Genome Sequence Resource of ‘*Candidatus Liberibacter asiaticus*’ Strain Myan16 from Myanmar

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Abstract

‘*Candidatus Liberibacter asiaticus*,’ an uncultured α -proteobacterium, is associated with citrus huanglongbing (HLB, yellow shoot disease), a destructive disease threatening citrus production worldwide. Here, we reported the draft genome sequence of ‘*Ca. L. asiaticus*’ strain Myan16 from an HLB-affected lime tree in Myitkyina, Kachin State, Myanmar. The strain Myan16 genome is 1,229,102 bp with an average G+C content of 36.4%, along with a circular prophage: P-Myan16-2 (36,303 bp, type 2). This is the first genome sequence of a ‘*Ca. L. asiaticus*’ strain from Myanmar, which will enrich the current ‘*Ca. L. asiaticus*’ genome sequence database and facilitate HLB epidemiology research in Asia and world.

‘*Candidatus Liberibacter asiaticus*,’ an uncultured, phloem-limited α -proteobacterium, is associated with citrus huanglongbing (HLB, yellow shoot disease), a devastating citrus disease worldwide (Bové 2006; Jagoueix et al. 1994; Lin 1956). In Myanmar, the presence of HLB was first confirmed by PCR detection of ‘*Ca. L. asiaticus*’ in 1996 (Garnier and Bové 2000). ‘*Ca. L. asiaticus*’ was detected in the blotchy mottle or yellow leaves of lemon, lime, mandarin, pummelo, rough lemon, and sweet orange collected in Mandalay, Pindaya, Kalaw, Inle, and Pegu areas in Myanmar (Garnier and Bové 2000), indicating the prevalence of HLB in Myanmar. However, the HLB occurrence in Myanmar could be earlier than 1996, especially because the Asian citrus psyllid (*Diaphorina citri*), the insect vector of ‘*Ca. L. asiaticus*,’ already existed in Myanmar since 1933 (Clausen 1933). As part of Indochina, Myanmar is located between the two historical epidemic centers of HLB, the Indian subcontinent and continental China (Asana 1958; Bové 2014; Lin 1956). To better understand the biology, population diversity, and epidemics of ‘*Ca. L. asiaticus*’ in Asia, genomic information for the ‘*Ca. L. asiaticus*’ strain from Myanmar is needed. In this study, we sequenced the whole genome of ‘*Ca. L. asiaticus*’ strain Myan16 from Myanmar.

The mottled leaves of an HLB-affected lime tree (*Citrus aurantium*) were collected from Myitkyina, Kachin State, Myanmar (25°23′0″N, 97°24′0″E). Total DNA were extracted from midribs of leaves sample (named Myan16) using a E.Z.N.A. High-Performance Plant DNA Extraction Kit (Omega Bio-Tek Co., China). The presence of ‘*Ca. L. asiaticus*’ was confirmed by a quantitative Taq-Man real-time PCR with primer set CLas-4G/HLBr (AGTCGAGCGCGTATGCGAAT/GCGTTATCCCGTAGAAAAAGGTAG) (Bao et al. 2020) with cycle threshold value = 22.64. Genome sequencing was carried out on an Illumina HiSeq 3000 platform with 150-bp paired-end output (Illumina Inc., San Diego, CA, U.S.A.) through a commercial source.

In total, 37,941,638 reads (150 bp per read) were generated from the Myan16 sample. All reads were initially mapped to the *C. maxima* genome (MKYQ00000001.1), *C. reticulata*

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Keywords

‘*Candidatus Liberibacter asiaticus*,’ citrus huanglongbing, genome, Myanmar

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Table 1. Mapping result of '*Candidatus Liberibacter asiaticus*' strain Myan16

Parameters	Reference (accession number, size in base pairs)			
	A4 (CP010804, 1,230,251)	SC1 (HQ377372.1, 40,048)	SC2 (HQ377373.1, 38,997)	P-JXGC-3 (KY661963.1, 31,449)
Total mapped reads	198,247	3,407	5,966	3,188
Covered of reference (%)	99	54	94	60
Average nucleotide coverage (X)	24.0	12.7	23.0	15.2
Consensus (bp)	1,226,034	21,595	36,598	18,784

genome (NIHA00000000.1), *C. sinensis* genome (AJPS00000000.1), *C. sinensis* mitochondrion (NC_037463.1), and *C. reticulata* chloroplast (KU170678.1) using Bowtie 2 (v 2.4.1) with default settings (Langmead and Salzberg 2012). All mapped reads were removed and, in total, 1,484,848 unmapped reads were retained for assembly. Both reference-based assembly and de novo assembly were performed. For reference-based assembly, the retained reads were mapped to strain A4 genome (CP010804.2) and three prophage genomes (type 1: SC1/ HQ377372.1, type 2: SC2/HQ377373.1, and type 3: P-JXGC-3/ KY661963.1) with CLC Genomic Workbench version 9.5 (default setting, length fraction = 0.5 and similarity fraction = 0.8). The prophage type of strain Myan16 was detected based on coverage levels of mapping results with three prophage sequences (Table 1). Based on the covered of reference, the prophage of type 2 (96%) but not type 1 (54%) and type 3 (60%) was detected in strain Myan16 (Table 1). The de novo assembly was performed by Velvet 1.2.0 (k-mer = 97, minimum contig length = 500 bp) (Zerbino and Birney 2008). The draft genome sequence was further assembled by combining both consensus sequences from reference-based assembly and contigs from de novo assembly through BLAST+ v2.10.0 (Camacho et al. 2009). Briefly, the de novo contigs were used as query to blast against (word size = 28, e-value = 1e-5) with reference-mapping contig sequences. Based on the blast result, the de novo contigs which connected two neighboring reference-mapping contigs were used for gap closure of Myan16 consensus sequence. For further gap closure, PCR experiments were performed with specific primer sets predicted to connect the adjacent sides of two contigs followed by Sanger sequencing of amplicons. All obtained contigs were further confirmed by remapping with the citrus-filtered reads (a total of 1,484,848 reads). These efforts generated the Myan16 whole-genome sequence with a total of 1,229,102 bp (with average G+C content of 36.4%). The Myan16 genome contained two contigs: a chromosomal region (1,192,799 bp, G+C content of 36.3%) and a type 2 prophage sequence (named P-Myan16-2, 36,303 bp, G+C content of 39.4%). The P-Myan16-2 was assembled from three de novo contigs, especially one contig that was found to connect both the 5' and 3' ends of P-Myan16-2, indicating the circular form of the P-Myan16-2 prophage sequence. Annotation with the RAST server (Aziz et al. 2008) revealed that strain Myan16 encoded a total of 1,180 open reading frames and 53 RNA genes.

The Myan16 strain genome is the first '*Ca. L. asiaticus*' whole genome reported from Myanmar. The Myan16 genome information will enrich the genome database of '*Ca. L. asiaticus*', contribute to the research in '*Ca. L. asiaticus*' population diversity, and facilitate HLB epidemiology study in Asia and the world.

Accession number. This whole-genome shotgun project has been deposited at DNA Data Bank of Japan/European Nucleotide Archive/GenBank under accession numbers CP060689 and CP060690. The version described in this article is version CP060689.1 and CP060690.1.

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