DATA NOTE

Whole genome sequence of five strains of *Spiroplasma citri* isolated from different host plants and its leafhopper vector

Raymond Yokomi1*, Rachel Rattner1, Fatima Osman2, Yogita Maheshwari1, Vijayanandraj Selvaraj1, Deborah Pagliaccia3, Jianchi Chen1 and Georgios Vidalakis4

Abstract

**Objectives:** *Spiroplasma citri* is a bacterium with a wide host range and is the causal agent of citrus stubborn and brittle root diseases of citrus and horseradish, respectively. *S. citri* is transmitted in a circulative, persistent manner by the beet leafhopper, *Nealoaliturus (Circulifer) tenellus* (Baker), in North America. Five strains of *S. citri* were cultured from citrus, horseradish, and *N. tenellus* from different habitats and times. DNA from cultures were sequenced and genome assembled to expand the database to improve detection assays and better understand its genetics and evolution.

**Data description:** The whole genome sequence of five strains of *S. citri* are described herein. The *S. citri* chromosome was circularized for all five strains and ranged from 1,576,550 to 1,742,208 bp with a G+C content of 25.4–25.6%. Characterization of extrachromosomal DNAs resulted in identification of one or two plasmids, with a G+C content of 23.3 to 27.6%, from plant hosts; and eight or nine plasmids, with a G+C content of 21.65 to 29.19%, from *N. tenellus*. Total genome size ranged from 1,611,714 to 1,832,173 bp from plants and 1,968,976 to 2,155,613 bp from the leafhopper. All sequence data has been deposited in DDBJ/ENA/GenBank under the accession numbers CP046368-CP046373 and CP047426-CP047446.

**Keywords:** Citrus stubborn disease, Brittle root disease, Leafhopper vectors, Genome sequencing and annotation, Spiroplasmas, *Circulifer*

Objective

Spiroplasmas are wall-less, gram-positive bacteria with mobile helical cells and can infect numerous organisms including plants, insects, mites, ticks, crustaceans, and mammals. Economically important spiroplasmas in the U.S.A. are plant pathogens such as *Spiroplasma citri*, causal agent of citrus stubborn disease (CSD) [1], brittle root of horseradish (*Armoracia rusticana*) [2], and *S. kunkelli*, causal agent of corn stunt [3]. In California, CSD is endemic [4] and can be a serious disease of citrus [5, 6]. Its incidence ranges from spotty to abundant due to reasons such as, but not limited to, abundance of the *S. citri* vector *Nealoaliturus tenellus* (Baker), also known as *Circulifer tenellus*, the beet leafhopper (BLH); proximity of citrus to annual crops and dichotomous hosts infected with *S. citri* which are hosts of the BLH; and semi-arid, hot climate/habitat [4, 7]. To determine any genomic differences that may occur between *S. citri* populations, the bacterium was isolated and cultured from different times and hosts, and *S. citri* DNA purified and subjected to PacBio sequencing. The whole genome sequence was assembled for five strains to add to the two *S. citri* sequences in the public database [8, 9]. The genome sequences reported here will be used for comparative genomics and to better understand the etiology, relationships and evolution...
among the spiroplasmas. In addition, this genomic data will be used to improve detection assays for S. citri from those previously published [4, 10, 11].

**Data description**

_Spiroplasma citri_ was isolated and grown in LD8 medium [12], triple cloned, and stored at −80 °C. _S. citri_ strain C189 was established in 1972 from a Navel orange tree (_Citrus sinensis_ (L.) Osb.) [1] in Riverside, California by grafting to Madam Vinous sweet orange seedlings and maintained _in planta_ at the Citrus Clonal Protection Program, University of California, Riverside, California. _S. citri_ strain BR-12 was obtained in 1981 from horseradish in Collinsville, Illinois [2]. _S. citri_ strain LB319 was isolated in 2007 from a Spring Navel orange tree in Ducor, California. _S. citri_ strain BLH-13 was isolated in 2010 from BLH collected from parsley (_Petroselinum crispum_) in Mettler, California. _S. citri_ strain BLH-MB was isolated in 2011 from BLH collected from Russian thistle (_Salsola tragus_) in Parlier, California.

