Draft Genome Sequence of "*Candidatus* Liberibacter americanus" Bacterium Associated with Citrus Huanglongbing in Brazil

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We report here the draft genome sequence of "*Candidatus* Liberibacter americanus" strain PW_SP. The 1,176,071-bp genome, with 31.6% G+C content, comprises 948 open reading frames, 38 tRNAs, and three complete rRNAs.

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"he genus of "Candidatus Liberibacter" belongs to the Gramnegative alphaproteobacteria. There are five pathogenic "Ca. Liberibacter" species in this genus. "Candidatus Liberibacter asiaticus," "Candidatus Liberibacter africanus," and "Candidatus Liberibacter americanus" are associated with citrus huanglongbing (HLB) (1). "Candidatus Liberibacter solanacearum" is associated with potato zebra chip disease and other diseases of solanaceous crops (2). In addition, "Candidatus Liberibacter europaeus" is associated with disease-like symptoms similar to those of Scotch broom in New Zealand (3). "Ca. Liberibacter asiaticus" and "Ca. Liberibacter americanus" were identified in São Paulo State, Brazil, in 2004 and 2005, respectively (4, 5). "Ca. Liberibacter americanus" was the prevalent species and occurred in almost 90% of symptomatic trees in citrus orchards compared with "Ca. Liberibacter asiaticus" during the first two years of the HLB outbreak in Brazil. However, this situation has gradually reversed during the last five years, with "Ca. Liberibacter asiaticus" now being found in the majority of HLB-affected citrus orchards in São Paulo State (6). While the reason for this is not clear, it has been suggested that the heat sensitivity of "Ca. Liberibacter americanus" and the high titer in "Ca. Liberibacter asiaticus"-infected symptomatic plants might account for a competitive advantage of the latter during the natural transmission by psyllids (6, 7). Hitherto, "Ca. Liberibacter americanus" has only been found in Brazil. Both "Ca. Liberibacter americanus" and "Ca. Liberibacter asiaticus" are transmitted by an insect vector, the Asian citrus psyllid (Diaphorina citri) (1).

To gain insight into its bacterial genomic information, we sequenced the genome of "*Ca.* Liberibacter americanus" strain PW_SP from São Paulo, Brazil. Genomic DNA was obtained from dodder-infected periwinkle plants (*Catharanthus roseus*) grown in a screen house at Centro de Citricultura, in Cordeiropolis, São Paulo, Brazil. The draft genome sequence of the "*Ca.* Liberibacter americanus" genome was obtained by an Illumina HiSeq 2000 with a 300-bp paired-end library that achieved $18.6 \times$ coverage for the "*Ca.* Liberibacter americanus" genome. *De novo* assembly was performed using the Velvet assembler version 1.1. Twenty-two contigs were identified as belonging to the "*Ca.* Liberibacter americanus" genome via BLASTn and BLASTx analyses against the reference genome of "Ca. Liberibacter solanacearum" (accession no. CP002371) (8). All 22 "Ca. Liberibacter americanus" contigs were confirmed by PCR. Final annotation was reconfirmed by the NCBI Prokaryotic Genomes Annotation Pipeline (PGAAP) (http://www.ncbi.nlm.nih.gov/genomes /static/Pipeline.html). The "Ca. Liberibacter americanus" strain PW_SP genome comprises 1,176,071 nucleotides with a G+C content of 31.6%, 948 predicted coding sequences, 38 tRNAs, 3 complete copies of ribosomal RNA genes (16S, 23S, and 5S) and 213 hypothetical genes. While "Ca. Liberibacter americanus" is associated with citrus HLB, comparative genome analyses of the "Ca. Liberibacter asiaticus," "Ca. Liberibacter solanacearum," and "Ca. Liberibacter americanus" genomes indicated that the "Ca. Liberibacter americanus" genome is more similar to that of "Ca. Liberibacter solanacearum" (8) than to that of "Ca. Liberibacter asiaticus" (9), with respect to their genomic structure and sequence similarity. Phylogenetic analysis using 52 orthologous genes from pathogenic "Ca. Liberibacter asiaticus," "Ca. Liberibacter solanacearum," and "Ca. Liberibacter americanus" species, along with the members of Rhizobiaceae and other alphaproteobacteria, shows that "Ca. Liberibacter americanus" is closely positioned proximal to the basal node, followed by "Ca. Liberibacter solanacearum" and "Ca. Liberibacter asiaticus," indicating an early divergence of "Ca. Liberibacter americanus." An additional "Ca. Liberibacter americanus" genome sequence extends comparative genome analyses and advances the understanding of the reductive genome evolution in "Ca. Liberibacter."

Nucleotide sequence accession number. The whole-genome sequence of "*Ca*. Liberibacter americanus" strain PW_SP has been deposited in GenBank under the accession no. AOFG00000000.

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REFERENCES

- 1. Bové JM. 2006. Huanglongbing: a destructive, newly-emerging, centuryold disease of citrus. J. Plant Pathol. 88:7–37.
- Liefting LW, Weir BS, Pennycook SR, Clover GR. 2009. "Candidatus Liberibacter solanacearum," associated with plants in the family Solanaceae. Int. J. Syst. Evol. Microbiol. 59:2274–2276.
- Thompson S, Fletcher JD, Ziebell H, Beard S, Panda P, Jorgensen N, Fowler SV, Liefting LW, Berry N, Pitman AR. 2013. First report of "*Candidatus* Liberibacter europaeus" associated with psyllid infested Scotch broom. New Dis. Rep. 27:6.
- 4. Coletta-Filho HD, Targon MLPN, Takita MA, De Negri JD, Pompeu J, Machado MA, do Amaral AM, Muller GW. 2004. First report of the causal agent of Huanglongbing "*Candidatus* Liberibacter asiaticus" in Brazil. Plant Dis. 88:1382.
- Texeira DC, Ayres J, Kitajima EW, Danet L, Jagoueix-Eveillard S, Saillard C, Bové JM. 2005. First report of a huanglongbing-like disease of citrus in São Paulo state, Brazil and association of a new Liberibacter spe-

cies, "*Candidatus* Liberibacter americanus," with the disease. Plant Dis. **89**:107.

- 6. Coletta-Filho HD, Carlos EF, Lotto LL, Luciane FC, Alves KCS, Pereira MAR, Machado MA. 2010. Prevalence of "*Candidatus* Liberibacter" spp. in HLB-diseased citrus plants in São Paulo State, Brazil. Proceedings of the 17th conference of the IOCV, 2010 Insect-transmitted prokaryotes. Centro APTA Citros Sylvio Moreira, IAC, Cordeiropolis, São Paulo, Brazil.
- Gasparoto MCG, Coletta-Filho HD, Bassanezi RB, Lopes SA, Lourenco SA, Amorim L. 2012. Influence of temperature on infection and establishment of "*Candidatus* Liberibacter americanus" and "*Candidatus* Liberibacter asiaticus" in citrus plants. Plant Pathol. 61:658–664.
- Lin H, Lou B, Glynn JM, Doddapaneni H, Civerolo EL, Chen C, Duan Y, Zhou L, Vahling CM. 2011. The complete genome sequence of "*Candidatus* Liberibacter solanacearum", the bacterium associated with potato zebra Chip disease. PLoS One 6:e19135.
- Duan Y, Zhou L, Hall DG, Li W, Doddapaneni H, Lin H, Liu L, Vahling DM, 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.