Complete Genome Sequence of the Fire Blight Pathogen Strain *Erwinia amylovora* Ea1189

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Abstract

Erwinia amylovora causes fire blight, the most devastating bacterial disease of apples and pears in the United States and worldwide. The model strain *E. amylovora* Ea1189 has been extensively used to understand bacterial pathogenesis and molecular mechanisms of bacterial-plant interactions. In this work, we sequenced and assembled the de novo genome of Ea1189, using a combination of long Oxford Nanopore Technologies and short Illumina sequence reads. A complete gapless genome assembly of Ea1189 consists of a 3,797,741-bp circular chromosome and a 28,259-bp plasmid with 3,472 predicted genes, including 78 transfer RNAs, 22 ribosomal RNAs, and 20 noncoding RNAs. A comparison of the Ea1189 genome to previously sequenced *E. amylovora* complete genomes showed 99.94 to 99.97% sequence similarity with 314 to 946 single nucleotide polymorphisms. We believe that the availability of the complete genome sequence of strain Ea1189 will further support studies to understand evolution, diversity and structural variations of *Erwinia* strains, as well as the molecular basis of *E. amylovora* pathogenesis and its interactions with host plants, thus facilitating the development of effective management strategies for this important disease.

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Keywords

genome, plant-microbe interactions, pathogenesis, *Erwinia amylovora*

Genome Announcement

Fire blight was first described more than 250 years ago and is the first plant disease discovered to be caused by a bacterium (Smits et al. 2017). It is undoubtedly one of the most serious and perplexing bacterial diseases on pomaceous fruit trees, including pear, apple, quince, and several other members of the family Rosaceae (van der Zwet 1979). The causal agent *Erwinia amylovora* is a gram-negative bacterium, belonging to the Enterobacteriaceae family, which also includes many important human and animal pathogens (Wang et al. 2010). Currently, 135 assemblies of *E. amylovora* at different levels are publicly available in GenBank, including eight with complete genome sequences, three at the chromosome level, 99 at the scaffold level, and 25 at the contig level (National Center for Biotechnology Information [NCBI] *Erwinia amylovora* database). Previous comparative genomic analyses confirmed a low level of genetic diversity between *E. amylovora* genomes from commercial orchards (Parcey et al. 2020; Singh and Khan 2019; Smits et al. 2010). Genome sequences

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*The *e-Xtra* logo stands for “electronic extra” and indicates there are supplementary materials published online.

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with relatively low integrity are generally sufficient for taxonomical analysis (Smits 2019). However, to fully understand the diversity of virulence among these highly conserved *E. amylovora* strains, complete genome assemblies with fewer contigs and improved sequencing quality are essential (Smits 2019; Wang et al. 2010; Zeng et al. 2018). In this study, we report the complete genome sequence of strain *E. amylovora* Ea1189, which has been extensively used as a model strain in understanding bacterial pathogenesis and plant-microbe interactions in the past decades (Burse et al. 2004; Khan et al. 2012; Yang et al. 2014; Zhao 2014; Zhao and Qi 2011; Zhao et al. 2005).

E. amylovora Ea1189 was originally isolated from pear (*Pyrus communis*) in Göttingen, Germany in 1989 (Burse 2003; Burse et al. 2004). After we obtained the isolate, the isolate was stored at -80°C in 15% glycerol. A single colony was picked from a Luria-Bertani (LB) agar plate for overnight culture in LB broth at 28°C . Genomic DNA was extracted from overnight bacterial cells using MasterPure complete DNA and RNA purification kit (Epicenter, Madison, WI, U.S.A.). A Nanopore library was prepared using the Rapid Barcoding SQK-RBK004 library kit. The library was sequenced with a SpotON R9.4.1 RevC FLO-MIN106 flowcell for a 48-h run on a GridIONx5 sequencer (Oxford Nanopore Technologies (ONT), Oxford). FAST5 files containing raw Nanopore signal data were base-called using Guppy 2.0.5 and were converted to FASTQ format using Guppy 2.3.7 (Wick et al. 2019). The program Porechop 0.2.3 was used to demultiplex the sequences and to trim sequencing adapters. In total, 53,503 reads representing 8.93×10^8 bases were generated. A following quality filtering was conducted to retain $>3\text{-kb}$ longer sequences with quality scores higher than 10, using BBDMap 38.36 (Bushnell 2014). A final set of high-quality 46,761 ONT long reads with a total of 8.51×10^8 bases and approximately 218 $\times$ coverage of the *E. amylovora* genome was assembled along with previously sequenced Illumina data (Singh and Khan 2019), using the Unicycler hybrid assembly pipeline 0.4.8 (Wick et al. 2017). Canu assembly pipeline 1.9 (Koren et al. 2017) with merely long ONT reads as input was also used to manually confirm Unicycler output. The resulting assembly consisted of two circular contigs of 28,259 bp and 3,797,982 bp in length, which corresponded to the plasmid and chromosome sequences of Ea1189, respectively.

