Genome Sequence Resource of *Xanthomonas citri* pv. *citri* from Formalin-Fixed Citrus Leaves Specimen Showing Canker Lesions Collected in 1982

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Abstract

Xanthomonas citri pv. *citri* is the causative agent of citrus canker, one of the most devastating diseases threatening global citrus production. Here, we present the genome sequence of *X. citri* pv. *citri* strain GD82 from a formalin-fixed citrus leaf specimen showing canker lesions collected in 1982 in Guangdong Province, China. The GD82 genome consisted of 5,197,217 bp with G+C content of 64.8%, along with four circular plasmids: pXAC33 (32,377 bp), pXAC64 (63,972 bp), pXAC47 (47,810 bp), and pGD82.1 (219,560 bp). This is the oldest *X. citri* pv. *citri* genome from historical citrus canker specimens in China, which will enrich the current *X. citri* pv. *citri* genome database and facilitate genomic evolution research of *X. citri* pv. *citri*.

The bacterial pathogen *Xanthomonas citri* pv. *citri* is the causal agent of citrus canker, one of the most destructive diseases in citrus production (Graham et al. 2004). *X. citri* pv. *citri* invades citrus plants via stomata or wounds and causes erumpent lesions on fruit, leaves, and young stems of susceptible citrus cultivars (Gottwald et al. 2002). In China, citrus canker was first reported in *Fortunella hindsii* located in Guangdong Province in 1918 (Lee 1918). The evolutionary history, origin, and diversification of different *X. citri* pv. *citri* pathotypes have been analyzed previously but with a small number of *X. citri* pv. *citri* strains from China (Patané et al. 2019; Zhang et al. 2015). This was mainly because of the limited genome resource of *X. citri* pv. *citri* strains from China available in public databases, especially for the *X. citri* pv. *citri* strains isolated from historical citrus canker specimens collected decades ago. The use of historical specimen collections contributes to the study of the origin, evolution, and diversity of *X. citri* pv. *citri*. Based on the genetic analysis of *X. citri* pv. *citri* strains from historical herbarium specimens, a previous study demonstrated that the source of the original outbreak of citrus canker disease in Florida was introduced from Japan (Li et al. 2007). Here, we report the whole-genome sequence of an *X. citri* pv. *citri* strain GD82 derived from a formalin-fixed sweet orange (*Citrus sinensis*) leaves specimen showing canker lesions symptoms collected in 1982 in Guangzhou City, Guangdong Province, China (Fig. 1A and 1B).

The formalin-fixed leaves with canker lesions (Fig. 1B) were initially washed with phosphate-buffered saline (PBS) (0.01 M, pH 7.4) and dried with sterile filter paper. The erumpent brown lesions with chlorotic margins were removed from the leaf and chopped into small segments (approximately 5 by 5 mm) with sterile scissors. All segments were soaked in PBS (0.01 M, pH 7.4) for 3 months, with solution refreshing every 2 days. Thereafter, all segments were rinsed with 70, 80, 90, and 100% ethanol gradient and re-soaked in PBS (0.005 M, pH 7.4) for 48 h. Total DNA was extracted by using the E.Z.N.A. High Performance Plant DNA Extraction Kit (Omega Bio-Tek Co.). The quality and quantity of the GD82

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Keywords

citrus canker, formalin-fixed specimen, genome sequencing, historical specimen, *Xanthomonas citri* pv. *citri*

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Fig. 1. Canker symptom and PCR detection result of a historical formalin-fixed *Citrus sinensis* specimen (named GD82). **A**, Formalin-fixed citrus samples collected in Guangzhou City in 1982. **B**, Formalin-fixed citrus leaves showed canker erumpent lesions. **C**, Agarose gel electrophoresis result showed expected PCR product sizes for *X. citri* pv. *citri* in the GD82 DNA samples with primer set JYF5/JYR5. Lane M = DNA ladder (top to bottom: 2,000, 1,000, 750, 500, and 250 bp), + indicates positive control, and GD82-1 and GD82-2 = two DNA samples extracted from the GD82 sample.

DNA sample was evaluated by using agarose gel electrophoresis (1%) and NanoDrop One (Thermo Fisher Scientific). The GD82 DNA sample had a concentration of approximately 25 ng/μl with absorbance ratio of 260/280 = 1.85. The presence of *X. citri* pv. *citri* was confirmed by the *X. citri* pv. *citri*-specific SYBR Green Real-Time PCR (primer set XACF07/XACR07) with a cycle threshold value of 17.39 (Yin et al. 2007) and the conventional PCR (primer set JYF5/JYR5) with an expected band of 413 bp (Fig. 1C) (Wang et al. 2004). Genome sequencing was carried out on an Illumina HiSeq 3000 platform with 150-bp paired-end output (Illumina Inc.) through a commercial source.

