Complete Genome Sequence of *Spiroplasma helicoides* TABS-2T (DSM 22551), a Bacterium Isolated from a Horsefly (*Tabanus abactor*)

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*Spiroplasma helicoides* TABS-2T (DSM 22551) was isolated from the gut of a horsefly (*Tabanus abactor*) collected near Ardmore, Oklahoma, USA, in 1987. Here, we report the complete genome sequence of this bacterium to facilitate the investigation of its biology and the comparative genomics among *Spiroplasma* species.

*S. helicoides* is a horsefly-associated bacterium found in North America (1). The type strain TABS-2T was isolated from the gut of a horsefly (*Tabanus abactor*) collected near Ardmore, Oklahoma, USA, in 1987 and was assigned to group XXXII within the genus. Biochemical characterization of this strain demonstrated that it is capable of catabolizing glucose but does not hydrolyze arginine or urea (1). To facilitate future investigation on the biology of this bacterium, as well as to improve the taxonomic sampling of available *Spiroplasma* sequences for comparative and evolutionary studies (2), we determined the complete genome sequence of *S. helicoides* TABS-2T.

The strain was acquired from the German Collection of Microorganisms and Cell Cultures (catalogue number DSM 22551). The freeze-dried cell sample was processed according to the manufacturer’s instructions and cultured in M1D medium (3) prior to DNA extraction using the Wizard Genomic DNA purification kit (~4,200-bp insert, 2,828,554 reads). The initial de novo assembly was performed using ALLPATHS-LG release 52188 (14). Subsequently, PAGIT version 1 (15) was used to assist an iterative process for improving the assembly. For each iteration, the raw reads were mapped to the assembly using BWA version 0.7.12 (16), programatically checked using the MPILEUP program in SAMtools package version 1.2 (17), and visually inspected using IGV version 2.3.57 (18). Polymorphic sites and gaps were corrected based on the mapped reads. The process was repeated until the complete genome sequence was obtained. The programs RNAmmer (19), tRNAscan-SE (20), and Prodigal (21) were used for gene prediction. The gene names and product descriptions were first annotated based on the homologous genes in other *Spiroplasma* genomes (4–13) as identified by OrthoMCL (22). Subsequent manual curation was based on BLASTp (23) searches against the NCBI nonredundant database (24) and the KEGG database (25, 26). Putative clustered regularly interspaced short palindromic repeats (CRISPRs) were identified using CRISPRFinder (27).

The circular chromosome of *S. helicoides* TABS-2T is 1,326,546 bp in size and has a G+C content of 26.8%; no plasmid was found. The first version of annotation includes one set of 16S-23S-5S rRNA genes, 29 tRNA genes (covering all 20 amino acids), 1,151 protein-coding genes, four pseudogenes, and one CRISPR locus (chromosomal positions 1,068,189 to 1,071,129; containing 44 spacers).

**Accession number(s).** The complete genome sequence of *S. helicoides* TABS-2T has been deposited at DDBJ/EMBL/GenBank under the accession number CP017015.

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REFERENCES

1. Whitcomb RF, French FE, Tully JG, Gasparich GE, Rose DL, Carle P, Bove JM, Henegar RB, Konai M, Hackett KJ, Adams JR, Clark TB, Williamson DL. 1997. Spiroplasma chrysipae sp. nov., Spiroplasma gladiator sp. nov., Spiroplasma helicoides sp. nov., and Spiroplasma taitanidaica sp. nov., from tabanid (Diptera: Tabanidae) flies. Int J Syst Bacteriol 47:713–719. http://dx.doi.org/10.1099/00207713-47-3-713.

2. Lo W-S, Huang Y-Y, Kuo C-H. 2016. Winding paths to simplicity: genome evolution in facultative insect symbionts. FEMS Microbiol Rev [Epub ahead of print]. http://dx.doi.org/10.1093/femsre/fuw028.

3. Whitcomb RF, Tully JG, McCawley P, Rose DL. 1982. Application of the growth inhibition test to Spiroplasma taxonomy. Int J Syst Bacteriol 32:387–394. http://dx.doi.org/10.1099/00207713-32-4-387.

4. Lo W-S, Chen L-L, Chung W-C, Gasparich GE, Kuo C-H. 2013. Comparative genome analysis of Spiroplasma melliferum IPMB4A, a honeybee-associated bacterium. BMC Genomics 14:22. http://dx.doi.org/10.1186/1471-2164-14-22.

5. Ku C, Lo W-S, Chen L-L, Kuo C-H. 2013. Complete genomes of two dipteran-associated spiroplasmas provided insights into the origin, dynamics, and impacts of viral invasion in Spiroplasma. Genome Biol Evol 5:1151–1164. http://dx.doi.org/10.1093/gbe/evt084.

6. Lo W-S, Ku C, Chen L-L, Chang T-H, Kuo C-H. 2013. Comparison of metabolic capacities and inference of gene content evolution in mosquito-associated Spiroplasma dininium and S. taiwannense. Genome Biol Evol 5:1512–1523. http://dx.doi.org/10.1093/gbe/evt108.

