Complete Genome Sequence Data of Three *Xylella fastidiosa* subsp. *multiplex* Strains Isolated from Olive Trees in California, U.S.A.

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Abstract

Xylella fastidiosa is a xylem-limited bacterial plant pathogen that causes disease on numerous hosts. Additionally, *X. fastidiosa* asymptomatically colonizes a wide range of plant species. *X. fastidiosa* subsp. *multiplex* has been detected in olive (*Olea europaea*) trees grown in California, U.S.A., as well as in Europe. Strains of *X. fastidiosa* subsp. *multiplex* isolated from California olive trees are not known to cause disease on olive, although some can induce leaf-scorch symptoms on almond (*Prunus dulcis*). No genome assemblies currently exist for olive-associated *X. fastidiosa* subsp. *multiplex* strains; therefore, a hybrid assembly method was used to generate complete genome sequences for three *X. fastidiosa* subsp. *multiplex* strains (Fillmore, LM10, and RH1) isolated from olive trees grown in Ventura and Los Angeles counties of California.

Keyword

bacteriology

Resource Announcement

Xylella fastidiosa is a xylem-limited plant pathogenic bacterium that causes disease on numerous plant hosts (Jeger et al. 2018). *X. fastidiosa* is divided into five genetically distinct subspecies (i.e., *fastidiosa*, *multiplex*, *morus*, *sandyi*, and *pauca*) that are further divided into sequence types (STs) according to a multilocus sequencing typing (MLST) classification scheme (Yuan et al. 2010). In California, U.S.A., *X. fastidiosa* subsp. *multiplex* is associated with almond (*Prunus dulcis*), where it causes almond leaf scorch disease, and ornamental hosts, where it may induce leaf scorch symptoms or colonize asymptomatically (Hernandez-Martinez et al. 2007). Several *X. fastidiosa* subsp. *multiplex* strains, e.g., Fillmore, LM10 (alias Xf10 LaMirada), RH1 (alias Xf11 RollingHills), and Oceanside, were isolated from different mature (i.e., at least 30 years old) ornamental olive (*Olea europaea*) trees with leaf scorch symptoms in Ventura, Los Angeles, and San Diego counties, respectively. However, these strains did not induce symptoms in seven olive cultivars ('Arbequina', 'Arbosana', 'Barouni', 'Koroneiki', 'Manzanillo', 'Mission', and 'Sevillano') after mechanical inoculation, and experimental infections did not persist after 1 year (Krugner et al. 2014). In contrast, three of these strains (i.e., Fillmore, LM10, and RH1) induced typical almond leaf scorch disease symptoms in mechanically inoculated almond cultivar Sonora (Krugner et al. 2014).

X. fastidiosa subsp. *multiplex* strains belonging to STs 6, 7, 81, and 87 have been identified in Europe in association with a wide variety of hosts (Arias-Giraldo et al. 2020; Denance et al.

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2017; Giampetruzzi et al. 2019a; Jeger et al. 2018; Landa et al. 2020; Olmo et al. 2017). Genome sequences have been assembled for several almond-infecting strains of *X. fastidiosa* subsp. *multiplex* isolated in Spain, including the complete genome of an ST 6 strain, IVIA5901, assembled using a hybrid method (Arias-Giraldo et al. 2020; Giampetruzzi et al. 2019b; Landa et al. 2020). In addition to causing disease on almond, STs 6 and 81 have been detected in association with olive trees in Spain, although they are not reported to cause disease on olive (Jeger et al. 2018; Landa et al. 2020); in contrast, *X. fastidiosa* subsp. *pauca* strains cause widespread disease in olive in Italy and have been detected in olive in Ibiza, Spain (Saponari et al. 2019). *X. fastidiosa* subsp. *multiplex* strains isolated in Spain and France are related to *X. fastidiosa* subsp. *multiplex* strains isolated from ornamental hosts in California, including LM10 and RH1, based on ST and core-genome alignment (Landa et al. 2020; Vanhove et al. 2019). To facilitate comparison of *X. fastidiosa* subsp. *multiplex* strains isolated in different regions, we produced high-quality complete genome sequences for *X. fastidiosa* subsp. *multiplex* strains Fillmore, LM10, and RH1, using a hybrid assembly approach.

