

# Complete Genome Sequence of *Brucella melitensis* Biovar 3 Strain NI, Isolated from an Aborted Bovine Fetus

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## From an aborted bovine fetus in China, a bacterial strain named NI was isolated and identified as *Brucella melitensis* by a PCR assay. Strain NI was further characterized as *B. melitensis* biovar 3 using biochemical assays. Here we report the complete genome sequence of strain NI.

**B***animals.* Typical symptoms of brucellosis in humans and many animals. Typical symptoms of brucellosis are undulant fever, abortion, asthenia, endocarditis, and encephalitis (6). Based on host specificity, *Brucella* has been classified into 10 species. *Brucella melitensis* is mainly responsible for brucellosis in goats/sheep, and it is also the most pathogenic *Brucella* species in humans.

A host shift phenomenon (i.e., the ability of a pathogen to colonize or infect a new host) was observed in our study. Specifically, from an aborted cattle fetus at a farm that had about 300 sheep and 40 cattle, we atypically isolated a *Brucella* strain, NI, in Inner Mongolia, China, in 2007. A low level of prevalence of brucellosis was observed at the farm at that time. No vaccine against *Brucella* or other pathogens had ever been administered to the animals at the farm before the isolation of strain NI. The bacterium was characterized as being Gram negative, nonmotile, and nonencapsulated. Strain NI colonies are circular and convex with unbroken edges but not hemolytic. Subsequently, strain NI was identified as a *B. melitensis* strain by the nucleic acid recognition of repetitive genetic element IS711 (2). The biochemical profiling indicated that this is a *B. melitensis* biovar 3 strain.

Strain NI genomic DNA was sequenced using the Illumina/ Solexa sequencing analyzer through 100-fold (100×) genome coverage at the Huada Genomics Institute (Shenzhen, China). Sequence data were analyzed using SOAPdenovo, which resulted in large numbers of contigs (5). Assembled contigs were compared to the published genome sequence of B. melitensis strain 16M (NC\_003317 and NC\_003318). Whenever possible, the order and the orientation of the assembled contigs were determined in accordance with the control genome. Based on the extensive similarity between the published genomes of Brucella, PCR primers were designed to link the gaps between two neighboring contigs. The leftover gaps were sequenced separately by conventional Sanger sequencing. Putative protein coding sequences (CDSs) were predicted using GeneMarkHMM-P (1). Functional annotation of CDSs was performed by searching the NCBI protein database and KEGG protein database using BLSATP. tRNA was predicted using tRNAscan-SE (3). RNAmmer (4) was used to determine rRNA. ISFINDER was used to identify the transposon-related genes (7).

The whole-genome sequence of strain NI is 3.294 Mb and is

comprised of two single circular chromosomes, 2,117,879 bp (ChrI) and 1,176,816 bp (ChrII). The G+C contents of ChrI and ChrII are 57.16% and 57.35%, respectively. The genome includes 52 tRNA genes, 9 rRNA operons, and 35 transposons. ChrI and ChrII include 2,091 and 1,149 open reading frames (ORFs), respectively. Among these ORFs, 527 (25.20%) genes of ChrI and 255 (22.19%) genes of ChrII were classified as hypothetical proteins.

**Nucleotide sequence accession numbers.** The genome sequence of *B. melitensis* NI is available in GenBank under accession numbers CP002931 for ChrI and CP002932 for ChrII.

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#### REFERENCES

- Besemer J, Borodovsky M. 2005. GeneMark: web software for gene finding in prokaryotes, eukaryotes and viruses. Nucleic Acids Res. 33:W451–W454.
- Bricker BJ, Halling SM. 1994. Differentiation of Brucella abortus bv. 1, 2, and 4, Brucella melitensis, Brucella ovis, and Brucella suis bv. 1 by PCR. J. Clin. Microbiol. 32:2660–2666.
- Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23: 673–679.
- Lagesen K, et al. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35:3100–3108.
- Li R, Li Y, Kristiansen K, Wang J. 2008. SOAP: short oligonucleotide alignment program. Bioinformatics 24:713–714.
- Pappas G, Panagopoulou P, Christou L, Akritidis N. 2006. Brucella as a biological weapon. Cell. Mol. Life Sci. 63:2229–2236.
- 7. Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. 2006. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res. 34:D32–D36.

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