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RESOURCE ANNOUNCEMENT

e-Xtra*

Draft Genome Resources of Two Strains ("ESVL" and "IVIA5901") of *Xylella fastidiosa* Associated with Almond Leaf Scorch Disease in Alicante, Spain

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Abstract

An outbreak of *Xylella fastidiosa* subsp. *multiplex* sequence type ST6 was discovered in 2017 in mainland Spain affecting almond trees. Two cultured almond strains, "ESVL" and "IVIA5901," were subjected to high throughput sequencing and the draft genomes assembled. Phylogenetic analysis conclusively indicated they belong to the subspecies *multiplex*, and pairwise comparisons of the chromosomal genomes showed an average nucleotide identity higher than 99%. Interestingly, the two strains differ for the presence of the plasmids pXF64-Hb_ESVL and pUCLA-ESVL detected only in the ESVL strain. The availability of these draft genomes contribute to extend the European genomic sequence dataset, a first step toward setting new research to elucidate the pathway of introduction and spread of the numerous strains of this subspecies so far detected in Europe.

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Genome Announcement

In 2017, *Xylella fastidiosa* was detected for the first time in mainland Spain, on almond trees in the Guadalest Valley, Alicante province (Valencian Community). In this province, in spring 2018 infections had been detected in more than 170 almond orchards located in 27 municipalities (EFSA PLH Panel 2018). The number of affected orchards is currently higher as the official surveillance in the area is progressing. Infected trees showed noticeable symptoms of almond leaf scorch disease (ALSD), with typical yellow and brown lesions on leaf tips and margins, but also extensive branch and twig dieback. Infections have also been discovered on *Rosmarinus officinalis* and five additional ornamental species and scrubland Mediterranean vegetation.

Multilocus sequence typing analyses performed directly on DNA extracts from infected almonds identified isolates genetically related to subsp. *multiplex* and sequence type ST6 (EFSA PLH Panel 2018). Isolates of the subsp. *multiplex*, native to temperate North America, are generally associated to leaf scorch diseases in various hosts (almond, plum, blueberry, pecan, and shade trees). The current status on the presence and occurrence of strains of

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different *X. fastidiosa* subspecies in the three European countries (Italy, France, and Spain) indicates that strains of the subsp. *multiplex* are the most common, with the highest number of STs identified, including a new undescribed ST (ST81) (EFSA PLH Panel 2018). Previous reports in Europe indicated that strains of subsp. *multiplex* (ST6-ST7) are widespread in Corsica (Denancé et al. 2017) and in two Balearic Islands (ST7-ST81) (Olmo et al. 2017) in the outbreaks detected in southern France (PACA region, ST6-ST7) and more recently in those detected in mainland Spain, both in the province of Alicante (ST6) and Madrid (ST6).

In this report, we describe the draft genome sequences of *X. fastidiosa* strains "ESVL" and "IVIA5901", selected among the ST6-cultured isolates recovered from ALSD-affected almond trees from two orchards located in Bolulla and Benimantell municipalities (Alicante province), distant from each other by approximately 8 km. Genomic DNA of strains ESVL and IVIA5901 were extracted from pure cultures grown in PD2 agar medium using a commercial DNA purification kit. The whole genome sequencing (WGS) libraries were paired-end sequenced with a HiSeq4000 Illumina platform. WGS library for ESVL was run in three technical replicates on three different lanes, and WGS library of IVIA5901 was run in one replicate.

Illumina sequencing yielded a total of 1,369,695, 2 × 150 bp high quality paired reads for ESVL and a total of 3,956,773, 2 × 150 bp for IVIA5901. De novo genome assembly was done using SPAdes v3.9.0 (Antipov et al. 2016; Bankevich et al. 2012). Extremities of circular contigs annotated as plasmid sequence were trimmed and closed by PCR amplification with custom primers. The final assemblies of the bacterial chromosomes resulted in 131 contigs (>200 bp) for ESVL and in 141 contigs (>200 bp) for IVIA5901, with an equal GC content of 51.8% (Table 1). The nucleotide coverage of the chromosomal genome was 110× for ESVL and 309× for IVIA5901 (Table 1). The average nucleotide identity (ANI) between ESVL and IVIA5901 chromosomes calculated by ANI calculator tool (Rodriguez-R and Konstantinidis 2016) was 99.99%.