Cultures were re-established for this study and total genomic DNA was extracted by CTAB [13]. Sequencing was performed using PacBio (Menlo Park, CA, USA) RS II platform using single molecule real-time (SMRT) cell v3 with sequencing polymerase (P) and chemistry 4.0 v2©—P6C4 [9]. The library was prepared using PacBio Procedure-Preparing > 30 kb libraries using SMRTbell Express Template Preparation Kit according to manufacturer’s specifications. Adapter screening and quality filtering of raw sequencing data were performed using SMRT Analysis (PacBio) with default settings. The _S. citri_ genomes yielded between 40,816 and 122,010 reads encompassing a range of 5.4 Mb to 1.7 Gb. The N50 value was between 17,795 and 20,974 bp.

For each of the five _S. citri_ strains, filtered subreads established by PacBio were assembled into contigs using Canu 1.8 [14]. To check for contig circularity, ~500 bp segments from each end of a contig were used to BLASTn search the PacBio read data. Appropriate reads connecting both ends were used for enclosure. The chromosome and plasmid status of each contig were further confirmed by BLASTn analyses against the GenBank database. The _S. citri_ chromosome was circularized for all five strains and ranged from 1,576,550 to 1,742,208 bp, with an average coverage of 59-fold and an average G+C content of 25.4%. Total genome size ranged from 1,611,714 to 1,832,173 from plants and 1,968,976 to 2,155,613 from plants and 1,968,976 to 2,155,613 from plants and 1,968,976 to 2,155,613 from plants and 1,968,976 to 2,155,613 from plants and 1,968,976 to 2,155,613 from plants and 1,968,976 to 2,155,613 from plants and 1,968,976 to 2,155,613 from plants and 1,968,976 to 2,155,613 from plants and 1,968,976 to 2,155,613 from plants and 1,968,976 to 2,155,613 from

**Table 1 Overview of data files/data sets**

| Label          | Name of data file/data set                                      | File types (file extension) | Data repository and identifier (DOI or accession number) |
|----------------|----------------------------------------------------------------|----------------------------|--------------------------------------------------------|
| Bioproject [16] | Spiroplasma citri genome sequencing and assembly                | No file type                | PRJNA591027 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA591027) |
| Data set 1 [17] | SRX7514565:Whole genome sequencing of Spiroplasma citri       | fastq.gz                   | NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/SRR10849327) |
| Data set 2 [18] | SRX7196003:Whole genome sequencing of Spiroplasma citristrain LB 319 | fastq.gz                   | NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/SRR10507068) |
| Data set 3 [19] | SRX7196004:Whole genome sequencing of Spiroplasma citristrain BR12 | fastq.gz                   | NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/SRR10507067) |
| Data set 4 [20] | SRX7196005:Whole genome sequencing of Spiroplasma citristrain BLH-MB | fastq.gz                   | NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/SRR10507066) |
| Data set 5 [21] | SRX7196006:Whole genome sequencing of Spiroplasma citristrain BLH-13 | fastq.gz                   | NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/SRR10507065) |
| Data file 1 [22] | ASM1058717v1: Spiroplasma citri strain C189 Whole genome assembly data | FASTA                      | NCBI Genbank (Accession: GCA_010587175.1) (https://www.ncbi.nlm.nih.gov/assembly/GCA_010587175.1) |
| Data file 2 [23] | ASM1058710v1: Spiroplasma citri strain LB 319 Whole genome assembly data | FASTA                      | NCBI Genbank (Accession: GCA_010587105.1) (https://www.ncbi.nlm.nih.gov/assembly/GCA_010587105.1) |
| Data file 3 [24] | ASM1058705v1: Spiroplasma citri strain BR12 Whole genome assembly data | FASTA                      | NCBI Genbank (Accession: GCA_010587055.1) (https://www.ncbi.nlm.nih.gov/assembly/GCA_010587055.1) |
| Data file 4 [25] | ASM1058730v1: Spiroplasma citri strain BLH-MB Whole genome assembly data | FASTA                      | NCBI Genbank (Accession: GCA_010587305.1) (https://www.ncbi.nlm.nih.gov/assembly/GCA_010587305.1) |
| Data file 5 [26] | ASM1058725v1: Spiroplasma citri strain BLH-13 Whole genome assembly data | FASTA                      | NCBI Genbank (Accession: GCA_010587255.1) (https://www.ncbi.nlm.nih.gov/assembly/GCA_010587255.1) |
the BLH. Extrachromosomal DNAs were characterized, which resulted in identification of one or two plasmids from the plant hosts; and eight or nine plasmids from the BLH. The genome sequence data has been deposited in the NCBI database under Accession numbers CP046368-CP046373 and CP047426-CP047446 (Table 1; Bioproject; Data set 1–5). Annotation of each contig was performed by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [15] and predicted 38 RNA genes for all strains and between 1908 and 2556 coding sequences. These data extend the sequence database of S. citri and should help to improve detection assays for S. citri and provide insight on the evolution of plant pathogenic spiroplasmas.

