

Complete Genome Sequence of *Mycoplasma bovoculi* Strain M165/69^T (ATCC 29104)

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Bovine ocular infections compromise animal health and result in significant economic losses. *Mycoplasma bovoculi* is an etiological agent of conjunctivitis. Presented here is the 760,240-bp complete genome sequence of the *M. bovoculi* type strain M165/69^T. An analysis of the deduced proteome provides insights into the adherence and antigenic variation mechanisms of the strain.

Received 31 January 2014 Accepted 3 February 2014 Published 20 February 2014

Citation Calcutt MJ, Foecking MF. 2014. Complete genome sequence of *Mycoplasma bovoculi* strain M165/69^T (ATCC 29104). *Genome Announc.* 2(1):e00115-14. doi:10.1128/genomeA.00115-14.

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Mycoplasma bovoculi is an etiological agent of bovine conjunctivitis (1) and is also associated with the development of the more serious infection, bovine keratoconjunctivitis (IBK), caused by *Moraxella bovis* (2). Despite its importance, there is a paucity of molecularly characterized features beyond the identification of the adherence related antigen, P94 (3, 4). Presented here is the complete genome sequence of the M165/69^T type strain that was isolated in 1968 from a bovine eye in Canada (5, 6).

Total genomic DNA was prepared from *M. bovoculi* M165/69^T (obtained from the American Type Culture Collection as ATCC 29104) and subjected to 454 Titanium sequencing at The Genome Institute of Washington University, St. Louis, MO. The resulting reads were assembled *de novo* using Newbler software (Roche), yielding 11 contigs with 156× coverage. An analysis of contig ends together with PCR amplification and amplicon cloning enabled closing of the 760,240-bp genome. After initial automated annotation (PGAP pipeline at the National Center for Biotechnology Information), manual curation was performed, followed by verification of potential pseudogenes by PCR and Sanger sequencing. As a result, the genome comprises 626 genes (579 open reading frames [ORFs] and 12 pseudogenes), including those for 30 tRNAs and one copy for each rRNA (with 5S rRNA gene separated from the 16S-23S rRNA operon). The G+C content is 28.2%.

As anticipated from its 16S rRNA-based phylogeny, most genes in *M. bovoculi* M165/69^T exhibit the greatest similarity to those encoded by other sequenced members of the *Mycoplasma neurolyticum* cluster, with those from the caprine pathogen *Mycoplasma conjunctivae* typically being the best match. Although multiple short regions of synteny are present with the latter genome, none are longer than the 27 genes within the *rpsJ-rplQ* locus that predominantly encode ribosomal proteins.

In other sequenced taxa in this cluster, distinctive pairs of ORFs encode posttranslationally processed surface proteins that have been biochemically proven (7, 8) or implicated (9, 10) in mediating adherence to cellular receptors or extracellular matrix components. The *M. bovoculi* genome encodes 7 instances of such gene pairs that are candidate adherence factors for this agent. An array of five genes (*vpbA*, *vpbB*, *vpbC*, *vpbD*, and *vpbE*) encoding

paralogous surface proteins (52 to 66% protein identity) represents another potential adaptation feature. Each gene in this novel contingency locus is preceded by a putative promoter containing a homopolymeric thymidine tract that is indicative of phase variation and combinatorial expression patterns (11).

An analysis of the restriction-modification genes identified potentially phase-variable type I and type III systems, each containing a coding sequence harboring poly(GA) repeats that are predicted to undergo stochastic slipped-strand mispairing as reported in other systems (12, 13). A putative HpaI restriction-modification locus is also present, linked to a *repB* pseudogene that likely represents a plasmid integration event. No clustered regularly interspaced short palindromic repeat (CRISPR) units, multicopy insertion sequences, prophages, or integrative conjugative elements were identified.

The genomic sequence is the first for this species and provides a foundation for future investigations of pathoadaptations to the bovine eye. Ultimately, such analyses may allow the development of improved immunological products against conjunctivitis and IBK.

Nucleotide sequence accession number. This complete genome sequence has been deposited at DDBJ/EMBL/GenBank under the accession no. CP007154.

ACKNOWLEDGMENTS

This research was supported in part by grants from the University of Missouri Research Council and by USDA-ARS Animal Health Formula Funds from the University of Missouri College of Veterinary Medicine.

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