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Complete Genome Sequence Data of Two *Xanthomonas arboricola* Strains Isolated from Blueberry Plants Displaying Bacterial Leaf Blight in Poland

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Keywords
bacteria, blueberry, complete genome, genomics, *Xanthomonas arboricola*

Genome Announcement

The cultivation of blueberry (*Vaccinium corymbosum* L.) is becoming increasingly important due to the high content of beneficial nutrients in the fruit, its attractiveness, and its high profitability. Both the worldwide total area harvested and production of blueberries have increased significantly since 1961 (FAOSTAT 2021). Poland is among the top 10 producers of blueberries, with an annual production that has reached the unprecedented amount of 34,770 tons in 2019 (FAOSTAT 2021) and, in 2021, the harvest was at the level of 55,300 tons (GUS 2021). For many years, this plant species remained rarely infected by bacterial pathogens. Hitherto described, but not present at a large intensity were tumorigenic *Agrobacterium* spp. causing crown gall (Alippi et al. 2012), *Burkholderia andropogonis* causing bacterial leaf spot (Kobayashi et al. 1995), and bacterial leaf scorch caused by the new emerging pathogen *Xylella fastidiosa* (Chang et al. 2009). Recently, blueberry plantations have been increasingly affected by *Pseudomonas* spp. (Kałużna et al. 2013), the causal agent of bacterial canker. New pathogenic bacteria—the subject of this study—were discovered that were never recorded on blueberry plantations. The strains reported here constitute the first report of a *Xanthomonas* sp. causing symptoms on this plant which might be economically relevant. The two strains were sequenced for further analysis of evolution within the species *Xanthomonas arboricola*, for determining whether the strains constitute a new pathovar within the species and improving the molecular diagnostics of this new pathogen.

In 2013, blueberry cultivars Toro and Duke growing in a nursery located in Central Poland presented russet-brown, irregular spots on leaves (Fig. 1A). From these leaf spots, fluorescent and yellow bacteria were isolated. Colony morphology of yellow isolates resembling that of the *Xanthomonas* genus were obtained on yeast extract nutrient agar (YNA) medium (Schaad et al. 2001). Two yellow isolates, 1311a and 1314c, obtained from 'Toro' and 'Duke', respectively (Table 1), were positive in a PCR assay using primers X1 and X2 specific for bacteria belonging to the genus *Xanthomonas* (Maes 1993). The pathogenicity tests performed on blueberry cultivar Bluecrop confirmed their pathogenic ability (Fig. 1B). Based on partial sequence analysis of *gyrB*, the strains were not closely related to each other; however, both were placed within *X. arboricola* strains (Fig. 1C), a species known to cause symptoms on several fruit trees but never reported on blueberry, like any other *Xanthomonas* sp. so far. A further taxonomic study relying on multilocus sequence analysis of partial sequence analysis of *gyrB*, *fuyA*, and *rpoD* (totaling 1,635 bp) confirmed the definitive classification of these isolates (data not shown). The isolates were stored at –80°C in a mixture of glycerol 20% (vol/vol) and phosphate-buffered saline buffer

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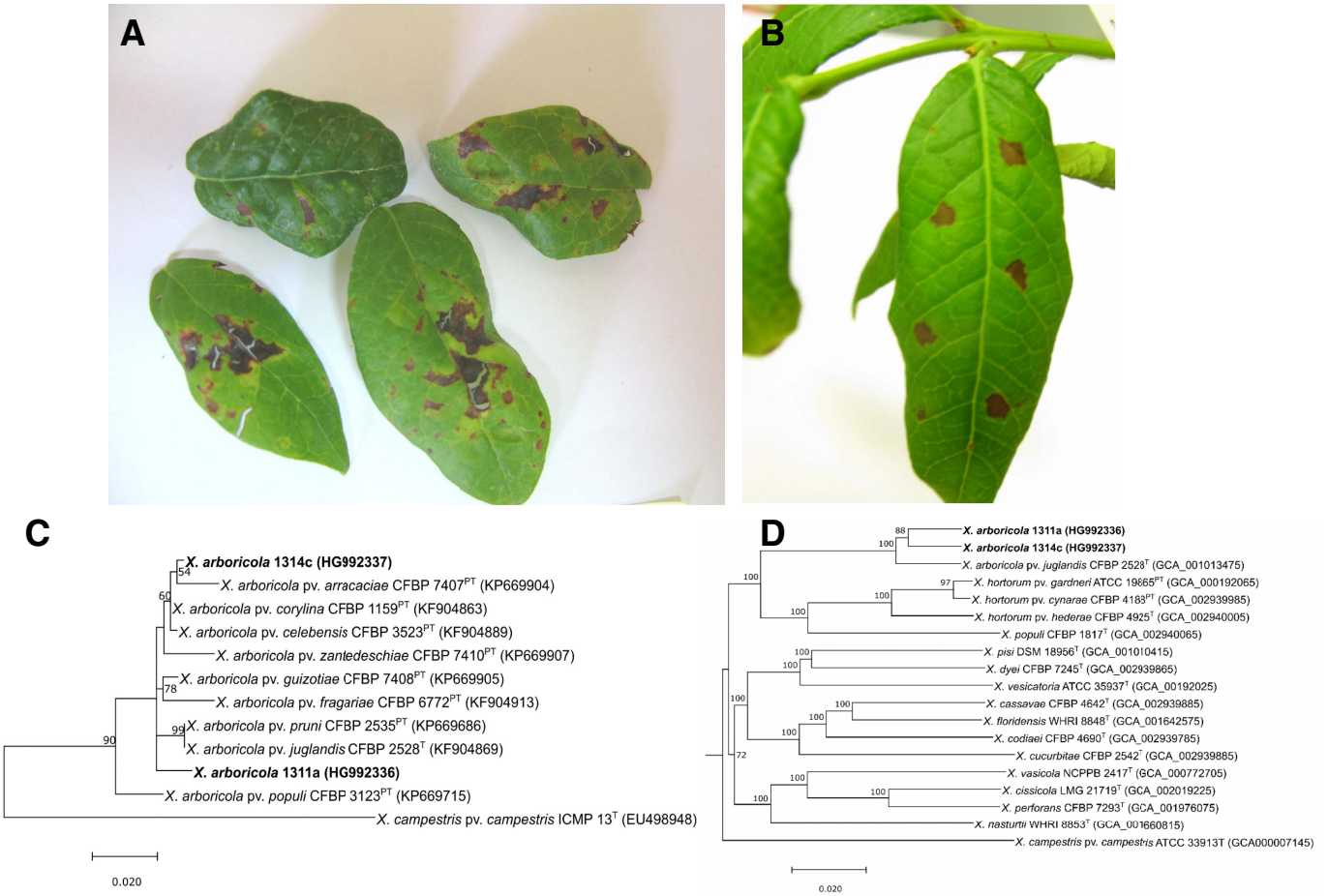