7. Ku C, Lo W-S, Chen L-L, Kuo C-H. 2014. Complete genome sequence of Spiroplasma apis (ATCC 33834), a bacterium associated with May disease of honeybees (Apis mellifera). Genome Announc 2(1):e00115-13. http://dx.doi.org/10.1128/genomeA.00115-13.

8. Chang T-H, Lo W-S, Ku C, Chen L-L, Kuo C-H. 2014. Molecular evolution of the substrate utilization strategies and putative virulence factors in mosquito-associated Spiroplasma species. Genome Biol Evol 6:500–509. http://dx.doi.org/10.1093/gbe/evu033.

9. Paredes JC, Herren JK, Schüper F, Marin R, Claverol S, Kuo C-H, Lemaître B, Béven L. 2015. Genome sequence of the Drosophila melanogaster male-killing Spiroplasma strain MSRO endosymbiont. mBio 6:e02437-14. http://dx.doi.org/10.1128/mBio.02437-14.

10. Lo W-S, Gasparich GE, Kuo C-H. 2015. Found and lost: the fates of horizontally acquired genes in arthropod-symbiotic Spiroplasma. Genome Biol Evol 7:2458–2472. http://dx.doi.org/10.1093/gbe/evv160.

11. Lo W-S, Lai Y-C, Lien Y-W, Wang T-H, Kuo C-H. 2015. Complete genome sequence of Spiroplasma litorale TN-1 (DSM 21781), a bacterium isolated from a green-eyed hornfly (Tabanus nigrovittatus). Genome Announc 3(5):e01166-15. http://dx.doi.org/10.1128/genomeA.01166-15.

12. Lo W-S, Gasparich GE, Kuo C-H. 2015. Complete genome sequence of Spiroplasma cantharica CC-1 (DSM 21588), a bacterium isolated from a soldier beetle (Cantharis carolinus). Genome Announc 3(5):e01253-15. http://dx.doi.org/10.1128/genomeA.01253-15.

13. Lo W-S, Gasparich GE, Kuo C-H. 2016. Complete genome sequence of Spiroplasma turonicum Tab4c3, a bacterium isolated from horse flies (Haematopota spp.). Genome Announc 4(5):e01010-16. http://dx.doi.org/10.1128/genomeA.01010-16.

14. Gnerre S, MacCallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, Sharpe T, Hall G, Shea TP, Sykes S, Berlin AM, Aird D, Costello M, Davo R, Williams L, Nikol R, Gnarke A, Nusbaum C, Lander ES, Jaffe DB. 2011. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. Proc Natl Acad Sci U S A 108:1513–1518. http://dx.doi.org/10.1073/pnas.1017351108.

15. Swain MT, Tsai JJ, Assfale SA, Newbold C, Berriman M, Otto TD. 2012. A post-assembly genome-assembly improvement toolkit (PAGIT) to obtain annotated genomes from contigs. Nat Protoc 7:1260–1264. http://dx.doi.org/10.1038/nprot.2012.068.

16. Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25:1754–1760. http://dx.doi.org/10.1093/bioinformatics/btp324.

17. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25:2078–2079. http://dx.doi.org/10.1093/bioinformatics/btp352.

18. Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP. 2011. Integrative genomics viewer. Nat Biotechnol 29:24–26. http://dx.doi.org/10.1038/nbt.1754.

19. Lagesen K, Hallin P, Rodland EA, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35:3100–3108. http://dx.doi.org/10.1093/nar/gkm160.

20. Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP. 2011. Integrative genomics viewer. Nat Biotechnol 29:24–26. http://dx.doi.org/10.1038/nbt.1754.

21. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25:2078–2079. http://dx.doi.org/10.1093/bioinformatics/btp352.

22. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25:2078–2079. http://dx.doi.org/10.1093/bioinformatics/btp352.

23. Li W, Stoeckert CJ, Roos DS. 2003. OrthoMCL-ID: identification of ortholog groups for eukaryotic genomes. Genome Res 13:2178–2189. http://dx.doi.org/10.1101/gr.1224503.

24. 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/10.1186/1471-2105-11-421.

25. Clark K, Karshis-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2016. GenBank. Nucleic Acids Res 44:D67–D72. http://dx.doi.org/10.1093/nar/gkv1276.

26. Kanehisa M, Goto S. 2000. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28:27–30. http://dx.doi.org/10.1093/nar/28.1.27.

27. Kanehisa M, Goto S, Furumichi M, Tanabe M, Hirakawa M. 2010. KEGG for representation and analysis of molecular networks involving diseases and drugs. Nucleic Acids Res 38:D355–D360. http://dx.doi.org/10.1093/nar/gkp896.

28. Grissa I, Vergnaud G, Pourcel C. 2007. CRISPRefinder: a web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res 35:W52–W57. http://dx.doi.org/10.1093/nar/gkm360.