In addition, for the strain ESVL, two plasmids were also assembled: pXF64-Hb_ESVL of 59,678 bp, with a GC content of 52.6%, and pUCLA-ESVL of 1,289 bp, with a GC content of 54.7%. Average nucleotide coverage was 27,741× for pUCLA-ESVL and 241× for pXF64-Hb_ESVL. To confirm, the presence/absence of both plasmids primers were designed (Supplementary Table S1) and used in PCR tests carried out on the corresponding cultured strains, and on several infected samples collected in the almond orchards. PCR results, sequencing, and BLAST analysis of amplified products confirmed the WGS data and disclosed the occurrence in the fields of ST6-infected plants either positive or negative for the presence of the two plasmids.

Functional annotation by submission to the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) resulted in the identification of 6 rRNA genes (two operons), 51 tRNA loci, 2,464 genes, 2,236 protein-encoding genes, 4 noncoding RNAs in the chromosome of IVIA5901 strain and 6 rRNA genes (two operons), 50 tRNA loci, 2,515 genes, 2,275 protein-encoding genes, 4 noncoding RNAs in the chromosome of ESVL strain; 61 protein-encoding genes in the pXF64-Hb_ESVL plasmid.

The plasmid pXF64-Hb_ESVL showed a high sequence similarity with the conjugative plasmid pXF64-HB (NZ_CP009886.1) reported in *X. fastidiosa* strain Hib4 (ST70), which belongs to the subsp. *pauca.* The plasmid pUCLA-ESVL showed highest sequence similarity with the rolling-circle plasmids pUCLAb and pUCLAc of the grape-infecting *X. fastidiosa* strain UCLA (Guilhabert et al. 2006).

A major difference found between the ST6 strains from Alicante is the absence of the two entire plasmids in strain IVIA5901.

In a previous work, the first genomic sequences of European strains of the subsp. *multiplex* harboring ST6 and ST7 genotypes, associated with outbreaks in Corsica and mainland France, were determined (Denancé et al. 2017). The genome sequences of the ST6 strains ESVL and

Table 1. Summary statistics of the genome assemblies of *Xylella fastidiosa* strains IVIA5901 and ESVL[a]

Strain	Number of supercontigs (≥50,000 bp)	Number of contigs	Number of contigs plasmid related	Total length (bp)	Genome coverage	N50 (bp)	N75 (bp)	Max (bp)	Min (bp)	GC content (%)	Predicted genes
ESVL	18	133	2	2,554,495	110×	103,116	67,809	335,582	207	51.8	2,515
IVIA5901	16	141	–	2,493,558	309×	116,584	67,790	335,598	218	51.8	2,464

[a] N50 = median length of more than 50% of the supercontigs in the entire assembly; N75 = median length of more than 75% of the supercontigs in the entire assembly; Max and Min = maximum and minimum lengths, respectively, of supercontigs found in the entire assembly; GC content = percentage of guanine-cytosine bases in the entire assembly; and Predicted genes = number of genes predicted by PGAAP (NCBI).

IVIA5901 from mainland Spain further contribute to extend the genomic data available on the European strains of *X. fastidiosa* subsp. *multiplex*. The utility of such data for phylogenetic inference is critically to advance the investigations on the pathway of introduction(s), spread, genetic relationship among the European outbreaks, genomic population evolution, and determine divergence times from previously characterized strains in America and Europe.

Accession numbers. The genome sequences of *X. fastidiosa* strains ESVL and IVIA5901 have been deposited in GenBank under accession numbers QPQV01000000 and QPQW01000000, respectively. *X. fastidiosa* strains ESVL and IVIA5901 are being deposited at the Spanish Type Culture Collection (CECT).

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