Fig. 1. A, Leaves of *Vaccinium corymbosum* 'Duke' naturally infected by *Xanthomonas arboricola* 1314c and obtained from a nursery in Central Poland in 2013 displaying leaf spot symptoms. **B,** Leaf spots symptoms developing on *V. corymbosum* 'Bluecrop' after syringe infiltration with *X. arboricola* 1314c after 2 to 3 weeks postinoculation in the greenhouse under natural daylight conditions. **C,** Maximum-likelihood unrooted phylogenetic tree based on the analysis of 508 bp of *gyrB* partial sequences of *Xanthomonas* spp. The dendrogram was constructed based on the Tamura-Nei model. A discrete γ distribution was used to model evolutionary rate differences among sites (five categories; +*G*, parameter = 0.2776). Analyses were conducted using MEGA X version 10.0.5 (Kumar et al. 2018). Bootstrap values (expressed as percentages of 500 replicates) are indicated at each node and displayed only when over 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. Superscripts following strain names: T indicates the type strain of a species and PT indicates the pathotype strain for a pathovar. **D,** Whole-genome BLAST distance phylogeny (GBDP) inferred with the Type (Strain) Genome Server (TYGS) (Meier-Kolthoff and Göker 2019). Results were provided by the TYGS on 27 October 2021 using the two genomes sequenced in this study in addition to 17 best-matching *Xanthomonas* type strains as determined by the TYGS platform. The tree was inferred with FastME version 2.1.6.1 from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d_5. Numbers above branches are GBDP pseudobootstrap support values >60% from 100 replicates, with an average branch support of 97.3%. The tree was rooted at the midpoint. Genome assembly accessions or accession numbers for genome sequences are indicated within parentheses next to the species name, with strains sequenced in this study marked in bold. Superscripts following strain names: T indicates the type strain of a species and PT indicates the pathotype strain for a pathovar.

(0.27% Na_2HPO_4, 0.04% NaH_2PO_4, and 0.8% NaCl) until further use. Before extraction of DNA for genomic analysis, the strains were cultured on YNA medium and incubated at 26°C for 48 h.

Genomic DNA for short- and long-read sequencing was isolated according to the salt-extraction method described by Aljanabi and Martinez (1997), with slight modifications (Kałużna et al. 2012). A paired-end library (with insert size of approximately 350 bp) was prepared with the NEBNext DNA Library Prep Master Mix Set for Illumina (NEB, Ipswich, MA, U.S.A.). 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.

The library for the MinION sequencing was prepared with the ligation sequencing kit (catalog number SQK-LSK1O8; Oxford Nanopore Technologies, Oxford, United Kingdom) and run on an R9.4.1 flow cell with a MinION sequencer. The native barcoding expansion kit (catalog number XP-NBD114) was used for multiplexing. Reads were base called and demultiplexed using Guppy version 3.3.3.

Short- and long-read library preparation and sequencing were outsourced at Genomed S.A. (Warsaw, Poland).

