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Complete Genome Sequence of *Mycoplasma canadense* Strain HAZ 360_1 from Bovine Mastitic Milk in Japan

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Bovine mycoplasmal mastitis is spreading quickly among cows. *Mycoplasma canadense*, a causal species of bovine mastitis, reduces milk quality and quantity via the infiltration of numerous inflammatory cells. Presented here is the complete 693,241-bp genome sequence of *M. canadense* strain HAZ 360_1, which was isolated in Japan.

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Mycoplasma canadense is a causal bacterium of bovine mastitis, and it is also isolated from the respiratory and reproductive tracts of cattle (1, 2). An intramammary *M. canadense* infection results in an immediate and sharp decrease in milk yield, a massive amount of inflammatory cells infiltrating the milk of all quarters, and persistent infection for long periods (2). The clinical picture is that of a mild outbreak of *Mycobacterium bovis* mastitis (1–3). Despite the importance of *M. canadense*, little genetic information on *M. canadense* is available. The entire genome sequence of *M. canadense* strain HAZ 360_1, isolated in 2008 from bovine mastitic milk in Japan, is presented here.

Total genomic DNA was prepared from *M. canadense* strain HAZ 360_1 and subjected to 454 Titanium sequencing at Hokkaido System Science Co. (Sapporo, Japan). The resulting reads were assembled *de novo* using the GS *de novo* Assembler software version 2.7 (Roche), yielding 41 contigs with 96.7× coverage. An analysis of the contig ends, together with polymerase chain reaction (PCR) amplification and amplicon cloning, showed that the 693,241-bp genome has a closed-ring structure. After the initial automated annotation performed using Microbial Genome Annotation Pipeline version 2.18 at the DNA Data Bank of Japan (http://migap.ddbj.nig.ac.jp/mgap/jsp/index.jsp) (4–6), manual curation was performed, followed by the verification of potential pseudogenes by PCR and Sanger sequencing. As a result, 484 open reading frames, 15 pseudogenes, 32 tRNAs, and two sets of each rRNA (5S rRNA, 16S rRNA, and 23S rRNA) were confirmed in this genome sequence. The G+C content is 24.34%.

As anticipated based on its 16S rRNA-based phylogeny, most genes in *M. canadense* strain HAZ 360_1 exhibit high similarity to the amino acid sequences of the genes carried by members of the *Mycoplasma alkalescens* cluster, with the best similarity shown with genes from *Mycoplasma arginini* (7).

The enzymes involved in the arginine hydrolysis pathway and acetate kinase have an important role in the generation of energy in arginine-metabolizing mycoplasmas, such as *M. alkalescens*, and genes coding for these enzymes were found in the genome sequence of *M. canadense* (8, 9). However, the genes of proteins involved in the synthesis of capsular polysaccharides and the production of active oxygen-containing molecules, which are suggested to be important mycoplasmal etiologic agents (e.g., UTP-glucose-1-phosphate-uridyltransferase and glycerol-3-phosphate oxidase) were not confirmed (10, 11).

A part of the amino acid sequences of the hypothetical proteins MCAN 360_0280, MCAN 360_0281, and MCAN 360_0504 showed certain similarities to the surface proteins involved in the antigenic variation shift in other mycoplasma species (11, 12). Moreover, the discriminative homopolymeric tract of contiguous adenines [poly(A)] is located upstream of the repetitive regions in these hypothetical proteins (11, 12). These genes contain homologous regions, which consist of 81 periodic amino acid sequences.

The genomic sequence of *M. canadense* will provide a foundation for further investigations of this species, and it is hoped that this study will contribute to the reduction of bovine diseases, such as mastitis.

Nucleotide sequence accession number. The whole-genome sequence has been registered at DDBJ/EMBL/GenBank under the accession no. AP014631.

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