Cultures of strains Fillmore, LM10, and RH1 were grown on PD3 agar medium at 28°C for 7 to 10 days; cells were subsequently collected and genomic DNA was extracted with a DNeasy Blood & Tissue Kit (Qiagen) using the manufacturer's protocol for Gram-negative bacteria, including a 10-minute incubation with 20 µl of RNase A at 20 mg/ml. DNA was assessed for quality and quantity using a Nanodrop spectrophotometer (ThermoFisher Scientific), Qubit fluorometer (Invitrogen), and agarose gel imaging. The same DNA sample was used to prepare both long read and short read libraries, for sequencing on the Oxford Nanopore Technologies MinION and Illumina Novoseq platforms, respectively. For long read libraries, 1 µg of DNA from strains Fillmore, LM10, and RH1 was fragmented using a g-TUBE (Covaris, Woburn, MA) following the manufacturer's instructions for 8- or 20-kbp average fragment size; for strains LM10 and RH1, an additional library was prepared with unfragmented DNA. Long read sequencing libraries were prepared with a Ligation Sequencing Kit (SQK-LSK109, Oxford Nanopore Technologies) and Native Barcoding Expansions (EXP-NDB104, Oxford Nanopore Technologies), following the manufacturer's instructions, and sequenced on a MinION device using an R9.4.1 flow cell (Oxford Nanopore Technologies). A corresponding short read library consisting of 150 bp paired-end reads with inserts of 350 to 400 bp was prepared by the UC Davis Genome Center DNA Technologies Core, and sequencing using the Illumina Novoseq platform, yielding 355,324,924 short read pairs for Fillmore, 328,887,528 for LM10, and 358,608,868 for RH1. Nanopore long reads were base-called, filtered for quality, demultiplexed, and trimmed using Guppy v3.3.0 (Oxford Nanopore Technologies). Illumina reads were trimmed and filtered with fastp v0.20.0, then assessed for quality using fastqc v0.11.9 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and multiqc v1.8 (Chen et al. 2018; Ewels et al. 2016). Analysis of all resulting fastq files with Kraken2 v2.0.8-beta using the MiniKraken2_v1 8Gb database assigned 98.6% to 99.6% of sequenced reads from each file to *Xylella*, verifying that contaminant (i.e., non-*Xylella*) sequences were not introduced during library preparation or demultiplexing steps (Wood et al. 2019). Due to the excessive short read sequencing depth (i.e., approximately 40,000x per strain), randomly subsampled sets of short read pairs, with an estimated genome coverage of 200x, were taken with Seqtk v1.3 (<https://github.com/lh3/seqtk>) and used for subsequent assembly and analysis steps.

To generate complete genome assemblies, multiple combinations of assemblers, assembly parameters, and input reads were evaluated. Unicycler v0.4.8-beta was used to generate hybrid assemblies with both long and short reads, whereas Flye v2.7 was used to construct long read only assemblies (Kolmogorov et al. 2019; Wick et al. 2017). Assembly graphs were evaluated using Bandage v0.8.1 (Wick et al. 2015). For all three strains, both Unicycler and Flye failed to resolve a complete circular assembly using long reads solely from a library fragmented to 8 kbp. For Fillmore, a complete assembly was successfully generated with Flye using combined reads from 8- and 20-kbp libraries (Table 1). For strains LM10 and RH1, several combinations of assembly parameters and read sets (e.g., reads obtained from 8- and 20-kbp libraries) failed to generate complete assemblies, similar to initial failure to obtain a complete assembly of *X. fastidiosa* subsp. *multiplex* strain IVIA5906 encountered in a previous study, which the authors tentatively attributed to the presence of long repetitive sequences (Arias-Giraldo et al. 2020). Combining reads from the 8-kbp, 20-kbp, and unfragmented libraries successfully generated complete assemblies with Flye for strains LM10 and RH1, in conjunction with limiting the read length with Filtlong v0.2.0 (<https://github.com/rwwick/Filtlong>) or the '-asm-coverage' option of Flye (Table 1). For strain RH1, a 14.9-kbp circular contig was frequently obtained from incomplete assemblies; however, long read alignment with minimap2 v2.16

TABLE 1. Summary of methods used and characteristics of genome assemblies of Fillmore, LM10, and RH1, and comparison with *Xylella fastidiosa* subsp. *multiplex* strain M12

Strain	Statistics	Fillmore	LM10	RH1	M12
Year isolated		2009	2011	2008	2003
Long read summary ^a	Total reads	80,778	94,611	164,649	NA
	Total bases (bp)	408,589,596	584,164,405	924,523,400	NA
	Read N50 (bp)	7,802	9,344	9,556	NA
Short read summary ^b	Total read pairs	10,000,002	6,666,668	8,333,335	NA
	Total bases (bp)	3,000,000,600	2,000,000,400	2,500,000,500	NA
	Total depth	1,189.0	749.8	934.3	NA
Assembly method	Software	Flye ^c + Pilon (6) ^d	Flye ^e + Pilon (4) ^d	Filtlong + Flye ^e + Pilon (5) ^d	NA
Assembly characteristics and analysis	Input long-read N50 (bp)	7,802	19,589	31,629	NA
	Total sequencing depth	1,346.0	962.9	1,005.7	NA
	Length (bp)	2,526,544	2,669,650	2,678,425	2,475,130
	CDS	2,240	2,419	2,434	2,041
	rRNA operons	2	2	2	2
	tRNAs	50	49	49	48
	GC%	52.0%	52.1%	52.2%	51.9%
	ST	81	7	7	7
	CheckM completeness	99.64%	99.64%	99.64%	99.64%
	IDEEL results <0.9 ^f	170	224	224	168
Data availability	BioSample accession number	SAMN14680500	SAMN14680501	SAMN14680502	SAMN02598402
	GenBank WGS accession number	CP052855	CP052854	CP052853	CP000941

