Complete Genome Sequences of Four *Brucella* Strains Isolated from China

Xiaowen Yang,[a] Xiaofang Cao,[a] Ning Wang,[a] Jiawei Wang,[b] Pengfei Bie,[a] Yanli Lyu,[a] Qingmin Wu[a]

Key Laboratory of Animal Epidemiology and Zoonosis of the Ministry of Agriculture, College of Veterinary Medicine, China Agricultural University, Beijing, China[a]; Animal Science and Technology College, Beijing University of Agriculture, Beijing, China[b]

ABSTRACT *Brucella* spp. are facultative intracellular pathogens that cause a contagious zoonotic disease. Twelve different species are currently identified. This study presents the complete genome sequences of four *Brucella* strains. These complete genomes were annotated and the contents compared to those of other strains isolated from China.

Address correspondence to Yanli Lyu, luyanli@cau.edu.cn, or Qingmin Wu, wuqm@cau.edu.cn.

Brucella spp., facultative intracellular pathogens that can persistently colonize animal host cells, cause zoonosis that can result in outcomes such as abortion or sterility in susceptible animal hosts (1). Twelve different species are currently identified, *B. melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, *B. ceti* (2), *B. pinnipedialis* (3), *B. neotomae* (4), *B. microti* (5), *B. inopinata* (4), *B. papionis* (6), and *B. vulpis* (7). Until now, there were nine strains isolated from China. In this study, we performed whole-genome sequencing of two *B. melitensis* strains and two *B. abortus* strains isolated from China.

The whole genomes of these strains were sequenced using the Illumina sequencing platform using a 250-bp paired-end library, with at least 100-fold (100×) coverage to obtain the raw data. Raw data were used with FastQC to test the sequencing quality, and redundant and low-quality sequences were removed. SAMtools (8) was used to output the depth and coverage of sequencing. By comparing the sequencing depth and coverage, the closed genomes were identified (9). The paired-end reads were assembled *de novo* using the Velvet (10) software. The contigs were marked by NCBI BLAST to confirm the sites in the closed genomes. Meanwhile, the gaps were amplified using primers designed by Vector NTI (Invitrogen). These data were submitted to the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) for annotation (11).

B. melitensis BY38 was isolated from sheep in Bayingolin Mongol Autonomous Prefecture, Xinjiang Uygur Autonomous Region, and identified in 2013. The complete genome had two chromosomes, and the total genome was 3,311,862 bp, the average GC content was 57.2%, the coverage was 99.99%, and the sequencing depth was 181.65×. A total of 3,305 coding sequences (CDSs), 9 rRNAs, 55 tRNAs, and 4 noncoding RNAs (ncRNAs) were annotated. The most closely related genome was that of *B. melitensis* M28.

B. melitensis BL was isolated from a dairy cow in Altay Prefecture, Xinjiang Uygur Autonomous Region, and identified in 2012. The complete genome also had two chromosomes, and the total genome was 3,312,673 bp, the average GC content was 57.2%, the coverage was 99.95%, and the sequencing depth was 195.91×. A total of 3,310 CDSs, 9 rRNAs, 55 tRNAs, and 4 ncRNAs were annotated. The most closely related genome was that of *B. melitensis* 63/9.

B. abortus BD was isolated from a dairy cow in Baoding city, Hebei Province, and identified in 2008. The total genome was 3,271,067 bp, the average GC content was 57.2%, the coverage was 99.84%, and the sequencing depth was 158.28×. A total of

3,271 CDSs, 9 rRNAs, 55 tRNAs, and 4 ncRNAs were annotated. The most closely related genome was that of *B. abortus* BAB8416.

B. abortus MC was isolated from a dairy cow in Mancheng city, Hebei Province. The total genome was 3,312,673 bp, which was shorter than that of *B. abortus* BD. The average GC content of genome was 57.2%, the coverage was 99.79%, and the sequencing depth was 157.65×. A total of 3,267 CDSs, 9 rRNAs, 55 tRNAs, and 4 ncRNAs were annotated. The most closely related genome was also that of *B. abortus* BAB8416.

