Genome Sequence Resource for *Spiroplasma citri*, Strain CC-2, Associated with Citrus Stubborn Disease in California

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Abstract

Spiroplasma citri is a bacterium that causes stubborn disease of citrus and infects other crops, ornamentals, and weeds. It is transmitted by leafhoppers in a circulative manner. Due to limited sequence data on *S. citri*, the bacterium was isolated from naturally infected Chinese cabbage grown on a farm in Fresno County, CA. DNA from *S. citri* CC-2 was extracted from a pure culture in LD8 and subjected to PacBio sequencing. Four contigs were obtained with a single circular chromosome of 1,709,192 bp and three plasmids of 40,210, 39,313, and 2,921 bp in size. The genome developed herein extends the sequence database of *S. citri* and is the first whole-genome sequence record of *S. citri* from California.

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Genome Announcement

Spiroplasma citri (Bacteria; Tenericutes; Mollicutes; Entomoplasmatales; Spiroplasmataceae; Spiroplasma) is a phloem-limited, cell-wall-less, Gram-positive plant-pathogenic bacterium and has a wide host range. It infects many weed and ornamental plants; for example, London rocket (*Sisymbrium irio*), mustard (*Brassica* spp.), wild radish (*Raphanus raphanistrum*),periwinkle (*Vinca rosea*), Chinese aster (*Callistephus chinensis*), and Shasta daisy (*Leucanthemum × superbum*) (Calavan and Bové 1989). Crop hosts include citrus (*Citrus* spp.) (Calavan and Bové 1989), horseradish (*Armoracia rusticana*, syn. *Cochlearia armoracia*) (Fletcher et al. 1981), carrot (*Daucus carota* subsp. *sativus*) (Lee et al. 2006), and sesame (*Sesamum indicum*) (Zarei et al. 2017), where it induces diseases such as citrus stubborn disease (CSD), horseradish brittleroot, carrot purple leaf, and putative sesame yellows, respectively. *S. citri* also infects leafhoppers such as *Circulifer tenellus* (Liu et al. 1983), and *C. hematoceps* (Breton et al. 2010), which transmit the pathogen from plant to plant in a circulative manner (Liu et al. 1983). In California, CSD is endemic and causes variable damage in citrus. In sweet orange (*Citrus sinensis*), symptoms include stunted growth; shortened internodes; chlorotic leaves; small upturned leaves; off-season flowering; and small, misshaped, and stylar-end greening of fruit (Yokomi et al. 2008), resulting in reduced fruit yield and quality (Mello et al. 2010).

A genome sequence of *Spiroplasma citri* strain R8-A2, collected originally from Morocco, has been reported with a 1,599,709-bp circular chromosome and two plasmids of 26,182 and

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14,987 bp in size (Davis et al. 2017). The purpose of the present research was to sequence a *S. citri* strain from California.

Using LD8 medium (Lee and Davis 1984), *S. citri* strain CC-2 was isolated in August 2016 from Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) from a production farm in Fresno County, CA and maintained in culture. For sequencing, *S. citri* strain CC-2 was triple cloned and cultured in LD8 medium at 30°C for 48 h and repeated to obtain enough DNA for sequencing. Total genomic DNA was extracted using the cetyltrimethylammonium bromide method (Doyle 1991).

Whole-genome sequencing of *S. citri* CC-2 was performed on the PacBio (Menlo Park, CA, U.S.A.) RS II platform using single-molecule real-time (SMRT) cell v3 with sequencing polymerase (P) and chemistry 4.0 v2-P6C4. The library was prepared using the PacBio procedure for preparing >30-kb libraries using the SMRTbell Express Template Preparation Kit, according to the manufacturer's specifications. Adapter screening and quality filtering of raw sequencing data were performed using SMRT Analysis (PacBio) with default settings. The CC-2 genome yielded 92,698 reads encompassing 1,420,555,954 bp. The scaffold length at which 50% of the total assembly length is covered was 21,520 bp.

The assembly of contigs from subreads was generated using Canu 1.8 (Koren et al. 2017). Four contigs were obtained (Table 1). To check for contig circularity, approximately 500-bp segments from each end of a contig were used to BLAST search PacBio read data. Appropriate reads connecting both ends were used for enclosure. Contig 1 was determined to be a chromosome because of its size of 1,709,192 bp, similar to the *S. citri* R8-A2 strain (Davis et al. 2017). Contigs 2, 3, and 4 were assigned to be plasmids because of their sizes of 40,210, 39,313, and 2,921 bp, respectively (Table 1). The chromosome and plasmid status of each contig was further confirmed by BLAST analyses against the GenBank database. All four contigs had top hits to *S. citri* (Table 1). Annotation of each contig was performed by the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al. 2016). Related metrics are presented in Table 1. These data extend the *S. citri* genome sequence database from that previously reported (Davis et al. 2017) and is the first from California.

Data availability. The whole-genome sequences reported here have been deposited in DDBJ/ENA/GenBank under the accession numbers CP042472 for contig 1, CP042473 for contig 2, CP042474 for contig 3, and CP042475 for contig 4. The versions described in this article are the first versions. PacBio sequencing reads in this study have been deposited in the NCBI Sequence Read Archive under accession number SRR9903453 and in BioProject PRJNA558054. *S. citri* strain CC-2 has been deposited in the American Type Culture Collection under number SD-7278.

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Table 1. *Spiroplasma citri* CC-2 draft genome sequence

Contig	Status ^a	Size (bp)	Coverage	GC	ORF ^b	Gene ^c	GenBank BLAST results			
							Top hit	QC ^d	ID (%) ^e	Accession ^f
1	Chr	1,709,192	1,062	25.6	1,944	38	Strain R8-A2	97	99.91	CP013197.1
2	Plasmid	40,210	37	27.7	44	0	pSci5	82	95.71	AJ969073.1
3	Plasmid	39,313	16	26.0	43	0	pSci6	90	98.89	AJ969074.1
4	Plasmid	2,921	19	23.3	5	0	pSciA	95	97.76	AJ966734.1
Sum	...	1,791,636	...	...	...	...	...	...	...	...

^a Genomic status; Chr = chromosome.

^b Open reading frame.

^c RNA gene.

^d Query cover.

^e Identity.

^f Reference accession.

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