In total, 102,741,744 HiSeq reads (Q30 = 95.34%) were generated from the GD82 sample. All HiSeq reads were initially mapped to the *C. sinensis* genome (AJPS00000000.1), *C. maxima* genome (MKYQ00000001.1), *C. reticulata* genome (NIHA00000000.1), *C. sinensis* mitochondrion (NC_037463.1), and *C. sinensis* chloroplast (NC_008334.1) using Bowtie 2 (v 2.4.1) with default settings (Langmead and Salzberg 2012). All mapped reads were removed and, in total, 102,592,806 unmapped reads (99.86% of total reads) were retained for both reference-based assembly and de novo assembly. Reference-based assembly was performed by using the whole-genome sequence of *X. citri* pv. *citri* strain 306 (NZ_003919.1) and four *X. citri* pv. *citri* plasmid sequences: pXAC33 (NC_003921.3), pXAC64 (NC_003922.1), pXAC47 (NC_CP024080.1), and pLJ207-7.1 (NZ_CP018851.1), using CLC Genomic Workbench v9.5 with defaulting setting (length fraction = 0.5 and similarity fraction = 0.8). The de novo assembly was performed by Velvet v1.2.10 (*k*-mer = 75 and min_contig_length = 1,000) (Zerbino and Birney 2008). The draft genome sequence of *X. citri* pv. *citri* strain GD82 was further assembled by combining the reference-based assembly contigs and de novo assembly contigs through BLAST+ v2.10.0 (Camacho et al. 2009). Briefly, the contigs from de novo assembly was used as query to blast against (word_size = 28 and e-value = 1e-5) with contigs from the reference-based assembly. Based on the BLASTn result, the de novo assembly contigs that covered the gap region of two neighboring reference-mapping contigs were used for gap closure. For a gap region that could not be filled with de novo assembly contigs, the PCR experiments with specific primer sets predicted to connect the adjacent sides of two contigs were performed and followed by Sanger sequencing of amplicons. The amplicon sequences were used to close the gap and generate the final version of the *X. citri* pv. *citri* strain GD82 genome. The *X. citri* pv. *citri* strain GD82 whole-genome sequence had a size of 5,197,217 bp (approximately 1,120× coverage depth), with G+C content of 64.8% (Table 1). The GD82 genome was annotated to contain 4,811 open reading frames and 61 RNA genes using the RAST server (<https://rast.nmpdr.org/>) (Aziz et al. 2008). In addition, the assembly result showed that the *X. citri*

Table 1. Mapping result of *Xanthomonas citri* pv. *citri* strain GD82

Parameter	Genome 306	Plasmid			
		pXAC33	pXAC64	pXAC47	pLJ207-7.1
Accession number (size [bp])	NC_003919.1/5 (175,554)	NC_003921.3 (33,700)	NC_003922.1 (64,920)	NZ_CP024080.1 (47,152)	NZ_CP018851.1 (213,743)
Total mapped reads	38,352,851	917,547	1,513,635	805,169	1,754,451
Covered of reference (%)	99	100	100	97	90
Average nucleotide coverage (×)	1,111	4,080	3,493	2,600	1,231
Consensus (bp)	5,136,479	33,703	64,923	45,928	192,938

pv. *citri* strain GD82 harbored four plasmids, including pXAC33 (32,377 bp), pXAC64 (63,972 bp), pXAC47 (47,810 bp), and pGD82.1 (219,560 bp) (Table 1).

The *X. citri* pv. *citri* strain GD82 genome is the first *X. citri* pv. *citri* genome sequenced from a historical formalin-fixed specimen of citrus-canker-infected citrus leaves in China. To date, the *X. citri* pv. *citri* strain GD82, originally collected in 1982, was the oldest *X. citri* pv. *citri* strain to be sequenced or assembled in China. The *X. citri* pv. *citri* strain GD82 genome information will enrich the *X. citri* pv. *citri* genome database and contribute to further research of *X. citri* pv. *citri* evolution in China and the world.

Data Availability

This whole-genome shotgun project has been deposited at DNA Data Bank of Japan/European Nucleotide Archive/GenBank under the accession numbers CP075325, CP075326, CP075327, CP075328, and CP075329. The versions described in this article are versions CP075325.1, CP075326.1, CP075327.1, CP075328.1, and CP075329.1.

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