Accession number(s). The complete genome sequences were deposited in NCBI GenBank under accession numbers CP022827 and CP022828, CP022875 and CP022876, CP022877 and CP022878, and CP022879 and CP022880 for *B. melitensis* BY38, *B. melitensis* BL, *B. abortus* BD, and *B. abortus* MC, respectively.

REFERENCES

1. Keriel A, Botella E, Estrach S, Bragagnolo G, Vergunst AC, Feral CC, O'Callaghan D. 2015. *Brucella* intracellular life relies on the transmembrane protein CD98 heavy chain. J Infect Dis 211:1769–1778. https://doi.org/10.1093/infdis/jiu673.

2. Foster G, Whatmore AM, Dagleish MP, Baily JL, Deaville R, Davison NJ, Koylass MS, Perrett LL, Stubberfield EJ, Reid RJ, Brownlow AC. 2015. Isolation of *Brucella ceti* from a long-finned pilot whale (*Globicephala melas*) and a Sowerby's beaked whale (*Mesoploden bidens*). J Wildl Dis 51:868–871. https://doi.org/10.7589/2014-04-112.

3. Foster G, Osterman BS, Godfroid J, Jacques I, Cloeckaert A. 2007. *Brucella ceti* sp. nov. and *Brucella pinnipedialis* sp. nov. for *Brucella* strains with cetaceans and seals as their preferred hosts. Int J Syst Evol Microbiol 57:2688–2693. https://doi.org/10.1099/ijs.0.65269-0.

4. Wahab T, Ferrari S, Lindberg M, Bäckman S, Kaden R. 2014. Draft genome sequences of *Brucella suis* biovar 4 strain NCTC 10385, *Brucella ceti* strain NCTC 12891ᵀ, *Brucella inopinata* strain CAMP 6436ᵀ, and *Brucella neotomae* strain ATCC 23459ᵀ. Genome Announc 2(5):e00783-14. https://doi.org/10.1128/genomeA.00783-14.

5. Rónai Z, Kreizinger Z, Dán Á, Drees K, Foster JT, Bányai K, Marton S, Szeredi L, Jánosi S, Gyuranecz M. 2015. First isolation and characterization of *Brucella microti* from wild boar. BMC Vet Res 11:147. https://doi.org/10.1186/s12917-015-0456-z.

6. Whatmore AM, Davison N, Cloeckaert A, Al Dahouk S, Zygmunt MS, Brew SD, Perrett LL, Koylass MS, Vergnaud G, Quance C, Scholz HC, Dick EJ, Jr, Hubbard G, Schlabritz-Loutsevitch NE. 2014. *Brucella papionis* sp. nov., isolated from baboons (*Papio* spp.). Int J Syst Evol Microbiol 64:4120–4128. https://doi.org/10.1099/ijs.0.065482-0.

7. Scholz HC, Revilla-Fernández S, Al Dahouk S, Hammerl JA, Zygmunt MS, Cloeckaert A, Koylass M, Whatmore AM, Blom J, Vergnaud G, Witte A, Aistleitner K, Hofer E. 2016. *Brucella vulpis* sp. nov., isolated from mandibular lymph nodes of red foxes (*Vulpes vulpes*). Int J Syst Evol Microbiol 66:2090–2098. https://doi.org/10.1099/ijsem.0.000998.

8. 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. https://doi.org/10.1093/bioinformatics/btp352.

9. Shallom SJ, Tae H, Sarmento L, Preston D, McIver L, Franck C, Dickerman A, Adams LG, Garner HR. 2012. Comparison of genome diversity of *Brucella* spp. field isolates using Universal Bio-signature Detection Array and whole genome sequencing reveals limitations of current diagnostic methods. Gene 509:142–148. https://doi.org/10.1016/j.gene.2012.07.073.

10. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res 18:821–829. https://doi.org/10.1101/gr.074492.107.

11. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.