

Genome Sequence Resource of ‘*Candidatus Liberibacter asiaticus*’ from *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) from La Réunion

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Abstract

‘*Candidatus Liberibacter asiaticus*’ is an insect-transmitted, phloem-restricted α -proteobacterium associated with huanglongbing. Here, we provide the whole genome sequence of ‘*Ca. L. asiaticus*’ strain ReuSP1 from its insect vector *Diaphorina citri* (Hemiptera: Liviidae) collected in La R  union. The genome is composed of 1,230,064 bp and has a 36.5% G+C content. This study reports the first ‘*Ca. L. asiaticus*’ genome sequence from La R  union, which will add to ‘*Ca. L. asiaticus*’ genome resources and help elucidate our understanding of the introduction pathway into La R  union.

Huanglongbing (HLB) or citrus greening is the most severe citrus disease. It is well established that HLB is associated with ‘*Candidatus Liberibacter asiaticus*’, ‘*Ca. L. americanus*’, and ‘*Ca. L. africanus*’ (Bov   2006; Lin 1956). ‘*Ca. L. asiaticus*’ occurs in many citrus-growing regions around the world, whereas ‘*Ca. L. africanus*’ is associated with commercial citrus orchards in Africa (Ajene et al. 2020; Bov   2006; Pietersen et al. 2010; Saponari et al. 2010), Saudi Arabia, Yemen, the Comoros Islands, Madagascar, La R  union, and Mauritius in the Indian Ocean (Bov   2006; EPPO 2014) and the island of St. Helena in the Atlantic Ocean (EPPO 2014; van den Berg and Greenland 2000).

HLB was recorded in La R  union (France: Indian Ocean) in 1967 (Bov   and Cassin 1968; Moreria 1967). Both ‘*Ca. L. asiaticus*’ and ‘*Ca. L. africanus*’, as well as their associated insect vectors *Diaphorina citri* Kuwayama (Hemiptera: Sternorrhyncha: Liviidae) and *Trioza erytreae* Del Guercio (Sternorrhyncha: Triozidae), are present in La R  union (Aubert 1977; Bov   and Cassin 1968; Catling 1970, 1972; Garnier et al. 1996). Sequencing the genome of the bacterium may advance our understanding of the genetic diversity of ‘*Ca. L. asiaticus*’ and the nature of host–pathogen interactions, thereby assisting in the development of more effective disease control measures. In this study, we report the first whole-genome sequence for a strain of ‘*Ca. L. asiaticus*’ from La R  union.

D. citri was collected from *Citrus* (Rutaceae: Aurantioideae: Aurantieae) in Pierrefond, Saint Pierre, La R  union (21  17’S, 55  26’E) in October 2017. DNA was extracted from a single adult using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, U.S.A.). Real-time PCR targeting the 16S ribosomal RNA gene was used to confirm the presence of ‘*Ca. L. asiaticus*’ (Li et al. 2006). A double-index Illumina sequencing library was prepared

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Keywords

Asian citrus psyllid, ‘*Candidatus Liberibacter asiaticus*’, citrus greening disease, genome

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Table 1. Mapping information of prophage in '*Candidatus Liberibacter asiaticus*' strain ReuSP1

Types of prophage	Reference sequence (bp)	Consensus (bp)	Total reads count	Average nucleotide coverage
Type 1	SC1 (40,048)	34,455	2,970	10.82
Type 2	SC2 (38,997)	17,455	1,104	3.29
Type 3	P-JXGC-3 (31,449)	17,529	1,354	5.82

from DNA extracts with an average insert size of 300 bp using an Illumina TruSeq, version 2 paired-end library preparation kit (300-bp insert size) (Illumina, San Diego, CA, U.S.A.). Genome sequencing was performed using a HiSeq PE150 Illumina platform (Illumina Inc.).

In total, 115,572,582 raw reads with an average length of 150 bp were generated. Adapters were trimmed and bases below a quality threshold of 30 were discarded using Trimmomatic v0.36 (Bolger et al. 2014). From all filtered reads, a preliminary metaassembly was created using MEGAHIT v1.2.9 (Li et al. 2015). The resulting contigs were queried against the genome sequences of '*Ca. L. asiaticus*' strain A4 (GenBank: CP010804) and three types of prophage sequences, represented by SC1 (GenBank: HQ377372), SC2 (GenBank: HQ377373), and P-JXGC-3 (GenBank: KY661963) using BLAST+ 2.9.0 (Camacho et al. 2009). Filtered reads were aligned to the contigs identified as '*Ca. L. asiaticus*' using the Burrows-Wheeler Aligner (BWA v0.7.15) (Li and Durbin 2009). Only reads matching those contigs were used to create a refined assembly with SPAdes v3.14.1 (Bankevich et al. 2012). Gap closure was performed using methods described by Silva et al. (2021). In total, 21 contigs over 1,000 bp were obtained that ranged from 1,434 to 341,352 bp. MUMmer v4.0 (Kurtz et al. 2004) was used to align the assembled contigs to the genome of '*Ca. L. asiaticus*' strain A4. For prophage identification, filtered reads were mapped to the three types of prophage sequences using the Burrows-Wheeler Aligner (BWA v0.7.15) (Li and Durbin 2009). The study strain, ReuSP1, was confirmed as having type 1-like prophage (Table 1). The draft genome sequence of ReuSP1, including one prophage, is 1,230,064 bp in length, with a GC content of 36.5%, an average nucleotide coverage of 8.3x, N₅₀ of 111,047 bp, and L₅₀ of 3. The genome was annotated using the RAST server (<https://rast.nmpdr.org>) (Aziz et al. 2008) and contains 1,184 open reading frames and 53 RNA genes.

In this study, we report the first draft genome sequence of '*Ca. L. asiaticus*' in La Réunion and France, thereby providing a valuable resource for understanding genetic diversity and for further comparative genomics of the '*Ca. L. asiaticus*'.

Accession number. This whole-genome shotgun project has been deposited at DNA Data Bank of Japan/European Nucleotide Archive/GenBank under the accession number CP061535.

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