

Draft Genome Sequence of “*Candidatus Liberibacter asiaticus*” from *Diaphorina citri* in Guangdong, China

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The draft genome sequence of “*Candidatus Liberibacter asiaticus*” strain YCPsy from an Asian citrus psyllid (*Diaphorina citri*) in Guangdong, China, is reported here. The YCPsy strain has a genome size of 1,233,647 bp, 36.5% G+C content, 1,171 open reading frames (ORFs), and 53 RNAs.

Received 22 September 2015 Accepted 28 September 2015 Published 5 November 2015

Citation Wu F, Zheng Z, Deng X, Cen Y, Liang G, Chen J. 2015. Draft genome sequence of “*Candidatus Liberibacter asiaticus*” from *Diaphorina citri* in Guangdong, China. *Genome Announc* 3(6):e01316-15. doi:10.1128/genomeA.01316-15.

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“*Candidatus Liberibacter asiaticus*,” an unculturable alphaproteobacterium, inhabits both Asian citrus psyllids (*Diaphorina citri* Kuwayama) and citrus plants. Infection of the bacterium is associated with citrus huanglongbing (HLB) (yellow shoot disease), a destructive disease in citrus production. *D. citri* transmits “*Ca. Liberibacter asiaticus*” (1). HLB was observed in Guangdong, China, >100 years ago (2). Transmission of the HLB pathogen by *D. citri* was reported in 1977 (3) before the detection of “*Ca. Liberibacter asiaticus*” in 1996 (4, 5). Because of the lack of *in vitro* culture, research on “*Ca. Liberibacter asiaticus*” has been challenging. Thanks to the development of next-generation sequencing technology, genomes of “*Ca. Liberibacter asiaticus*” strains can be sequenced directly from psyllid or plant hosts for biological study. Zheng et al. (6) sequenced the genome of “*Ca. Liberibacter asiaticus*” strain A4 from periwinkle in Guangdong. Here, we report a draft genome sequence of “*Ca. Liberibacter asiaticus*” from *D. citri* in the same geographical location.

A mandarin citrus tree (*Citrus reticulata* cv. Shatangju) infected with “*Ca. Liberibacter asiaticus*” was maintained in a growth chamber (RXZ-380A; Jiangnan Instrument, Inc., Ningbo, China) with the settings of 28 ± 1°C, 60% ± 5% rH, and 14:10 h light/dark (L:D) at South China Agricultural University in Guangzhou, China. The original source of “*Ca. Liberibacter asiaticus*” was from an HLB Shatangju tree in Boluo City of Guangdong (23°26′07″N, 114°29′56″E). Psyllids were fed on the infected citrus tree for 2 months. An individual psyllid adult was collected for DNA extraction using the DNeasy blood and tissue kit (Qiagen, Shanghai, China). Infection of “*Ca. Liberibacter asiaticus*” was monitored by the PCR method of Li et al. (7). DNA from a single psyllid sample (threshold cycle [C_T], 18.1) was amplified using illustra GenomiPhi version 2 DNA amplification kits (GE Healthcare, Inc., Waukesha, WI, USA). The amplified DNA was sequenced using an Illumina MiSeq format (Illumina, Inc., San Diego, CA).

A total of 3.98×10^7 reads with a mean of 251 bp per read were generated from the psyllid DNA sample. Using the whole genomes of “*Ca. Liberibacter asiaticus*” strains psy62 (8) and A4 (6) as

references, a total of 5,854,876 and 5,886,489 reads, respectively, were identified using the standalone BLAST software (version 2.2.30; e-value, $<10^{-20}$) (9). The “*Ca. Liberibacter asiaticus*” reads were collected using a Perl script. The combination of *de novo* assembly using Velvet (version 1.2.10) (10) and reference assembly using Bowtie2 (version 2.2.6) (11) generated 9 contigs ranging from 1,587 bp to 755,458 bp, with an average coverage of 1,120×. The draft genome of “*Ca. Liberibacter asiaticus*” strain YCPsy comprises 1,233,647 bp, with a G+C content of 36.5%. Annotation was performed using the RAST server (<http://rast.nmpdr.org/>) (12), and the YCPsy genome was predicted to have 1,171 open reading frames (ORFs) and 53 RNAs.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. [LIIM00000000](https://www.ncbi.nlm.nih.gov/nuclink/LIIM00000000). The version described in this manuscript is the first version LIIM01000000.

ACKNOWLEDGMENTS

This research was supported by Chinese Modern Agricultural Technology Systems (CARS-27) and the Special Fund for Agro-Scientific Research in the Public Interest, China (grant 2010003067) and the California Citrus Research Board.

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REFERENCES

1. Bové JM. 2006. Huanglongbing: a destructive, newly emerging, century-old disease of citrus. *J Plant Pathol* 88:7–37.
2. Lin KH. 1956. Observations on yellow shoot of citrus. Etiological study of yellow shoot of citrus. *Acta Phytopathol Sinica* 2:1–11.
3. Plant Pathology Teaching and Research Group of Guangdong Agricultural and Forestry College. 1977. Preliminary report on huanglongbing transmission by citrus psyllid. Guangdong. *J Agric Sci* 6:50–53.
4. Deng X, Tang W. 1996. The studies on detection of citrus huanglongbing pathogen by polymerase chain reaction. *J South China Agric Univ* 17: 119–120.

5. Tian Y, Ke S, Ke C. 1996. Detection and quantitation of citrus huanglongbing pathogen by polymerase chain reaction. *Acta Phytopathol Sin* 26:243–250.
6. Zheng Z, Deng X, Chen J. 2014. Whole-genome sequence of “*Candidatus Liberibacter asiaticus*” from Guangdong, China. *Genome Announc* 2(2): e00273-14. <http://dx.doi.org/10.1128/genomeA.00273-14>.
7. Li W, Hartung JS, Levy L. 2006. Quantitative real-time PCR for detection and identification of *Candidatus Liberibacter* species associated with citrus huanglongbing. *J Microbiol Methods* 66:104–115. <http://dx.doi.org/10.1016/j.mimet.2005.10.018>.
8. Duan Y, Zhou L, Hall DG, Li W, Doddapaneni H, Lin H, Liu L, Vahling CM, Gabriel DW, Williams KP, Dickerman A, Sun Y, Gottwald T. 2009. Complete genome sequence of citrus huanglongbing bacterium, “*Candidatus Liberibacter asiaticus*” obtained through metagenomics. *Mol Plant Microbe Interact* 22:1011–1020. <http://dx.doi.org/10.1094/MPMI-22-8-1011>.
9. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. *BMC Bioinformatics* 10:421. <http://dx.doi.org/10.1186/1471-2105-10-421>.
10. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
11. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with bowtie 2. *Nat Methods* 9:357–359. <http://dx.doi.org/10.1038/nmeth.1923>.
12. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>.