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John Dustin Loy
University of Nebraska-Lincoln, jdloy@unl.edu

Aaron M. Dickey
USDA, Agricultural Research Service

Michael L. Clawson
USDA, Agricultural Research Service

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Complete Genome Sequence of *Moraxella bovis* Strain Epp-63 (300), an Etiologic Agent of Infectious Bovine Keratoconjunctivitis

 John Dustin Loy,^a Aaron M. Dickey,^b Michael L. Clawson^b

^aSchool of Veterinary Medicine and Biomedical Sciences (SVMBS), University of Nebraska—Lincoln (UNL), Lincoln, Nebraska, USA

^bU.S. Department of Agriculture, Agricultural Research Service (ARS), U.S. Meat Animal Research Center (USMARC), Clay Center, Nebraska, USA

ABSTRACT We report here the complete closed genome sequence of *Moraxella bovis* strain Epp-63 (300) (Epp63). This strain was isolated from an infectious bovine keratoconjunctivitis (IBK) case in 1963. Since then, Epp63 has been used extensively for IBK research. Consequently, the genome sequence of Epp63 should help elucidate IBK host-pathogen interactions.

Infectious bovine keratoconjunctivitis (IBK) is a significant disease of cattle worldwide, and nearly 50% of herds in the United States are affected. The disease can cause considerable impact on afflicted animals, including blindness (1, 2).

Moraxella bovis is an etiologic agent of IBK. One strain, Epp-63 (300) (Epp63), has been studied in a variety of clinical models (3–5). Virulence factors such as pili (including phase variations thereof), hemolysins, phospholipases, and plasmids have been characterized in Epp63 (6–10). However, the genome of this important strain had not been sequenced, and no complete, closed genome sequence of any *M. bovis* isolate was available in GenBank as recently as July 2018.

Strain Epp63 had been stored lyophilized from 1987 until its revival in 2018. Following two passages on 5% sheep blood Trypticase soy agar, the isolate was grown overnight in brain heart infusion broth at 37°C with 5% CO₂, and the DNA was purified over 20/G gravity-flow anion-exchange columns (Qiagen, Valencia, CA, USA). The same DNA source was used to construct both a single-molecule real-time (SMRT) DNA library (10 to 20 kb) with SMRT Bell version 1.0 (Pacific Biosystems, Menlo Park, CA, USA) and a paired-end (2 × 151-bp) DNA library with TruSeq PCR-free LT (Illumina, San Diego, CA, USA), according to the manufacturers' instructions. The SMRT Bell and TruSeq libraries were sequenced on a PacBio RS II sequencer with P6 chemistry and a 6-h movie and an Illumina NextSeq 500 sequencer with NextSeq V2 chemistry, respectively.

The PacBio sequencing yielded 59,873 reads with a mean read length of 16,744 nucleotides (nt). These reads passed filtering controls of a minimum subread length of 500 nt and a minimum polymerase read quality and length of 0.80 and 100 nt, respectively, and were assembled with the Hierarchical Genome Assembly Process version 3 (HGAP3), which yielded three unique contigs. All three contigs had overlapping ends of redundant sequences that were identified with self-dotplots in Geneious version 11.1.2 (11) and removed, yielding a preliminary chromosome sequence and two plasmid sequences. The PacBio sequencing coverage exceeded 300- and 24-fold for the chromosome and plasmids, respectively. Sequences of the two plasmids had previously been generated and were available in GenBank; accordingly, the two plasmids sequenced in this study were oriented to start at the same base as their counterparts in GenBank. The chromosome was oriented to start at an origin of replication that was

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identified with Ori-Finder (12). The chromosome and plasmid sequences were then polished and error corrected with PacBio sequences using RS Resequencing version 1. The Illumina TruSeq library sequencing yielded 3.8×10^6 million reads that were mapped onto the chromosome and plasmid sequences in Geneious with “map to reference” and used to correct homopolymer errors. This yielded an *M. bovis* Epp63 chromosome of 2,839,913 bp and two plasmids, pMBO-1 and pMBO-2, of 44,217 and 27,077 bp, respectively.

The chromosome and plasmids were annotated with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok). A total of 2,816, 34, and 38 protein-coding genes were identified in the chromosome, pMBO-1, and pMBO-2, respectively.

Data availability. The genome sequence of the chromosome and plasmids of strain Epp63 have been deposited in DDBJ/ENA/GenBank under the accession numbers CP030241 to CP030243. The version of the chromosome described in this paper is the first version. The plasmid sequences were previously reported in GenBank under accession numbers AB169976 and AB169977.

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