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

	Strain	
Parameters[a]	**1311a**	**1314c**
Origin (year)	Poland (2013)	Poland (2013)
Host	*Vaccinium corymbosum* 'Toro'	*Vaccinium corymbosum* 'Duke'
Genome size (bp)	4,889,021	4,891,115
GC content (%)	65.71	65.7
Total number of genes	4,049	4,069
Illumina data		
Total number of reads	3,579,290	3,308,462
Average read length (bp)	251	251
Average coverage (×)	171	155
Oxford Nanopore data		
Total number of reads	68,923	432,617
Read length N_{50} (bp)	23,765	11,399
Average coverage (×)	149	300
SRA accession number (MinION/MiSeq)	ERR5260057/ERR5260084	ERR5260058/ERR5260086
ENA accession number	HG992336	HG992337
ANI (%)	96.54	96.54
dDDH (%)	70.5	70.5
BUSCO scores (%)	99.8	99.9

[a] SRA = Sequence Read Archive, ENA = European Nucleotide Archive, ANI = average nucleotide identity, dDDH = digital DNA-DNA hybridization, and BUSCO = benchmarking universal single-copy ortholog. ANI using BLAST (ANIb) and dDDH using the d_4 formula are relative to *X. arboricola* pv. *juglandis* CFBP 2528T (GenBank genome assembly accession GCA_001013475.1). BUSCO used the xanthomonodales_odb10 (2020-03-06) lineage dataset.

De novo hybrid assemblies using the MiSeq and MinION reads were conducted with Unicycler version 0.4.8 (Wick et al. 2017) and Trycycler version 0.3.3 for comparison purpose (Wick et al. 2021). In total, three and eight nucleotide changes for 1311a and 1314c, respectively, were performed during the first short-read polishing round 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.

A single chromosomal scaffold of 4.9 Mbp with a G+C content of 65.7% was obtained for both strains (Table 1). Similar assembly results were provided by the second hybrid assembler. Genome completeness was 99.8% and 99.9% (Table 1) when assessed using the benchmarking universal single-copy ortholog (BUSCO) version 5.2.1 (Manni et al. 2021) and the xanthomonodales_odb10 (2020-03-06) lineage dataset. 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 two strains had high degree of synteny (data not shown) to other *X. arboricola* genomes (data not shown) but particularly to *X. arboricola* pv. *juglandis* CFBP 2528^T, the type strain of the *X. arboricola* species (Table 1). Digital DNA-DNA hybridization and genome phylogeny inferred with the Type (Strain) Genome Server (Meier-Kolthoff and Göker 2019) also clearly assigned the two strains sequenced here to the *X. arboricola* species (Table 1; Fig. 1D).

To assess or highlight a relevant phytosanitary feature encoded by the two *X. arboricola* strains studied here, we analyzed the presence of genes involved in resistance to copper (Behlau et al. 2011). Results revealed the presence of the *copAB* operon and the *copL*, *cutC*, and *pCuAC* genes encoded by both strains. This observation suggests that these strains would survive at high copper concentration, as reported for other members of the species *X. arboricola* (Kałużna et al. 2021; Pothier et al. 2022).

To limit the risk of introduction to other countries, three of the nine known *X. arboricola* pathovars (Kałużna et al. 2021) were listed by the European Plant Protection Organization as A2 quarantine pathogens and as Regulated Non-Quarantine Pests (Picard et al. 2018) since the end of 2019 (European Union 2019). Molecular methods are currently available for the detection and diagnostics of two of these three regulated *X. arboricola* pathovars (Catara et al. 2021); namely, *X. arboricola* pv. *pruni* (Bühlmann et al. 2013; Palacio-Bielsa et al. 2011; Pothier et al. 2011) and *X. arboricola* pv. *juglandis* (Fernandes et al. 2017; Martins et al. 2019). Nevertheless, for the remaining regulated pathovar (*X. arboricola* pv. *corylina*), amplicons are also obtained with the species- and pathovar-level primer sets designed for *X. arboricola* pv. *pruni* (Kałużna et al. 2021; Pothier et al. 2011; Webber et al. 2020), thus allowing the detection of both regulated pathogens. Here, once PCR was performed with the DNA of strains 1311a

and 1314c, an amplicon was only observed with the species-level-specific primer set whereas no amplicon was observed with the pathovar-level primer set (pathovars *pruni* and *corylina*) or with the loop-mediated isothermal PCR set designed for *X. arboricola* pv. *pruni* (Bühlmann et al. 2013). Because our results suggest that the 1311a and 1314c strains belong to the *X. arboricola* species, we conclude that they also show the actual lack of a suitable molecular diagnostic for the strains causing symptoms on blueberry. Such molecular methods are required because further inspections confirmed the presence of *X. arboricola* on blueberry in other geographic localizations in Poland since the isolation of these two strains (M. Kałużna, *unpublished data*).

The sequenced genomes discussed here will be used for further analysis of evolution within the species *X. arboricola*, as well as for the development of improved diagnostics tools for this possibly relevant pathogen of blueberry to enable an effective disease control strategy.

Data Availability

The raw data and assembled or annotated genome sequences have been deposited in the European Nucleotide Archive under BioProject number PRJEB42845. Genome data and raw read accession numbers for strains 1311a and 1314c are listed in Table 1.

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