Genome Sequence Resource of ‘*Candidatus Liberibacter asiaticus*’ from Thailand

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Abstract

‘*Candidatus Liberibacter asiaticus*’ (Las) is an unculturable α -proteobacterium associated with citrus huanglongbing (HLB), a devastating disease currently threatening the citrus industry worldwide. Here, we present the genome sequence of Las strain TaiYZ2 from an HLB-affected pomelo tree in Hat Yai district, Songkhla Province, Thailand. The TaiYZ2 genome is composed of 1,230,623 bp with G+C content of 36.4%. This is the first Las genome sequence from Thailand, which will enrich current Las genome resource and facilitate HLB research and management.

‘*Candidatus Liberibacter asiaticus*’ (Las) is associated with citrus huanglongbing (HLB or yellow shoots disease, also known as citrus greening disease), a highly destructive disease currently threatening citrus production worldwide (Bové 2006; Jagoueix et al. 1994; Lin 1956). In Thailand, HLB was first observed in the 1960s and was limited to citrus orchards from the northern and central regions before 1970s (Schwarz et al. 1973a, 1973b). However, as of 1993, HLB was prevalent in most citrus orchards throughout Thailand and caused a significant decline of citrus production (Koizumi et al. 1997). All cultivated citrus varieties in Thailand were found to be affected with HLB and developed typical symptoms, including leaf mottling, yellowing, greening at the stylar end of mature fruit, abortive seeds, and tree decline (Koizumi et al. 1997). A previous study evaluated the genetic variation of the Las population in Thailand using two hypervariable prophage effector genes derived from a Florida Las strain (Puttamuk et al. 2014). Recent research showed that there existed significant genomic differences between Las strains from Florida and those from Asia (Liu et al. 2011; Puttamuk et al. 2014). Additional genomic information is needed for better understanding HLB and its pathogen in Thailand and around the world. There are currently 15 whole-genome sequences of Las strains available in GenBank (release 232.0). They are limited to the United States, China, and Japan. In this study, we performed whole-genome sequencing of a Las strain from Thailand.

The leaves of a pomelo tree (*Citrus maxima* [Burm.] Merr. cv. Tub Tim Siam) showing typical HLB symptoms in Hat Yai district, Songkhla Province, Thailand, were collected in August 2018. Total DNA was extracted from midribs using a cetyltrimethylammonium bromide method (Murray and Thompson 1980) with modifications (Deng et al. 2008). The presence of Las was confirmed by a real-time polymerase chain reaction (PCR) method

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Keywords

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

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Table 1. HiSeq sequencing and mapping information of '*Candidatus Liberibacter asiaticus*' strain TaiYZ2

Sequencing data	Total reads count	Read mapping ^a			
		A4 (1,233,514 bp)	SC1 (40,048 bp)	SC2 (38,997 bp)	P-JXGC-3 (31,449 bp)
Run 1 (reads)	81,123,432	99,545	1,711	2,959	1,591
Run 2 (reads)	68,878,490	47,799	865	1,511	828
Total (reads)	150,001,922	147,344	2,576	4,470	2,419
Consensus (bp)	–	1,230,340 (99.7%)	21,477 (53.6%) ^b	38,686 (99.2%)	19,027 (60.5%) ^b

^a The whole-genome sequence of strain A4 (GenBank accession CP010804) and three prophage sequences (SC1, HQ377372; SC2, HQ377373; and P-JXGC-3, KY661963) were used as reference for read mapping with TaiYZ2 HiSeq reads.

^b Consensus sizes were significantly less than the reference sizes, suggesting the absence of the reference prophage types.

targeting the 16S rRNA gene (Li et al. 2006) and ribonucleotide reductase gene (Zheng et al. 2016a). A previously developed procedure (Zheng et al. 2014) was followed to enrich Las DNA in the Hat Yai sample (strain TaiYZ2). Briefly, bacterial DNA was firstly enriched by removing the methylated host plant DNA with the NEBNext microbiome DNA enrichment kit (New England BioLabs, Ipswich, MA). The enriched DNA was then enlarged through multiple displacement amplification using an Illustra GenomiPhi version 2 DNA amplification kit (GE Healthcare, Waukesha, WI). Library construction for sequencing was carried out with the Illumina TruSeq version 2 paired-end library preparation kit (300-bp insert size) (Illumina, San Diego, CA). Genome sequencing was performed on the Illumina HiSeq platform. Two sequencing runs were used to ensure adequate genome coverage.

A total of 1.5×10^8 reads with an average size of 150 bp per read were generated. Referenced by the whole-genome sequence of Las strain A4 (GenBank accession CP010804) (Zheng et al. 2014, 2016b) and three Las-associated prophage sequences (SC1[HQ377372], SC2[HQ377373] [Zhang et al. 2011], and P-JXGC-3[KY661963] [Zheng et al. 2018]), a total of 147,364 nonredundant reads were identified using Bowtie2 software (version 2.3.4.3) with default settings (Langmead and Salzberg 2012) (Table 1). De novo assembly of Las reads was performed using Velvet 1.2.0 (kmer = 75, min_contig_lgth = 1,000) (Zerbino and Birney 2008). The assembly generated a total of eight contigs, ranging from 14,057 to 604,300 bp. Ordering of contigs was guided by the whole-genome sequence of Las strain A4. Gap closure was conducted by PCR with specific primer sets designed based on the adjacent sides of two contigs and Sanger sequencing. This generated a single contig consisting of 1,230,623 bp as the whole genome of Las strain TaiYZ2. The strain TaiYZ2 genome had a G+C content of 36.4% and average nucleotide coverage of 18.5x.

Genome annotation was performed with the RAST server (<http://rast.nmpdr.org/>) (Aziz et al. 2008). The TaiYZ2 genome was predicted to have 1,123 open reading frames (ORFs) and 53 RNA genes. These are similar as the published Las genomes from China: strain A4 with 1,110 ORFs and 53 RNA genes (Zheng et al. 2016b). To further identify and confirm the prophage type of Las strain TaiYZ2, a read mapping method was performed by using TaiYZ2 HiSeq data to map with three types of prophages (SC1, SC2, and P-JXGC-3). The result showed the partial cover of SC1 (type 1) and P-JXGC-3 (type 3) prophage by TaiYZ2 HiSeq reads was located at the homologous early gene region of SC1 and P-JXGC3 prophage instead of type-specific late genes region, which suggested the absence of type 1 and type 3 prophages in strain TaiYZ2 (Table 1). However, 99.2% mapping of SC2 prophage (type 2 prophage) with TaiYZ2 HiSeq data indicated strain TaiYZ2 harbored a type 2 prophage (designated as P-TaiYZ2-2) (Table 1). The total size of P-TaiYZ2-2 prophage was identified as 38,686 bp. Sequence comparison revealed the integration site of P-TaiYZ2-2 prophage was located at nucleotide position 1,189,888 of strain TaiYZ2 genome.

The genome sequence of Las strain TaiYZ2 is the first from Thailand and southeastern Asia, where the Las whole-genome sequence has not been reported. In addition to serving as a reference of HLB research in Thailand, the strain TaiYZ2 sequence (both chromosome and prophage) will contribute to research in global Las diversity and improve current efforts in HLB management.

Accession Number

This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number CP041385. The version described in this paper is version CP041385.1.

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