

Draft Genome Sequence of “*Candidatus Phytoplasma pruni*” Strain CX, a Plant-Pathogenic Bacterium

I.-M. Lee,^a J. Shao,^a K. D. Bottner-Parker,^a D. E. Gundersen-Rindal,^b Y. Zhao,^a R. E. Davis^a

Molecular Plant Pathology Laboratory, U.S. Department of Agriculture, Beltsville, Maryland, USA^a; Invasive Insect Biocontrol and Behavior Laboratory, U.S. Department of Agriculture, Beltsville, Maryland, USA^b

“*Candidatus Phytoplasma pruni*” strain CX, belonging to subgroup 16SrIII-A, is a plant-pathogenic bacterium causing economically important diseases in many fruit crops. Here, we report the draft genome sequence, which consists of 598,508 bases, with a G+C content of 27.21 mol%.

Received 14 August 2015 Accepted 11 September 2015 Published 15 October 2015

Citation Lee I-M, Shao J, Bottner-Parker KD, Gundersen-Rindal DE, Zhao Y, Davis RE. 2015. Draft genome sequence of “*Candidatus Phytoplasma pruni*” strain CX, a plant-pathogenic bacterium. *Genome Announc* 3(5):e01117-15. doi:10.1128/genomeA.01117-15.

Copyright © 2015 Lee et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to I.-M. Lee, ingming.lee@ars.usda.gov.

Phytoplasmas are cell wall-less plant-pathogenic prokaryotes, which inhabit both insects (e.g., leafhoppers, planthoppers, and psyllids) and >1,000 plant species (1–3), causing numerous economically important diseases worldwide. In nature, insects serve as vectors that transmit phytoplasmas and spread diseases among plants. There are a vast number of diverse phytoplasma strains that are distributed on all continents. Phylogenetic analysis based on 16S rRNA gene sequences has indicated that phytoplasmas form a large discreet monophyletic clade paraphyletic to the genus *Acholeplasma* in the class *Mollicutes* (4). Because of the inability to readily cultivate phytoplasmas in cell-free medium, the provisional genus “*Candidatus Phytoplasma*” and also “*Candidatus Phytoplasma* spp.” were proposed to accommodate their classification (5). For finer classification of phytoplasmas, a scheme was proposed based on restriction fragment length polymorphism (RFLP) analysis of the 16S rRNA sequence that thus far includes 32 16S ribosomal (16Sr) groups and >200 subgroups (6, 7). Group 16SrIII represents one of the most diverse groups (8–10). “*Candidatus Phytoplasma pruni*” strain CX belongs to subgroup 16SrIII-A; strains in this subgroup cause severe disease of decline in many stone fruit trees (10). In order to understand the pathogenic nature of phytoplasmas, genomic sequencing has been the focus. Thus far, five phytoplasma genomes (belonging to 16SrI, 16SrX, and 16SrXII groups) have been fully sequenced (11–15). Draft genome sequences of four phytoplasmas belonging to 16SrIII-B (Italian clover phyllody, “*Candidatus Phytoplasma*” strain MA), 16SrIII-F (milkweed witches’ broom, “*Candidatus Phytoplasma*” strain MWI and *Vaccinium* witches’ broom “*Candidatus Phytoplasma*” strain VAC), and 16SrIII-H (poinsettia branch-inducing “*Candidatus Phytoplasma*” strain JR1 = PoiBI), were published recently (16). Here, we report the draft genome sequence of “*Ca. Phytoplasma pruni*” strain CX.

“*Candidatus Phytoplasma pruni*” strain CX DNA was extracted from preparations of sieve cells isolated from infected periwinkle plants (*Catharanthus roseus*), as previously described (17), with the addition of RNase A digestion prior to the final phenol-chloroform extraction. Whole-genome paired-end sequencing

was performed using the 454-GS Junior system (Roche Diagnostics, Indianapolis, IN). The original 177,537 reads were filtered using the BLAST(p/n). The reads were searched against custom BLAST databases containing “*Candidatus Phytoplasma*” sequences. This resulted in 132,205 reads that were assembled using the Newbler Assembler 2.9. The number of aligned reads was 130,704, and the number of aligned bases was 43,684,964. The average read coverage was 65×. The assembly resulted in 46 contigs >534 bp, with a total base value of 598,508 bases, which is in agreement with the sizes of other published group 16SrIII “*Candidatus Phytoplasma*” partial genomes (583 to 670 kb) (16, 18). The G+C content was 27.21 mol%. The N_{50} was 38,825 bases, and the largest contig was 93,855 bases. Use of the gene finder GeneMark.hmm on the largest 46 contigs resulted in the identification of 602 protein-coding genes.

The availability of the 16SrIII-A CX draft genome sequence combined with the other existing four draft genome sequences of group 16SrIII strains will facilitate the identification of specific genomic features of this group that may be responsible for the pathogenesis inflicted by various 16SrIII group “*Candidatus Phytoplasma*” strains. All five strains exhibit characteristic symptoms in their common host, *C. roseus*.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under accession no. [LHCF00000000](https://www.ncbi.nlm.nih.gov/nuclink/LHCF00000000). The version described in this paper is the first version, LHCF01000000.

ACKNOWLEDGMENT

Funding for this project was provided by the U.S. Government.

