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Complete genome sequences and characterization of *Xanthomonas arboricola*, the novel causal agent of bacterial leaf blight of blueberry

INTRODUCTION

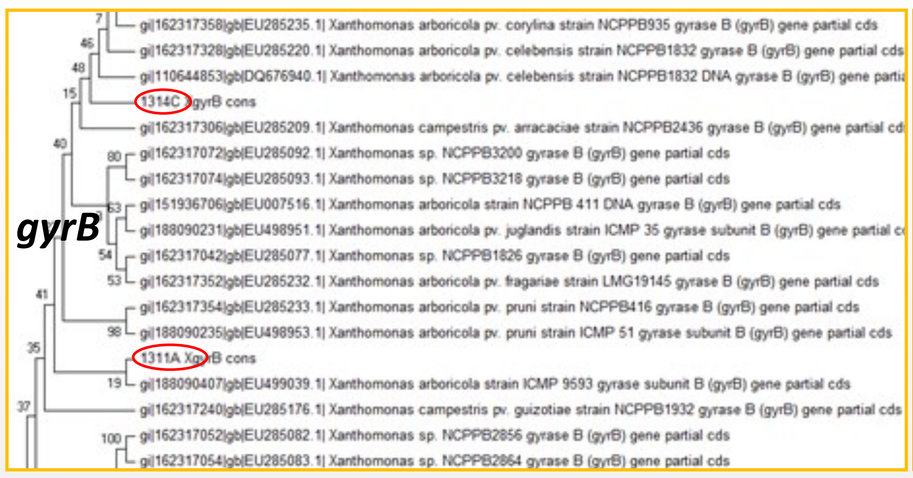
The cultivation of highbush blueberry (*Vaccinium corymbosum* L.) due to high content of pro-health values in the fruit, its attractiveness, followed by the high demand of the Polish and foreign markets is still increasing. For many years the blueberry was sporadically infected by bacterial pathogens. Hitherto described, but not present in a high intensity, were tumorogenic *Agrobacterium* spp. causing crown gall, *Burkholderia andropogonis* and the bacterial leaf scorch caused by the new emerging pathogen *Xylella fastidiosa*. Recently more and more the blueberry plantations are affected by *Pseudomonas* spp. – the causal agent of bacterial canker. Besides them, the novel pathogenic bacteria, never reported before in the blueberry plantations, were isolated.

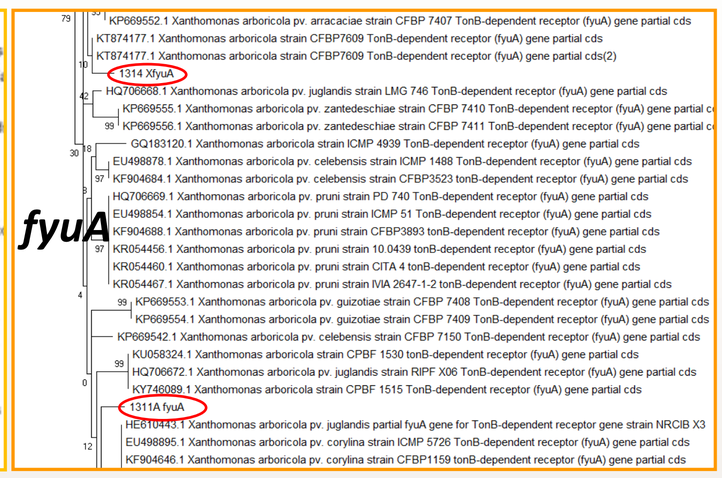
STUDIES

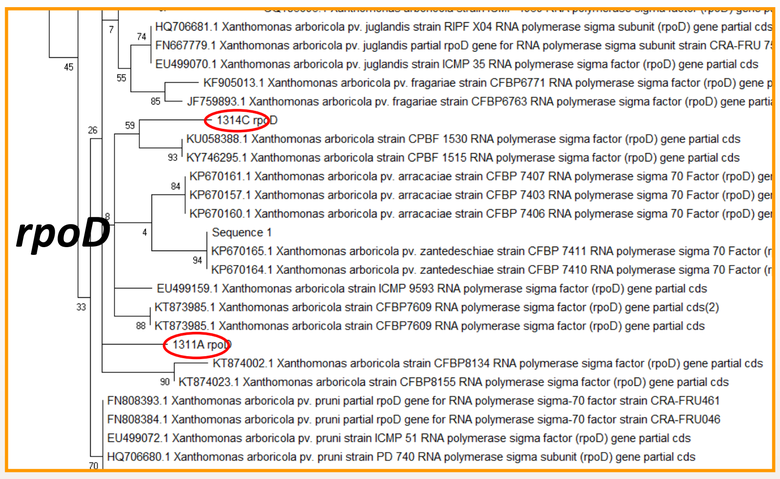
In 2013, on the blueberry plants cvs. Toro and Duke growing in a nursery located in Central Poland russet brown and irregular spots on leaves were observed. From these leaf spots, fluorescent and yellow pigmented bacteria were isolated. Two yellow isolates, named 1311a and 1314c, were positive in a PCR assay using primers X1 and X2 specific for bacteria belonging to the genus *Xanthomonas*. The pathogenicity test performed on blueberry plant confirmed their pathogenic ability.

Symptoms of bacterial leaf blight of blueberry

Based on partial sequences analysis of *gyrB*, *fuyA* and *rpoD* (total 1,635 bp), the strains were not closely related to each other, however, both were classified to *X. arboricola*, a species known to cause symptoms on several fruit trees but never reported on blueberry like any other *Xanthomonas* species so far.







The genomes size of the strains 1311a and 1314c, determined using short (MiSeq, Illumina) and long-read technologies (MinION, Oxford Nanopore) are 4,889,189bp and 4,891,143bp, respectively, with a G+C content of 65.7%. Whole genome-based taxonomic analysis using the Type (Strain) Genome Server (TYGS; https://tygs.dsmz.de) confirmed the affinity of these two strains to *X. arboricola*.

The further inspections of various plantations confirmed the presence of *Xanthomonas* on blueberry also in other geographic locations in Poland.

Additional analysis to determine if they constitute a new taxon within X. *arboricola* are being conducted.

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

Parameters[a]	Strain	
	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.

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