^a Combined values for all fragment size libraries used per strain, e.g., 8 kbp, 20 kbp, unfragmented.

^b Summary of all randomly subsampled reads used.

^c Run with ‘–plasmids’ option.

^d Indicates the number of times Pilon was run.

^e Run with ‘–plasmids’ and ‘–asm-coverage 80’ options.

^f Length ratio of each protein coding sequence to the best match in UniProt TREMBL. A ratio <0.9 suggests a protein coding sequence is smaller than expected and may be erroneously disrupted.

supports the inclusion of this sequence on the chromosome as a 31-kbp region (positions 1505426 to 1536384) containing two overlapping 16.1-kbp repeats (Li 2018). Once complete assemblies were obtained, each chromosome was rotated to begin at the first nucleotide position of the *dnaA* gene, and the assembly polished with Pilon multiple times to correct insertion/deletion errors common in long read assemblies that can interfere with accurate open reading frame prediction and downstream analysis (Walker et al. 2014; Watson and Warr 2019). Each polishing iteration used a new random subsample of Illumina read pairs, generated with Seqtk.

Polished assemblies were evaluated for completeness with CheckM v1.1.2, and for disrupted protein coding sequences with IDEEL (Parks et al. 2015; Stewart et al. 2019). As a control, both analyses included the complete genome assembly of *X. fastidiosa* subsp. *multiplex* ST 7 strain M12 (GenBank accession CP000941.1), isolated from almond in 2003 and assembled with data generated from Sanger sequencing (Chen et al. 2010). CheckM estimates the genome assemblies of Fillmore, LM10, and RH1 to be 99.64% complete, and assigns an identical score to M12. IDEEL identified 170 to 224 potentially truncated protein coding sequences (i.e., ratio of coding sequence length to that of the best match in UniProt TREMBL database <0.9) in Fillmore, LM10, and RH1, whereas IDEEL identified 168 in M12 (Table 1). Polished assemblies were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al. 2016).

A summary of assembly methods used and features for the polished genome assembly of each strain is shown in Table 1. No plasmids were identified in these strains. MLST analysis of housekeeping genes utilizing the *X. fastidiosa* PubMLST database (<https://pubmlst.org/xfastidiosa/>) identified LM10 and RH1 as ST 7, whereas Fillmore is ST 81. Strains of ST 7 and ST 81 have a similar MLST allelic profile, differing at only at CysG (allele 7 and allele 32, respectively). *X. fastidiosa* subsp. *multiplex* strains belonging to ST 7 and ST 81 have been previously identified in Europe, and draft genomes exist for a small number of European ST 7 (e.g., CFBP8416) and ST 81 (e.g., XF3348, XYL1752, and XYL1981) strains (Denance et al. 2017; Landa et al. 2020). Strains belonging to ST 81 have been detected in association with, or

isolated from, numerous hosts in the Balearic Islands of Spain, including almond, fig (*Ficus carica*), olive, plum (*Prunus domestica*), and several landscape or ornamental hosts (Jeger et al. 2018; Landa et al. 2020). The genome assembly of *X. fastidiosa* subsp. *multiplex* strain Fillmore represents the first genome sequence of an ST 81 strain originating from North America. Several recent studies have suggested whole-genome sequencing data for *X. fastidiosa* are informative for characterizing of strains involved in outbreaks, as the resolution provided by MLST analysis may be limited (Arias-Giraldo et al. 2020; Landa et al. 2020); comparison between Fillmore and Spanish ST 81 strains may provide additional insight into the origins of those strains.

Data availability. Data for the genome assemblies of *X. fastidiosa* subsp. *multiplex* strains Fillmore, LM10, and RH1 are associated with BioProject PRJNA627683 and BioSamples SAMN14680500 to SAMN14680502. Sequencing reads used to generate assemblies were deposited into the Sequence Read Archive (SRA) under accession numbers SRR11638828 to SRR11638838. Genome assemblies have been deposited into GenBank under accession numbers CP052853 to CP052855.

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