR5260054, ERR5260059	ERR5260055, ERR5260060	ERR5260056, ERR5260083
ENA accession number	HG992341 (chr)	HG992342 (chr), HG992343 (p24)	HG992338 (chr), HG992339 (p24)
ANI[b]	100	99.7	99.93
BUSCO score (%)	99.8	99.7	99.7

[a] The culture collection providing strains is abbreviated in the strain name as CFBP (Collection Française de Bactéries Associées aux Plantes, Beaucouzé, France). Superscript PT following the strain name indicates the pathotype strain for the pathovar. Abbreviations: SRA = Sequence Read Archive, ENA = European Nucleotide Archive, and chr = chromosome.
[b] Average nucleotide identity (ANI) using BLAST (ANIb) is relative to CFBP 1159[PT].

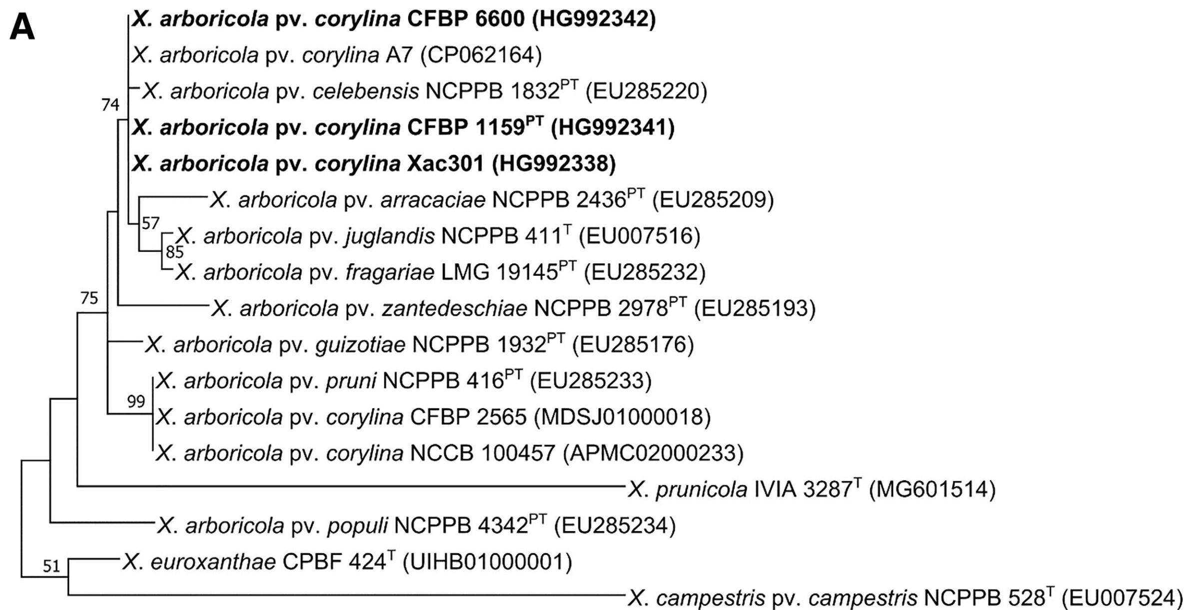
gene fragment sequence analysis (Puławska et al. 2010). The *gyrB* sequence of Xac301 was most similar to the sequence of the *X. arboricola* pv. *corylina* pathotype strain (Fig. 1A). Koch's postulates were validated with strain Xac301 using leaf inoculation of hazelnut cultivars 'Webb's Prize Cob', 'Cosford', and 'Merveille de Bollwiller' (Fig. 1B). The isolate was stored in a –80°C ultrafreezer in a mixture of 20% glycerol (vol/vol) and phosphate-buffered saline buffer (0.27% Na_2HPO_4, 0.04% NaH_2PO_4, and 0.8% NaCl) until further use. Before extraction of DNA, Xac301 was revived and cultured on YNA medium and incubated at 26°C for 48 to 72 h.

