Genome Sequence Resource of ‘*Candidatus Liberibacter asiaticus*’ strain 9PA From Brazil

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Abstract

‘*Candidatus Liberibacter asiaticus*’, an unculturable α -proteobacterium, is associated with citrus huanglongbing (HLB), a devastating disease threatening citrus production in Brazil and worldwide. In this study, a draft whole-genome sequence of ‘*Ca. L. asiaticus*’ strain 9PA from a sweet orange (cultivar Pera) tree collected in São Paulo State, Brazil, is reported. The 9PA genome is 1,231,881 bp, including two prophages, with G+C content of 36.7%. This is the first report of a whole-genome sequence of ‘*Ca. L. asiaticus*’ from Brazil or South America. The 9PA genome sequence will enrich ‘*Ca. L. asiaticus*’ genome resources and facilitate HLB research and control in Brazil and the world.

Huanglongbing (HLB) is a destructive disease with a potential of significantly reducing citrus production in Brazil and the world (Bassanezi et al. 2011; Bové 2006). In Brazil, HLB was associated with two unculturable α -proteobacteria: ‘*Candidatus Liberibacter asiaticus*’ (Coletta-Filho et al. 2004) and ‘*Ca. L. americanus*’ (Teixeira et al. 2005). Whole-genome sequencing and analyses have been playing key roles in understanding the bacterial biology and HLB control. The whole-genome sequence of ‘*Ca. L. americanus*’ strain São Paulo (NC_022793) has been published (Wulff et al. 2014). The whole-genome sequence of ‘*Ca. L. asiaticus*’ from Brazil has not yet been reported. ‘*Ca. L. asiaticus*’ is currently the predominant species found in HLB-affected citrus trees in Brazil (Lopes et al. 2017). Based on analyses in selected genomic loci, ‘*Ca. L. asiaticus*’ populations in São Paulo State of Brazil were highly homogeneous regardless of sampling year or geographic regions (da Silva et al. 2019). To further facilitate HLB research in pathogen genetics, population diversity, and other biological perspectives, this study sequenced the whole genome of a representative ‘*Ca. L. asiaticus*’ strain from Brazil.

A sweet orange tree (*Citrus sinensis* ‘Pera’) infected with ‘*Ca. L. asiaticus*’ through graft transmission in August of 2010 was maintained in a greenhouse at the Fundecitrus research facility in Araraquara, São Paulo State, Brazil. The ‘*Ca. L. asiaticus*’ strain originated from a production orchard (*C. sinensis* ‘Pera’) located in the municipality of Matão (district of Silvânia: 21°38′24.0″ S, 48°18′07.8″ W), São Paulo State and was designated as strain 9PA (da Silva et al. 2019). The infected citrus tree showed typical HLB symptoms of leaf blotchy mottling, yellow veins, and vein corking.

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The author(s) declare no conflict of interest.

Accepted for publication 20 July 2020.

Funding

This study was supported by São Paulo Research Foundation (FAPESP Process: 2019/17735-0); Fundecitrus, Araraquara, SP, Brazil; California Citrus Research Board project number 5300-188; and United States Department of Agriculture–Agricultural Research Service appropriated project number 2034-22000-012-00D.

Keywords

Brazil, ‘*Candidatus Liberibacter asiaticus*’, citrus greening, fruit, huanglongbing, pathogen diversity, prokaryotes, prophage, tree fruits, whole-genome sequence

Table 1. Summary of whole-genome sequence assembling of '*Candidatus Liberibacter asiaticus*' strain 9PA (JABDRZ000000000.1) from Brazil^a

Parameters	Mapping reference ^b			
	Contig 1-155 (chromosome)		Contig 156 (type 1)	Contig 157 (type 3)
	Psy62	A4	SC1	P-JXGC-3
Reference size (bp)	1,227,328	1,233,514	40,048	31,449
Reference accession	CP001677.5	CP010804	HQ377372	KY661963
Mapped (%)	99.7	97.4	86.5	99.5
9PA sequence size (bp)	1,160,312	NA	39,767	31,802

^a NA = not applied because the A4 sequence showed a smaller mapping percentage than the Psy62 sequence.

^b Contigs 156 and 157 are type 1 and type 3 prophages, respectively.

