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Draft Whole-Genome Sequence of "*Candidatus* Liberibacter asiaticus" Strain TX1712 from Citrus in Texas

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ABSTRACT The draft genome sequence of "*Candidatus* Liberibacter asiaticus" strain TX1712, obtained from a Texas citrus tree, is reported here. Strain TX1712 has a draft genome size of 1,203,333 bp, a G+C content of 36.4%, 1,230 predicted open reading frames, and 41 RNAs and comprises 97.4% of the psy62 reference genome.

The unculturable phloem-limited alphaproteobacterium "*Candidatus* Liberibacter asiaticus" causes Huanglongbing (HLB) disease on citrus (1, 2). HLB disease is one of the most serious citrus diseases and is responsible for major economic losses for the citrus industry across the world. "*Ca.* Liberibacter asiaticus" is the causal agent with worldwide distribution (3). In the United States, HLB disease associated with "*Ca.* Liberibacter asiaticus" was first identified in Florida in 2005 (4). Currently, the most widely used technique for detecting "*Ca.* Liberibacter asiaticus" is DNA-based analysis, due to the lack of *in vitro* culture. Whole-genome sequencing can be obtained only by using metagenomics. The first "*Ca.* Liberibacter asiaticus" genome sequence, isolated from a single infected psyllid (5), was released in 2009. HLB was first detected in Texas in 2012 from a Valencia sweet orange tree (6, 7), but the first genome sequence of a Texas "*Ca.* Liberibacter asiaticus" strain, isolated from infected psyllids, was released in 2017 (8). Here, we report a draft whole-genome sequence of "*Ca.* Liberibacter asiaticus" strain TX1712, obtained directly from an infected *Citrus sinensis* sample in Texas.

Total plant DNA of *Citrus sinensis* infected with "*Ca.* Liberibacter asiaticus" strain TX1712 was extracted from petiole and leaf midrib tissue using the DNeasy plant minikit (Qiagen, Valencia, CA). The concentration of "*Ca.* Liberibacter asiaticus" was relatively high using HLBaspr real-time quantitative PCR, giving a cycle threshold (CT) value of 19.36 (9). The 2 × 300-bp Illumina paired-end sequencing was performed on a MiSeq platform (Illumina, Inc., San Diego, CA, USA). The paired-end sequencing libraries were prepared using an Illumina TruSeq PCR-free DNA library prep kit. The DNA was fragmented by using a Covaris M220 sonicator with an average of size 550 bp.

A total of 5.16×10^7 reads, with a mean length of 296 bp per read, were generated from the MiSeq sequencing. Using the genome of "*Ca.* Liberibacter asiaticus" strain psy62 as a reference, a total of 44,844 reads with 14,281,900 bases were aligned using Geneious version 10.2.3 with the Bowtie 2 version 2.3.0 plugin tool (10), which covered 99.9% of the psy62 genome. The aligned reads were assembled using SPAdes version 3.10.0, which is available through Geneious plugin tools (11). The assembly generated 48 contigs ranging from 1,080 bp to 108,732 bp (N_{50} = 44,653) with ~10× average coverage. The contigs comprise a total length of 1,203,333 bp, with a G+C content of 36.4% and 97.4% coverage of the complete psy62 genome. Annotation was performed using the Rapid Annotations using Subsystems Technology (RAST) server (http://rast.nmpdr.org) (12). The TX1712 genome was predicted to have 1,230 open reading frames and 41 RNAs.

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Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number QEWL00000000. The version described in this paper is the first version, QEWL01000000.

REFERENCES

1. Jagoueix S, Bove J-M, Garnier M. 1994. The phloem-limited bacterium of greening disease of citrus is a member of the α subdivision of the *Proteobacteria*. Int J Syst Bacteriol 44:379–386. https://doi.org/10.1099/00207713-44-3-379.
2. Bové JM. 2006. Huanglongbing: a destructive, newly emerging, century-old disease of citrus. J Plant Pathol 88:7–37.
3. Gottwald TR, da Graça JV, Bassanezi RB. 2007. Citrus Huanglongbing: the pathogen and its impact. Plant Health Progr. https://doi.org/10.1094/PHP-2007-0906-01-RV.
4. Halbert SE. 2005. The discovery of Huanglongbing in Florida, p 50. *In* Proceedings of the International Citrus Canker and Huanglongbing Research Workshop. Florida Citrus Mutual, Orlando, FL.
5. 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. https://doi.org/10.1094/MPMI-22-8-1011.
6. Kunta M, Sétamou M, Skaria M, Li W, Nakhla MK, da Graça JV. 2012. First report of citrus Huanglongbing in Texas. Phytopathology 102:S4.66.
7. da Graça JV, Kunta M, Sétamou M, Rascoe J, Li W, Nakhla MK, Salas B, Bartels DW. 2015. Huanglongbing in Texas: report on the first detections in commercial citrus. J Citrus Pathol 2.
8. Kunta M, Zheng Z, Wu F, da Graca JV, Park J-W, Deng X, Chen J. 2017. Draft whole-genome sequence of "*Candidatus* Liberibacter asiaticus" strain TX2351 isolated from Asian citrus psyllids in Texas, USA. Genome Announc 5(15):e00170-17. https://doi.org/10.1128/genomeA.00170-17.
9. 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. https://doi.org/10.1016/j.mimet.2005.10.018.
10. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359. https://doi.org/10.1038/nmeth.1923.
11. Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, Prjibelski AD, Pyshkin A, Sirotkin A, Sirotkin Y, Stepanauskas R, Clingenpeel SR, Woyke T, McLean JS, Lasken R, Tesler G, Alekseyev MA, Pevzner PA. 2013. Assembling genomes and mini-metagenomes from highly chimeric reads. *In* Deng M, Jiang R, Sun F, Zhang X (ed), Research in computational molecular biology: RECOMB 2013. Springer, Berlin, Germany.
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. https://doi.org/10.1186/1471-2164-9-75.