Complete Genome Sequence Data for the Grapevine Crown Gall-Inhibiting Bacteria *Allorhizobium vitis* F2/5

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Genome Announcement

Crown gall disease in grapevine is caused by pathogenic strains of *Allorhizobium vitis*. *A. vitis* strain F2/5 is a nonpathogenic biocontrol agent that was previously shown to act as a biological control agent to crown gall disease and was first isolated from South Africa. Here, we present the complete assembled genome, which is 5.94 Mb in length with 5,414 predicted protein-coding sequences and has two circular chromosomes and five plasmids. The genome sequence has no detectable T-DNA border sequences and is missing key virulence genes, which is consistent with the bacteria being nonpathogenic. The F2/5 genome sequence could contribute to understanding the molecular basis underlying the biocontrol activity.

Grapevine crown gall caused by *Agrobacterium vitis* (now reclassified to the genus *Allorhizobium* [Mousavi et al. 2014]) is a serious chronic disease that can cause decreased production or early grapevine death (Gillings and Ophel-Keller 1995). Grape crown gall can be partially managed through varietal selections, cultural practices and can be suppressed to varying degrees in greenhouse experiments by biocontrol agents (Zheng and Burr 2016). Biocontrol agents include *A. vitis* strains ARK1, E26, VAR03-1, and F2/5 (Kawaguchi 2013; Kawaguchi et al. 2008; Liang 1990; Staphorst et al. 1985). Strain F2/5 is nontumorigenic and the antibiosis conferred against pathogenic *A. vitis* strains is thought to occur by inhibiting transformation of the grape tissue by the pathogen as opposed to inhibiting attachment of the pathogen to wounded cells or growth of the pathogen in the wounded tissue (Kaewnum et al. 2013). The antibiosis or grape tumor inhibition (GTI) conferred by F2/5 requires living cells, whereas heat-killed or sonicated-lysed cell preparations lose GTI ability (Burr et al. 1997). A number of genes were identified that are required for GTI, including *clpA* (*F-avi2537*), *alpP1* (*F-avi1696*), *aviR* (*F-avi4374*) (Zheng and Burr 2016). To date, no genome sequences of the potential biocontrol strains ARK1, E26, and F2/5 have been published, but the genome sequence for VAR03-1 has been recently published (Noutoshi et al. 2020).

A single colony of F2/5 (Burr collection, Cornell University, Ithaca, NY, U.S.A.) was inoculated into 6 ml of yeast mannitol broth and was grown overnight at 28°C. Genomic DNA purification was performed, using a PowerSoil kit (Qiagen). After shearing the purified DNA to 10 to 15 Kb with a 26-gauge needle, a Blue Pippin (Sage Science) was used to select fragments between 10 and 25 Kb. For PacBio library preparation and sequencing, DNA was processed with the SMRTbell template prep kit 1.0 (PacBio) and was sequenced on a PacBio Sequel instrument. A total of 2.25 Gb of sequencing data with a read N₅₀ of 6.20 Kb was obtained, representing approximately 374x coverage.

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Keywords

Agrobacterium vitis, *Allorhizobium vitis*, biological control, grapevine crown gall

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Table 1. Genomic features of the *Agrobacterium vitis* F2/5 genome^a

Name	Contig 1	Contig 2	Contig 3	Contig 4	Contig 5	Contig 6	Contig 7
Feature	Chr. 1	Chr. 2	Partial virulence region	Nopaline	Nopaline Acc operon	<i>ttuC</i>	...
Size (bp)	3,663,811	1,185,416	98,206	150,180	200,742	204,449	436,826
Circular	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Coding sequence	3,374	991	97	139	197	220	396
Ribosomal RNAs	9	3	0	0	0	0	0
Transfer RNAs	52	4	0	0	0	0	0

^a Chr. = chromosome; Acc = agrocinnopine uptake and catabolism.

A genome assembly was built from the PacBio sequence reads, using Flye v2.7 (Kolmogorov et al. 2019). The assembly consisted initially of eight contigs with an N₅₀ value of 3.07 Mb. The PacBio reads were mapped back to the draft genome assembly, using Minimap2 v2.22 (Li 2018), and the mapping rate was 98.6%. BUSCO v5.2.2 (Simão et al. 2015) identified 97.0% complete genes in the assembly of 1,937 markers in the database rhizobium-agrobacterium_group_odb10, suggesting high integrity and completeness of the assembly. LASTZ (Harris 2007) was used to identify the similarity of each contig with closely related bacterial species, using the default parameters (step length = 20, seed pattern = 12 of 19, HSP threshold score = 3,000 [Geneious Prime plugin LASTZ v1.02.00]). Two contigs were very similar to *A. vitis* S4 chromosome 1, were contiguous, and were manually merged to form contig 1. We showed contig 1 was contiguous in F2/5 by mapping the raw PacBio reads with Minimap2 (Geneious Prime v2021.2.2) and identified more than 50 reads spanning the junction. Contig 2 was very similar (average nucleotide identity [ANI] > 95%) to *A. vitis* S4 (GenBank accession GCA_000016285.1) chromosome 2. Accordingly, contig 1 was named chromosome 1 and contig 2 was named chromosome 2. ANI were calculated by using FastANI (v1.32) (Jain et al. 2018). All seven contigs were demonstrated as circular sequences by manually linking 10 Kb of the 3' and 5' ends and identifying spanning sequence reads, using Minimap2. Genome annotation was performed by using the DDBJ fast annotation and submission tool (DFAST) (Tanizawa et al. 2016).

The final, high-quality genome of seven contigs has a total length of 5.94 Mb, an N₅₀ value of 3.66 Mb, and GC content of 57.6%. DFAST identified 5,414 protein-coding sequences, 56 transfer RNAs and 12 ribosomal RNAs (Table 1). F2/5 is nonpathogenic on grapevine, and contig 3 harbors a partial virulence region with nine virulence-related genes (*VirA*, *VirB8*, *VirB11*, *VirC1*, *VirC2*, *VirD1*, *VirD2*, *VirD4*, and *VirG*). In contrast, pathogenic *Agrobacterium tumefaciens* C58 has 27 virulence genes on its Ti plasmid (Wood et al. 2001). Contigs 4 and 5 have genes for nopaline uptake and assimilation. In addition, contig 5 has an agrocinnopine uptake and catabolism operon. Contig 6 has a gene homologous to *ttuC* for tartrate utilization, typical of *A. vitis* (Xi et al. 2021). We searched the genome sequence for the consensus T-DNA border sequence NNTGGCAGGATATATNNNNNTGTAWN, and no identical sequence was found, which is consistent with the bacteria being nonpathogenic.

Data Availability

The complete genome sequence and associated data for *A. vitis* F2/5 were deposited under GenBank accession numbers CP081837 through CP081843, BioProject accession number PRJNA755483, Short Read Archive accession number SRR15522074, and BioSample accession number SAMN20826601.

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