Limitations

- Contigs that did not clearly associate with the chromosome were designated as putative plasmids.
- Plasmids that were not circularized were assumed to be linear.

Abbreviations

BLH: Beet leafhopper; bp: Base pair; CA: California; CSD: Citrus stubborn disease; CTAB: Cetyltrimethylammonium bromide; IL: Illinois; NCBI: National Center for Biotechnology Information; SMRT: Single-molecule real-time.

Acknowledgements

We thank Robert DeBorde of the United States Department of Agriculture–Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, Parlier, CA for technical assistance. Jackie Fletcher is gratefully acknowledged for genome assembly and bioinformatics. RR conducted data curation and analysis. RKY and GV wrote the manuscript and FO, DP, JC and GV edited the manuscript. All authors read and approved the final manuscript.

Authors’ contributions

RKY, GV, FO and DP were involved in conceptualization of the study and acquisition of S. citri cultures. Funds for this research were obtained from grants to RKY and GV. DP, YM, and VS cultured the pathogens and purified the DNA. FO supervised the PACBio submissions. JC provided technical assistance for genome assembly and bioinformatics. RR performed data curation and analysis. RKY and RR wrote the manuscript and FO, DP, JC and GV edited the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by USDA 2034-22000-013-10R and two grants from the Citrus Research Board: Project 5300-191 to R. Yokomi; and Project 5100-153 to G. Vidalakis. Additional support for this work was provided in part by the Citrus Research Board (Project 6100), the USDA National Institute of Food and Agriculture, Hatch Project 233744 and the National Clean Plant Network (AP17PPOSS&010C118 and AP18PPO5&020C107). The sequencing was carried out at the DNA Technologies and Expression Analysis Cores at the UC Davis Genome Center, supported by NIH Shared Instrumentation Grant 15100DD010786-01. The funding bodies played no role in the design of the study and collection, analysis, or interpretation of data or in writing of the manuscript.

Availability of data and materials

The data described in this Data note can be freely and openly accessed from the NCBI database https://www.ncbi.nlm.nih.gov/bioproject/PRJNA591027 [16]. All sequence data has been deposited in DDBJ/ENA/CentBank under the accession numbers CP046368-CP046373 and CP047426-CP047446. Please see Table 1 and references for details and links to the data. The versions described in this paper are the first versions. PacBio sequencing reads in this study have been deposited in BioProject PRJNA591027, SRR10843927 for C189, SRR10507068 for LB319; SRR10507067 for BR12; SRR10507065 for BLH13, and SRR10507066 for BLH-MB. S. citri cultures for BLH13, BLH-MB, BR12, and LB319 have been deposited in ATCC no. SD-7532-7533, respectively. However, ATCC Biorepository Service Agreement 2019-BRS-00049 maintains confidentiality of information regarding these cultures. Information on these cultures are available upon request from the corresponding author.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

1 United States Department of Agriculture - Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, Parlier, CA 93648, USA. 2 Department of Plant Pathology, University of California, Davis, CA 95616, USA. 3 Department of Botany & Plant Sciences, University of California, Riverside, CA 92521, USA. 4 Department of Microbiology and Plant Pathology, University of California, Riverside, CA 92521, USA.

