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

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**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...
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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REFERENCES

1. Webber JJ, Selby LA. 1981. Risk factors related to the prevalence of infectious bovine keratoconjunctivitis. J Am Vet Med Assoc 179: 823–826.
2. Brown MH, Brightman AH, Fenwick BW, Rider MA. 1998. Infectious bovine keratoconjunctivitis: a review. J Vet Intern Med 12:259–266. https://doi.org/10.1111/j.1939-1676.1998.tb02120.x.
3. Rogers DG, Cheville NF, Pugh GW, Jr. 1987. Pathogenesis of corneal lesions caused by Moraxella bovis in gnotobiotic calves. Vet Pathol 24:287–295. https://doi.org/10.1177/03009858702400401.
4. Hughes DE, Pugh GW, Jr, McDonald TJ. 1965. Ultraviolet radiation and Moraxella bovis in the etiology of bovine infectious keratoconjunctivitis. Am J Vet Res 26:1331–1338.
5. Gould S, Dewell R, Tofflemire K, Whitley RD, Millman ST, Opreisnig T, Rosenbusch R, Trujillo J, O’Connor AM. 2013. Randomized blinded challenge study to assess association between Moraxella bovoculi and infectious bovine keratoconjunctivitis in dairy calves. Vet Microbiol 164: 108–115. https://doi.org/10.1016/j.vetmic.2013.01.038.
6. Farn JL, Strugnell RA, Hoyné PA, Michalski WP, Tennent JM. 2001. Molecular characterization of a secreted enzyme with phospholipase B activity from Moraxella bovis. J Bacteriol 183:6717–6720. https://doi.org/10.1128/JB.183.22.6717-6720.2001.
7. Clinkenbeard KD, Thiessen AE. 1991. Mechanism of action of Moraxella bovis hemolysin. Infect Immun 59:1148–1152. https://iai.asm.org/content/59/3/1148.
8. Marrs CF, Schoolnik G, Koomey JM, Hardy J, Rothbard J, Falkow S. 1985. Cloning and sequencing of a Moraxella bovis pilin gene. J Bacteriol 163:132–139. https://jb.asm.org/content/163/1/132.
9. Kakuda T, Sarataphan N, Tanaka T, Takai S. 2006. Filamentous-hemagglutinin-like protein genes encoded on a plasmid of Moraxella bovis. Vet Microbiol 118:141–147. https://doi.org/10.1016/j.vetmic.2006.06.024.
10. Marrs CF, Ruehl WW, Schoolnik GK, Falkow S. 1988. Pilin-gene phase variation of Moraxella bovis is caused by an inversion of the pilin genes. J Bacteriol 170:3032–3039. https://doi.org/10.1128/jb.170.7.3032-3039.1988.
11. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. https://doi.org/10.1093/bioinformatics/bts199.
12. Guo F, Zhang C-T. 2008. Ori-Finder: a Web-based system for finding oriC in unannotated bacterial genomes. BMC Bioinformatics 9:79. https://doi.org/10.1186/1471-2105-9-79.