Due to the higher chances of sequence errors in long reads from ONT, we again used the Illumina short read sequences of Ea1189 previously generated by Singh and Khan (2019) to further improve the chromosome and plasmid assemblies of Ea1189. The Illumina data consisted of 303,997 paired-end 250-bp reads with 4.56×10^7 bases. The quality of the raw sequence reads was assessed using FastQC 0.11.8 (Andrews 2010), and Trimmomatic 0.38 (Bolger et al. 2014) was used to trim reads with a quality score <20 over a 4-bp sliding window. After trimming, the read sequences of less than 50 bp length were also eliminated. The remaining high-quality 272,471 paired-end reads with 4.13×10^7 bases were mapped to the Ea1189 assembly with Burrows-Wheeler Aligner 0.7.17 (Li and Durbin 2009) for error correction using Pilon 1.23 (Walker et al. 2014). We ran four iterations of Pilon until no further changes could be integrated. In total, 188 single nucleotide polymorphisms (SNPs), 87 small insertions (totaling 118 bases), and 33 small deletions (totaling 59 bases) were corrected in the improved Ea1189 assembly.

The gapless improved assembly consists of one 28,259-bp plasmid and one 3,797,741-bp circular chromosome, with G+C content at 50.24% and 53.61%, respectively. Annotation with the Prokaryotic Genome Annotation Pipeline (Tatusova et al. 2016) yielded a total of 3,472 genes, including 78 transfer RNA, 22 ribosomal RNA, and 20 noncoding RNA genes. To assess the similarity between Ea1189 and other *E. amylovora* strains, eight complete *E. amylovora* genome sequences (Supplementary Table S1) were downloaded from the NCBI database and were compared using MUMmer 4.0.0 (Kurtz et al. 2004). The genome similarities between Ea1189 and the selected eight strains range from 99.94 to 99.97% (Supplementary Table S2), confirming the low diversity of *E. amylovora* genomes. On the other hand, the number of SNPs between different comparisons with Ea1189 ranged from 314 to 946 (Supplementary Table S2). Regardless of different isolate hosts, there were fewer SNPs among the three European strains (Ea1189, CFBP 1430, and E-2) and among the five Korean strains (FB-86, FB-207, TS3238, FB-307, FB-20), suggesting that geographical origin rather than host of the strains played an important role in SNP variations across these *E. amylovora* strains. The nontransmissible plasmid pEA29 has been found in most *E. amylovora* strains, which appears to increase bacterial fitness after colonizing young host tissues (Parcey et al. 2020). A comparative analysis of plasmids from the nine complete

E. amylovora genomes (Supplementary Tables S1 and S3) confirmed the ubiquity of pEA29 in *E. amylovora* genomes. The pEA72 plasmid is present only in strain ATCC 4996 and was not detected in Ea1189. Moreover, the pEA29 sequences across the nine strains showed >99% identity. Consistent with genome-level SNP variations, pEA29 plasmids in strains from similar geographical locations have almost identical sequences with few SNPs.

The chromosome and plasmid assemblies have been deposited in GenBank under the accession numbers CP055227 and CP055228. The complete genome of Ea1189 is the first *E. amylovora* genome compiled using ONT and Illumina sequencing technologies. Hybrid assembly of long and continuous ONT reads and short Illumina reads produced a complete genome of the model strain *E. amylovora* Ea1189 with higher integrity and fewer sequencing errors. Additional complete genome assemblies of *E. amylovora* strains can facilitate a thorough investigation of nucleotide and structural variation across highly conserved *E. amylovora* strains, which could help further our understanding of *E. amylovora* pathogenesis and aid in the design of effective and sustainable management strategies for fire blight control.

Author-Recommended Internet Resources

NCBI *Erwinia amylovora* database: <https://www.ncbi.nlm.nih.gov/genome/genomes/1159>
 Porechop 0.2.3: <https://github.com/rwrick/Porechop>

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