Received: 7 February 2020   Accepted: 27 June 2020

Published online: 03 July 2020

References

1. El-Shafy A, Fuda-Allah A, Calavan EC, Igwegbe ECK. Culture of a mycoplasma-like organism associated with stubborn disease of citrus. Phytopathology. 1972;62:729–31.
2. Fletcher J, Schultz GA, Davis RE, Eastman CE, Goodman RM. Brittle root disease of horseradish: evidence for an etiological role of Spiroplasma citri. Phytopathology. 1981;71:1073–80.
3. Chen TA, Liao CH. Corn stunt spiroplasma: isolation, cultivation and proof of pathogenicity. Science. 1975;188(4192):1015–7.
4. Yokomi RK, Mello AFS, Saponari M, Fletcher J. Polymerase chain reaction-based detection of Spiroplasma citri associated with citrus stubborn disease. Plant Dis. 2008;92:253–60.
5. Mello AFS, Yokomi RK, Melcher U, Chen JC, Fletcher J. Citrus stubborn severity is associated with Spiroplasma citri titer but not with bacterial genotype. Plant Dis. 2010;94:75–82.
6. Mello AFS, Yokomi RK, Payton ME, Fletcher J. Effect of citrus stubborn disease on navel orange production in a commercial orchard in California. J Plant Path. 2010;92:429–38.
7. Yokomi RK, Sisterson M. Validation and comparison of a hierarchical sampling plan for estimating incidence of citrus stubborn disease. In: Proceedings of the 18th conference of the International Organization of Citrus virologists; 2011. https://oov.ucr.edu/proceeding/elevenht/Yokomi_and_Sisterson.pdf.
8. Davis RE, Shao J, Zhao Y, Gasparich GE, Gaynor BJ, Donofrio N. Complete genome sequence of Spiroplasma citri strain R8-A2T, causal agent of stubborn disease in Citrus species. Genome Announc. 2017;5:e00206–17. https://doi.org/10.1128/genomeA.00206-17.
9. Yokomi R, Chen J, Rattner R, Selvaraj V, Maheshwary Y, Osman F, Pagliaccia D, Vidalakis G. Genome sequence resource for Spiroplasma citri, strain CC-2, associated with citrus stubborn disease in California. Phytopathology. 2020;110:254–6. https://doi.org/10.1094/PHYTO-08-19-0304-A.
10. Wang X, Doddapaneni H, Chen J, Yokomi RK. Improved real-time PCR diagnosis of citrus stubborn disease by targeting prophage genes of Spiroplasma citri. Plant Dis. 2015;99:149–54.
11. Maheshwari Y, Selvaraj V, Hajeri S, Yokomi R. Application of droplet digital PCR for quantitative detection of Spiroplasma citri in comparison with real-time PCR. PLoS ONE. 2017;12(9):e0184751.
12. Lee I-M, Davis RE. New media for rapid growth of Spiroplasma citri and corn stunt Spiroplasma. Phytopathology. 1984;74:84–9.
13. Doyle JJ. DNA protocols for plants. In: G Hewitt, AWB Johnson, JPW Young, eds. Molecular techniques in taxonomy. NATO ASI Ser. H, Cell Biol, vol 57, 1991, p. 283–93. Springer-Verlag, Berlin.
14. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res. 2017;27:722–36. https://doi.org/10.1101/gr.215087.116.
15. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Navrotsky E, Zaslavsky L, Lomsadze A, Pruitt D, Borodovsky M, Ostell J. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res. 2016;44:6614–24. https://doi.org/10.1093/nar/gkw569.
16. https://www.ncbi.nlm.nih.gov/bioproject/PRJNAS91027.
17. https://www.ncbi.nlm.nih.gov/sra/SRR10843927.
18. https://www.ncbi.nlm.nih.gov/sra/SRR10507068.
19. https://www.ncbi.nlm.nih.gov/sra/SRR10507067.
20. https://www.ncbi.nlm.nih.gov/sra/SRR10507066.
21. https://www.ncbi.nlm.nih.gov/sra/SRR10507065.
22. https://www.ncbi.nlm.nih.gov/assembly/GCA_01087175.1.
23. https://www.ncbi.nlm.nih.gov/assembly/GCA_01087105.1.
24. https://www.ncbi.nlm.nih.gov/assembly/GCA_01087055.1.
25. https://www.ncbi.nlm.nih.gov/assembly/GCA_01087305.1.
26. https://www.ncbi.nlm.nih.gov/assembly/GCA_01087255.1.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.