For strain Xac301, genomic DNA (gDNA) for both short- and long-read sequencing was isolated using the modified method of Aljanabi and Martinez (1997) from cells grown overnight at 26°C on YNA, as described previously (Kałużna et al. 2012). For short- read sequencing, library preparation was done using a NEBNext DNA Library Prep Master Mix Set for Illumina (NEB, Ipswich, MA, U.S.A.). Pooled libraries were sequenced on a MiSeq sequencer (Illumina, San Diego, CA, U.S.A.) with 2 × 250-bp paired-end reads using a MiSeq reagent kit, version 2 (Illumina).

For the CFBP 1159[PT] and CFBP 6600 strains, gDNA for Illumina MiSeq short-read sequencing was extracted from cells grown overnight at 28°C in nutrient yeast extract glycerol broth using the NucleoSpin tissue kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's protocol. The quality of the gDNA was checked using a fragment analyzer (Advanced Analytical Technologies, Inc., Ankeny, IA, U.S.A.) and quantified using the Quant-iT PicoGreen double-stranded DNA quantification assay (Thermo Fisher Scientific, Waltham, MA, U.S.A.). Library preparation was done using the Nextera XT DNA library prep kit (Illumina) following the manufacturer's instructions. Sequencing of pooled libraries was performed on a MiSeq Illumina sequencer with 2 × 300-bp paired-end reads using a MiSeq reagent kit, version 3 (Illumina), according to the manufacturer's instructions.

For long-read sequencing, the gDNA of strains CFBP 1159[PT] and CFBP 6600 was extracted from overnight-grown cells using the Gentra PureGene Yeast/Bact kit protocol (Qiagen, Hilden, Germany). The gDNA quality was checked as described above and quantified using a high-sensitivity, double-stranded DNA quantitation kit (Allsheng, Hangzhou, China) and a Fluo-100B fluorometer (Allsheng).

For the three strains, long-read library preparation and sequencing were performed with the ligation sequencing kit (catalog number SQK-LSK109 for CFBP 1159[PT] and CFBP 6600 and catalog number SQK-LSK108 for Xac301; Oxford Nanopore Technologies, Oxford, United Kingdom) and run on an R9.4.1 flow cell with a MinION sequencer. The native barcoding



B

Fig. 1. A, Maximum-likelihood unrooted phylogenetic tree based on a 530-bp *gyrB* partial sequences of *Xanthomonas* strains. Phylogenetic and molecular evolutionary analyses were conducted using MEGA X, version 10.0.5 (Kumar et al. 2018). The alignment was obtained using the MUSCLE algorithm. The tree was constructed using the JTT matrix-based model. Percent bootstrap values calculated for 1,000 iterations are indicated near nodes and displayed only when >50. Accession numbers or source for *gyrB* sequences are indicated within parentheses next to the species name, with strains sequenced in this study marked in bold. Bar represents the expected number of substitutions per site. Superscripts following strain names: T indicates the type strain of a species and PT indicates the pathotype strain for a pathovar. **B,** Leaf spot symptoms developing on *Corylus avellana* 'Cosford' after syringe infiltration with *Xanthomonas arboricola* pv. *corylina* Xac301 and being kept for 10 weeks in the greenhouse under natural daylight conditions. Typical symptoms were already observed after 5 weeks postinoculation.

Table 2. Comparison of the improved hybrid assembly versus the existing assembly of *Xanthomonas arboricola* pv. *corylina* CFBP 1159^{PT}

Assembly name	GCA_905220785	ASM293984v1
Sequencing technology	Illumina MiSeq + Oxford Nanopore MinION	Illumina HiSeq
Assembler	Trycycler v.0.3.3 + Pilon v.1.22	Velvet v.1.2.07 + SOAPdenovo v.2.04
Coverage	121×	100×
Total sequence length (bp)	5,080,866	5,105,973
Number of contigs	1	124
N_{50}	5,080,866	135,548
G+C content (%)	65.56	65.50
Annotation pipeline	Prokka v.1.14.5	NCBI PGAP v.4.2
Number of		
Coding sequences	4,214	4,394
Ribosomal RNAs (5S, 16S, 23S)	2, 2, 2	1, 1, 1
Transfer RNAs	57	51
BUSCO score (%)	99.8	99.8

expansion kit (catalog number XP-NBD114) was used for multiplexing. Reads were basecalled and demultiplexed using Guppy, version 3.3.3.