REFERENCES

- Bertaccini A. 2007. Phytoplasmas: diversity, taxonomy, and epidemiology. *Front Biosci* 12:673–689. <http://dx.doi.org/10.2741/2092>.
- Hogenhout SA, Oshima K, Ammar E, Kakizawa S, Kingdom HN, Namba S. 2008. Phytoplasmas: bacteria that manipulate plants and insects. *Mol Plant Pathol* 9:403–423. <http://dx.doi.org/10.1111/j.1364-3703.2008.00472.x>.
- Lee I, Davis RE, Gundersen-Rindal DE. 2000. Phytoplasma: phytopatho-

- genic *Mollicutes*. *Annu Rev Microbiol* 54:221–255. <http://dx.doi.org/10.1146/annurev.micro.54.1.221>.
4. Gundersen DE, Lee I.-M, Rehner SA, Davis RE, Kingsbury DT. 1994. Phylogeny of mycoplasma-like organisms (phytoplasmas): a basis for their classification. *J Bacteriol* 176:5244–5254.
 5. IRPCM Phytoplasma/Spiroplasma Working Team--Phytoplasma Taxonomy Group. 2004. 'Candidatus Phytoplasma', a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects. *Int J Syst Evol Microbiol* 54:1243–1255.
 6. Lee I.-M, Gundersen-Rindal DE, Davis RE, Bartoszyk IM. 1998. Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. *Int J Syst Evol Microbiol* 48:1153–1169.
 7. Nejat N, Vadamalai G, Davis RE, Harrison NA, Sijam K, Dickinson M, Abdullah SNA, Zhao Y. 2013. "Candidatus Phytoplasma malaysianum", a novel taxon associated with virescence and phyllody of Madagascar periwinkle (*Catharanthus roseus*). *Int J Syst Evol Microbiol* 63:540–548. <http://dx.doi.org/10.1099/ijs.0.041467-0>.
 8. Wei W, Davis RE, Lee I.-M, Zhao Y. 2007. Computer-simulated RFLP analysis of 16S rRNA genes: identification of ten new phytoplasma groups. *Int J Syst Evol Microbiol* 57:1855–1867. <http://dx.doi.org/10.1099/ijs.0.65000-0>.
 9. Zhao Y, Wei W, Lee I.-M, Shao J, Suo X, Davis RE. 2009. Construction of an interactive online phytoplasma classification tool, *iPhyClassifier*, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). *Int J Syst Evol Microbiol* 59:2582–2593. <http://dx.doi.org/10.1099/ijs.0.010249-0>.
 10. Davis RE, Zhao Y, Dally EL, Lee I.-M, Jomantiene R, Douglas SM. 2013. "Candidatus Phytoplasma pruni", a novel taxon associated with X-disease of stone fruits, *Prunus* spp.: multilocus characterization based on 16S rRNA, *secY*, and ribosomal protein genes. *Int J Syst Evol Microbiol* 63:766–776. <http://dx.doi.org/10.1099/ijs.0.041202-0>.
 11. Oshima K, Kakizawa S, Nishigawa H, Jung H, Wei W, Suzuki S, Arashida R, Nakata D, Miyata S, Ugaki M, Namba S. 2004. Reductive evolution suggested from the complete genome sequence of a plant-pathogenic phytoplasma. *Nat Genet* 36:27–29. <http://dx.doi.org/10.1038/ng1277>.
 12. Bai X, Zhang J, Ewing A, Miller SA, Jancso Radek A, Shevchenko DV, Tsukerman K, Walunas T, Lapidus A, Campbell JW, Hogenhout SA. 2006. Living with genome instability: the adaptation of phytoplasmas to diverse environments of their insect and plant hosts. *J Bacteriol* 188:3682–3696. <http://dx.doi.org/10.1128/JB.188.10.3682-3696.2006>.
 13. Tran-Nguyen LTT, Kube M, Schneider B, Reinhardt R, Gibb KS. 2008. Comparative genome analysis of "Candidatus Phytoplasma australiense" (subgroup *tuf*-Australia I; *rp*-A) and "Ca. Phytoplasma asteris" strains OY-M and AY-WB. *J Bacteriol* 190:3979–3991.
 14. Kube M, Schneider B, Kuhl H, Dandekar T, Heitmann K, Migdoll AM, Reinhardt R, Seemüller E. 2008. The linear chromosome of the plant-pathogenic mycoplasma "Candidatus Phytoplasma mali". *BMC Genomics* 9:306. <http://dx.doi.org/10.1186/1471-2164-9-306>.
 15. Andersen MT, Liefting LW, Havukkala I, Beaver RE. 2013. Comparison of the complete genome sequence of two closely related isolates of "Candidatus Phytoplasma australiense" reveals genome plasticity. *BMC Genomics* 14:529. <http://dx.doi.org/10.1186/1471-2164-14-529>.
 16. Saccardo F, Martini M, Palmano S, Ermacora P, Scortichini M, Loi N, Firrao G. 2012. Genome drafts of four phytoplasma strains of the ribosomal group 16SrIII. *Microbiology* 158:2805–2814. <http://dx.doi.org/10.1099/mic.0.061432-0>.
 17. Lee I.-M, Davis RE. 1988. Detection and investigation of genetic relatedness among aster yellows and other mycoplasma-like organisms by using cloned DNA and RNA probes. *Mol Plant Microbe Interact* 1:303–310. <http://dx.doi.org/10.1094/MPMI-1-303>.
 18. Marcone C, Neimark H, Ragozzino A, Lauer U, Seemüller E. 1999. Chromosome sizes of phytoplasmas composing major phylogenetic groups and subgroups. *Phytopathology* 89:805–810. <http://dx.doi.org/10.1094/PHYTO.1999.89.9.805>.