Total DNA was extracted from the midrib of a symptomatic leaf using a cetyltrimethylammonium bromide method (Murray and Thompson 1980), with modifications used by the Fundecitrus Diagnostic Laboratory. Presence of '*Ca. L. asiaticus*' was confirmed by quantitative PCR (Li et al. 2006) with cycle threshold = 18.8. For whole-genome sequencing, 9PA DNA was enlarged by the GenomiPhi V2 DNA Amplification Kit (GE Healthcare) according to the manufacturer's instructions. Genome sequencing was carried out on an Illumina NextSeq 500 (1 × 75 cycle) format (Illumina, Inc.) through a commercial source. In total, 36,702,109,559 bp in 434,395,368 reads (read size = 35 to 85 bp) were generated with Q scores > 30.

The 9PA genome sequence was assembled from the NextSeq data by (i) reference mapping with CLC Genomic Workbench version 7.5 (Qiagen, Inc.) and Bowtie 2 (Langmead and Salzberg 2012) using the whole-genome sequences of '*Ca. L. asiaticus*' strains psy62 (CP001677.5) (Duan et al. 2009) and A4 (CP010804) (Zheng et al. 2014, 2016) (Table 1) and (ii) de novo assembling using the CLC Genomic Workbench. '*Ca. L. asiaticus*' de novo contigs were identified and extracted through BLAST search using psy62 and A4 as queries. Because higher mapping percentage was observed from psy62 query (99.7%) (Table 1), the psy62 reference-mapping contigs were used as the base to assemble the 9PA genome sequence. Gap closures were performed using (i) information from de novo contigs that connected 9PA reference-mapping contigs, (ii) read walking following the method previously published (Shih et al. 2019), and (iii) PCR with primer sets predicted to connect two different contigs followed by Sanger sequencing of amplicons for contig connections.

For prophage identification, the three published '*Ca. L. asiaticus*' prophage sequences SC1 (HQ377372, type 1) (Zhang et al. 2011), SC2 (HQ377373, type 2) (Zhang et al. 2011) and P-JXGC-3 (KY661963, type 3) (Zheng et al. 2018) were used for reference-mapping using CLC Genomic Workbench on the NextSeq data (Table 1). Based on coverage levels, prophages of type 1 (86.5%) and type 3 (99.5%) were detected. Further gap enclosures were performed with the methods described above (i.e., use of de novo contig information, read walking, and PCR-Sanger sequencing). The type 1 prophage was 39,767 bp and the type 3 prophage sequence was 31,802 bp. The partial coverage (43.9%) of SC2 (type 2) was only on the early gene region conserved to all prophages, suggesting the absence of type 2 prophage in strain 9PA.

The final 9PA whole-genome sequence, including the two prophages, had a total of 1,231,881 bp in 157 contigs of ≥1,000 bp, an average nucleotide coverage of 20×, N₅₀ of 13,826 bp, and G+C content of 36.7%. Average nucleotide identity (ANI), a standard index for bacterial genome comparison for species determination (Goris et al. 2007; Konstantinidis and Tiedje 2005), was calculated. ANIs between 9PA and Psy62, A4, and '*Ca. L. americanus*' were 99.9, 99.8, and 76.9, respectively, further confirming the '*Ca. L. asiaticus*' status of strain 9PA at the whole-genome level. The 9PA genome was annotated to have 1,393 open reading frames and 53 RNA genes using the RAST server (<https://rast.nmpdr.org/>) (Aziz et al. 2008).

The 9PA sequence is the first '*Ca. L. asiaticus*' whole-genome sequence including two prophages reported from Brazil, filling in the current gap of the absence of Brazilian or South American '*Ca. L. asiaticus*' genome sequences in the GenBank sequence database. In addition, this study utilized the Illumina NextSeq platform with a short sequencing turn-around time (11 h), in contrast to the much longer time (>3 days) of the more commonly used platforms (Illumina HiSeq/MiSeq). This could facilitate the speed of '*Ca. L. asiaticus*' identification or determination when whole-genome sequencing is required. The 9PA genome

sequence provides geographically unique genetic and genomic information for HLB research and control for Brazil and the world.

Accession number. This whole-genome shotgun project has been deposited at DNA Data Bank of Japan/European Nucleotide Archive/GenBank under the accession number JABDRZ000000000. The version described in this article is version JABDRZ000000000.1.

Acknowledgments

We thank Y. Chavez for technical assistance.

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