



Complete Genome Sequence of *Agrobacterium fabrum* Strain 1D159

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ABSTRACT This work reports the draft genome sequence of *Agrobacterium fabrum* strain 1D159 (also known as ATCC strain 27912). The assembled genome is composed of a 2,861,352-bp circular chromosome, a 2,058,040-bp linear chromosome, a 519,735-bp AT plasmid, and the 223,394-bp Ti virulence plasmid. The wild nondisarmed strain produces small gall-like structures in citrus.

Here, we present the novel genome from *Agrobacterium fabrum* strain 1D159 (formally known as *Agrobacterium tumefaciens*), obtained from the Kobe microbe collection at the University of California at Davis (UC Davis). This bacterium has been deposited at the ATCC as strain 27912. Strain 1D159 was obtained from soil around a gall-containing peach tree by the Kobe lab on 25 July 1969. Characterization of 1D159 by the Kobe lab in a 1972 notebook documented it to be pathogenic (gall forming) on sunflower. To verify this characterization, our lab demonstrated gall formation by 1D159 in citrus tissue along with the ability to transfer a binary plasmid-derived transfer DNA (T-DNA) producing DSRed expression in the tissue (Fig. 1). The binary plasmid and protocol used for citrus transformation was previously published (1). The strain was grown in Luria broth at 28 to 30°C with shaking at 200 rpm.

Genomic DNA was isolated from our strains (2) using the Qiagen blood and cell culture DNA maxikit (catalog number 13362) and genomic DNA buffer set (catalog number 19060) (3). DNA samples were evaluated using gel electrophoresis and quantified using both a NanoDrop 2100 spectrophotometer (Thermo Fisher Scientific) and a Qubit fluorimeter (Invitrogen) with the Qubit double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit (Invitrogen). The genomic DNA was sheared with a G-tube (Covaris). A 20-kb DNA library was constructed according to the manufacturer's instructions using the BluePippin size selection system and sequenced using single-molecule real-time (SMRT) sequencing technology on a PacBio RS system. SMRT sequencing data were generated at an average coverage of 59.94× with a mean read length of 23,913 bp. *De novo* genome assembly was conducted with the sequence reads using the Hierarchical Genome Assembly Process (HGAP) workflow (SMRT Portal; Pacific Biosciences) protocol RS_HGAP_Assembly.3 (4) and SMRT Analysis 2.3.0 software.

This allowed the generation of 4 polished contigs with an average N_{50} contig length of 23,193 bp and a sum of contig lengths of 5,726,826 bp. The linear DNA was manually circularized via chimeric overlap of 28,929 bp for the circular chromosome, which has a final composition of 2,861,352 bp with a GC content of 59.6% (367 subsystems, 2,868 coding sequences, and 50 rRNA genes). The linear chromosome was determined to be 2,058,040 bp long with a GC content of 59.7% (157 subsystems, 1,896 coding sequences, and 20 rRNA genes). The DNA was manually circularized via a chimeric overlap of 21,281 bp for the AT plasmid, giving a composition of 519,735 bp with a GC content of 57.5% (43 subsystems, 676 coding sequences, and 0 rRNA genes) and a chimeric overlap of 14,144 bp for the virulence vector pTi-1D159, providing 223,394 bp with a GC content of 56.9% (21 subsystems, 239 coding sequences, and 0 rRNA genes) for the final sequence.

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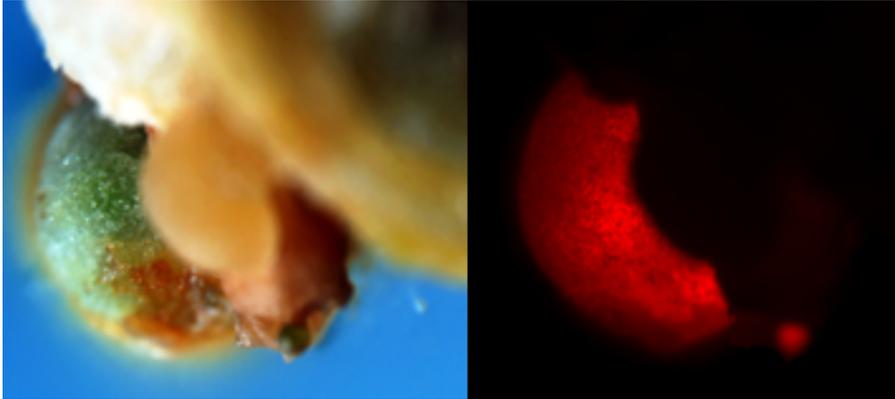


FIG 1 Gall formation and stable DSRed expression in citrus epicotyl tissue transformed with wild-type *Agrobacterium fabrum* strain 1D159 harboring the binary vector pCTAGV-KCN3. (Left) Gall viewed under white light; (right) same gall displaying DSRed expression.

The assembled and raw read sequences were entered into the National Center for Biotechnology Information (NCBI), and BLAST was used for identification (<http://blast.ncbi.nlm.nih.gov/>). Automated annotation was performed using the Rapid Annotations using Subsystems Technology (RAST) pipeline for annotation of the genome (5). *Agrobacterium fabrum* strain 1D159 contains 5,679 predicted coding sequences, 588 subsystems, and 70 predicted RNA-coding genes as curated by SEED data (<http://theSEED.org>). Genomic comparison shows 1D159 to be related to *Agrobacterium fabrum* strain C58 (formally known as *Agrobacterium tumefaciens* strain C58). DNA matrix analysis of the assembled contigs compared to the C58 sequences (GenBank accession numbers [NC_003065](#), [AE007869](#), [AE007870](#), and [AE007872](#)) was performed with MacVector version 17.0.0. The virulence Ti plasmid is nearly 100% identical to that of C58 with the exception of a single 850-bp deletion. The linear chromosome is syntenic for its entire length, with ~80% exhibiting 95% or better identity. There is almost no synteny at a 60% identity threshold for either the AT plasmid or the circular chromosome of C58. The ability of 1D159 to efficiently transform citrus makes it a novel tool for the improvement of citrus via biotechnology.

Data availability. The whole-genome assembly for *Agrobacterium fabrum* strain 1D159 (also known as *Agrobacterium tumefaciens* strain 1D159) has been deposited in DDBJ/ENA/GenBank under the BioProject accession number [PRJNA521804](#) and SRA accession number [SRX5367871](#).

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