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GENOME ANNOUNCEMENT

Genome Sequences of *Brucella melitensis* 16M and Its Two Derivatives 16M1w and 16M13w, Which Evolved *In Vivo*

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***Brucella melitensis* is an intracellular pathogen that induces chronic infection in humans. Here, we report the genome sequences of 16M and its two derivatives, 16M1w and 16M13w, which were allowed to adapt *in vivo* for 1 and 13 weeks, respectively. Our findings contribute to the investigation of adaptive mutations and mechanisms of chronic infection by *B. melitensis*.**

Brucella melitensis is an important intracellular bacterium that persists inside its host and results in chronic infection (8). The current view regarding chronic infection with *B. melitensis* presumes low replication and mutation rates when in the host. However, this cannot be easily tested in actual infections. Laboratory evolution studies of isolates of *Mycobacterium tuberculosis* showed accumulation of a large number of mutations during latency (4, 7). This implied that intracellular bacteria experience great adaptive mutations during survival in their hosts (2, 3). To investigate the mechanism of chronic infection for *Brucella melitensis*, we analyzed *in vivo* adaptive mutations in BALB/c mice. Here, we report the genome sequences of three *Brucella melitensis* strains: 16M, a laboratory strain, and two derivatives, 16M1w and 16M13w, which were isolated from BALB/c mice after infection for 1 (acute infection) and 13 (chronic infection) weeks, respectively.

Laboratory strain 16M was used to infect BALB/c mice, and 1 and 13 weeks postinfection, bacteria were isolated from the mice. Genomic DNA was directly extracted from the bacterial cultures. All the genomes were sequenced with an Illumina GA IIx sequencer with a paired-end protocol. Low-quality reads were removed, and the remaining reads were assembled with the Clcbio genomics workbench version 4.03. About 1.3 Gb of clear data was generated for each of the three strains, resulting in an average coverage of 450-fold. The clear data were assembled into 75, 83, and 83 contigs for 16M, 16M1w, and 16M13w, respectively. A total of 3.23, 3.23, and 3.21 Mb contig sequences were obtained for the three strains.

The genome sequences were then annotated with different tools. Open reading frames (ORFs) were identified by using the RAST (Rapid Annotation using Subsystem Technology) system (1). The rRNA and tRNA were predicted with RNAmmer (5) and tRNAscan-SE 1.21 (6), respectively. A total of 3,363, 3,363, and 3,335 coding sequences were predicted for 16M, 16M1w, and 16M13w. Each of the three genome sequences has one copy of 5S rRNA, two copies of large-subunit rRNA, and one copy of small-subunit rRNA.

Comparative genomic analysis was performed using the genome sequence of 16M as a reference. To our surprise, we found only 11 single-nucleotide polymorphisms (SNPs) for 16M1w, but we found 5,019 SNPs for 16M13w. Further analysis showed that, of the SNPs of 16M13w, 3,086 were nonsynonymous sites, 1,855 were synonymous sites, and 78 were nonsense mutations. A total of 3,184 SNPs are located on chromosome I, and 1,835 are located on chromosome II. The results showing that a large number of SNPs was observed in 16M13w implied that *Brucella melitensis* in chronic infection undergoes great adaptive mutations. This finding put forward great implications of adaptive mutations in the pathogenesis of intracellular bacteria. Further analysis of the adaptive mutations and their involvement in chronic infection will be provided in our future publications.

Nucleotide sequence accession numbers. The draft genome sequences of *B. melitensis* 16M, 16M1w, and 16M13w are available in GenBank under accession numbers AHWC00000000, AHWD00000000, and AHWE00000000, respectively. The version for strain 16M described in this paper, the first version, was assigned accession number AHWB01000000.

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