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Complete Genome Sequence of Spiroplasma sp. TU-14

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ABSTRACT *Spiroplasma* sp. TU-14 was isolated from a contaminated sample of *Entomoplasma lucivorax* PIPN-2^T obtained from the International Organization for Mycoplasmology collection. Here, we report the complete genome sequence of this bacterium to facilitate the investigation of its biology and the comparative genomics among *Spiroplasma* spp.

The genus *Spiroplasma* contains a group of host-associated bacteria mostly affiliated with various arthropods (1). The strain *Spiroplasma* sp. TU-14 was isolated by Gail Gasparich at Towson University in 2014 from a contaminated sample of *Entomoplasma lucivorax* PIPN-2^T (from the International Organization for Mycoplasmology collection; lyophilized on 25 April 1996 after 21 passes from the initial culture). The source of contamination and the ecology of this *Spiroplasma* strain are unknown. Nonetheless, this strain exhibits several intriguing genomic characteristics compared to its relatives. Based on the 16S rDNA sequence, this strain is most closely related to *Spiroplasma insolitum* M55^T. However, unlike *S. insolitum* and other lineages belonging to the Citri clade, which all contain a large number of plectroviral sequences in their genomes (2–6), our initial survey revealed that *Spiroplasma* sp. TU-14 lacks a prophage sequence and has a relatively small chromosome. To facilitate future investigation into the biology of this bacterium, as well as to improve the taxon sampling of available *Spiroplasma* sequences for comparative genomics and evolutionary studies (7), we determined its complete genome sequence.

The procedures for sequencing, assembly, and annotation were based on those described in our previous studies on *Spiroplasma* genomes (4–6, 8–15). Briefly, we used the Illumina MiSeq platform to generate 301-bp reads from one paired-end library (~550-bp insert, ~322 Mb, ~269-fold coverage). The initial *de novo* assembly was performed using Velvet version 1.2.10 (16). Subsequently, PAGIT version 1 (17) 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 (18), programmatically checked using the MPILEUP program in SAMTOOLS package version 1.2 (19), and visually inspected using IGV version 2.3.57 (20). 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 (21), tRNAscan-SE (22), and Prodigal (23) were used for gene prediction. The gene names and product descriptions were first annotated based on the homologous genes in other *Spiroplasma* genomes (4–6, 8–15) as identified by OrthoMCL (24). Subsequent manual curation was based on BLASTp (25) searches against the NCBI nonredundant database (26) and the KEGG

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database (27, 28). Putative clustered regularly interspaced short palindromic repeats (CRISPRs) were identified using CRISPRFinder (29).

The circular chromosome of *Spiroplasma* sp. TU-14 is 1,199,640 bp in size and has a G+C content of 28.7%, no plasmid was found. The first version of the annotation includes one set of 16S-23S-5S rRNA genes, 32 tRNA genes (covering all 20 amino acids), 1,036 protein-coding genes, and four pseudogenes. No putative plectroviral sequence or CRISPR element was found.

Accession number(s). The complete genome sequence of *Spiroplasma* sp. TU-14 has been deposited at DDBJ/EMBL/GenBank under the accession number CP017658.

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