Short- and long-read library preparation and sequencing were outsourced at Genomed S.A. (Warsaw, Poland) in the case of strain Xac301. For strains CFBP 1159^{PT} and CFBP 6600, these steps were outsourced at BSSE Genomics Facility (Basel, Switzerland) for short-read libraries and carried out in the Environmental Genomics and Systems Biology Research Group lab facilities (ZHAW) for long reads.

De novo hybrid assemblies using the MiSeq and MinION reads were conducted with Trycycler, version 0.3.3 (Wick et al. 2021). In total, 10,551, 10,533, and 199 nucleotide changes were performed during the first short-read polishing round for CFBP 1159^{PT}, CFBP 6600, and Xac301, respectively, using Pilon, version 1.22. The genomes were then annotated using Prokka, version 1.14.5 (Seemann 2014). All tools were run with default parameters unless otherwise specified.

The size of the hybrid assemblies ranged from 5,080,866 to 5,294,219 bp, a size range typically found in *Xanthomonas* genomes (Table 1). The G+C contents of the genomes varied from 65.37 to 65.56%, also comparable with other *Xanthomonas* spp. G+C contents. Whole-genome comparison based on average nucleotide identity using BLASTN (ANIb) implemented in pyANI, version 0.2.10 (Pritchard et al. 2016), confirmed that the three strains had high degree of synteny between them (Table 1) and to other *X. arboricola* genomes (data not shown). Genome completeness varied between 99.7 and 99.8% (Table 1) when assessed using the Benchmarking Universal Single-Copy Orthologs (BUSCO), version 5.2.1 (Manni et al. 2021) and the xanthomonodales_odb10 (2020-03-06) lineage dataset.

Because an assembly already existed for the pathotype strain CFBP 1159^{PT}, a comparison was performed versus the hybrid assembly presented here which revealed some improvements and few minor differences (Table 2).

A single 24-kb plasmid was present in the final assemblies of CFBP 6600 and Xac301. This plasmid contains the type three effector (T3E) XopAG (HopG1) and the avirulence protein XopE2. The presence of XopAG in two of the three strains and the plasmid-borne localization of this T3E agrees with previous observations from a draft genome sequence of this same pathovar (Ibarra Caballero et al. 2013). In the genome of CFBP 1159^{PT}, XopE2 was detected on the chromosome. The T3E AvrBs3 was also detected in the genome of the CFBP 1159^{PT} pathotype strain whereas it is absent from the two other genomes presented in this work, as previously reported from another draft genome of this same pathovar (Ibarra Caballero et al. 2013). The presence of the *copAB* operon and *copL* gene, whose products are involved in copper resistance in this pathogenic bacteria (Kałużna et al. 2021), was detected in the chromosome of all three strains. Similarly, *cutC* and *pCuAC* genes, whose product could be implicated in the survival of this bacterium at high copper concentration (Nuñez Cerda et al. 2022), were also found in all three strains.

The sequenced genomes discussed here will be used for further analysis of evolution within the species *X. arboricola*, producing a better understanding of the pathogenicity and virulence as well as development of improved tools for diagnostics of this relevant pathogen for the worldwide production of hazelnut.

Data Availability

The raw data and assembled and annotated genome sequences have been deposited in the European Nucleotide Archive under BioProject number PRJEB42844. The genome and raw read accession numbers for each strain are shown in Table 1.

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