Complete Genome Sequences of 16 Mycoplasma bovis Isolates from Canadian Bison and Cattle

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ABSTRACT Here, we report the complete genome sequences of 12 Mycoplasma bovis isolates cultured from Canadian bison and 4 cultured from Canadian cattle. The sequences are of value for understanding the phylogenetic relationship between cattle and bison isolates and will aid in elucidating the genetic basis for virulence and host specificity.

Mycoplasma bovis is a widespread cause of pneumonia, otitis media, arthritis, mastitis, and reproductive disorders in cattle and imposes a major economic burden on the beef and dairy industries around the world (1, 2). It was first recognized as a pathogen in the 1960s, when it caused an outbreak of mastitis in an American dairy herd (3), and has since spread to nearly all countries in which cattle are raised (1, 4). In the early 2000s, M. bovis began to appear as a primary disease agent in North American bison (Bison bison) and subsequently became one of the most serious and costly infectious disease threats faced by these animals (5). One theory suggests that the relatively recent appearance of M. bovis as a pathogen in bison is due to the emergence of unique, newly evolved genotypes with an expanded host range or heightened virulence. The complete genome sequences for 11 isolates from cattle have been reported (6–11) or are otherwise found in the NCBI genome or nucleotide databases, but until now, no genome assemblies for isolates from bison have been available. To better understand the phylogenetic relationship between isolates from cattle and bison and to generate a resource for exploring the genetic basis for virulence and host specificity in M. bovis, we sequenced the genomes of 12 bison isolates and 4 cattle isolates obtained from animals in western Canada.

Details pertaining to the M. bovis isolates included in this study are provided in Table 1. Isolates were sourced from tissue samples collected at necropsy. Samples were homogenized and cultured for 48 h at 37°C in an atmosphere of 5% CO2 in selective PPLO broth (PPLO broth base [BD Diagnostic Systems], 10 g/liter yeast extract [BD Diagnostic Systems], 20% heat-inactivated horse serum [Life Technologies], 0.05% thallium acetate [MP Biomedicals], and 500 IU/ml penicillin G [Sigma]), followed by plating onto selective PPLO agar. Axenic seed stocks generated from single, well-isolated colonies grown in selective PPLO broth were confirmed to be M. bovis using a diagnostic PCR (12). Genomic DNA was purified from broth cultures using the Gentra Puregene cell kit (Qiagen) according to the kit handbook. For isolate MJ1, PacBio and Illumina libraries were derived using DNA purified from two independent seed stock expansion cultures. Libraries for all remaining isolates were obtained using DNA prepared from a single culture per isolate. PacBio sequencing was completed by the
### Table 1: Isolates, geographic location, anatomic site and date of origin, sequencing metrics, and accession numbers of *M. bovis* isolates in this report

| Isolate name | Geographic origin (province) | Anatomic site of origin | Yr of origin | Length (bp) | PacBio reads | Illumina reads | Genome coverage (% of CDSs) | Total no. of pseudogenes | SRA accession no. for: | No. of RNA genes | GenBank accession no. |
|--------------|-----------------------------|-------------------------|-------------|-------------|--------------|----------------|-----------------------------|------------------------|---------------------|------------------|---------------------|
| NADCC18 | Alberta | Lung of aborted fetus | 2012 | 1,119,681 | 81,403 | 875,877 | 579 | 29.1 | 914 | 76 | SRX82368689 | SRX8236886 CP022589 |
| NADCC54 | Saskatchewan | Lung | 2012 | 1,143,450 | 84,172 | 977,092 | 209 | 29.0 | 929 | 74 | SRX82368870 | SRX8236887 CP022589 |
| NADCC55 | Manitoba | Lung | 2012 | 1,159,440 | 68,072 | 1,375,282 | 948 | 29.0 | 940 | 81 | SRX82368781 | SRX8236888 CP022591 |
| NADCC56 | Manitoba | Lung | 2012 | 1,085,052 | 116,898 | 1,177,141 | 267 | 29.1 | 884 | 66 | SRX82368724 | SRX8236889 CP022592 |
| NADCC57 | Alberta | Joint | 2013 | 978,015 | 100,885 | 1,120,174 | 287 | 29.3 | 806 | 50 | SRX82368735 | SRX8236890 CP022593 |
| NADCC60 | Alberta | Joint | 2013 | 1,157,131 | 72,345 | 1,097,492 | 545 | 29.1 | 951 | 90 | SRX82368777 | SRX8236894 CP022598 |
| NADCC58 | Saskatchewan | Lung | 2013 | 1,098,413 | 51,044 | 901,441 | 483 | 29.1 | 893 | 72 | SRX82368741 | SRX8236891 CP022594 |
| NADCC59 | Alberta | Lung | 2014 | 1,183,547 | 47,071 | 956,285 | 878 | 29.0 | 992 | 131 | SRX82368750 | SRX8236892 CP042939 |
| NADCC61 | Manitoba | Lung | 2012 | 1,119,681 | 92,537 | 1,219,230 | 726 | 29.1 | 909 | 72 | SRX82368767 | SRX8236893 CP022599 |
| NADCC67 | Alberta | Joint | 2013 | 1,113,699 | 29,650 | 855,320 | 305 | 29.1 | 902 | 83 | SRX82368778 | SRX8236895 CP022596 |
| NADCC68 | Alberta | Lung | 2013 | 1,107,634 | 31,736 | 962,980 | 423 | 29.1 | 835 | 56 | SRX82368786 | SRX8236896 CP022597 |
| NADCC70 | Alberta | Pericardium | 2014 | 1,103,068 | 45,377 | 1,084,481 | 403 | 29.0 | 904 | 99 | SRX82368808 | SRX8236898 CP022598 |
| MJ1 | Alberta | Joint | 2014 | 1,024,574 | 103,538 | 816,930 | 1,272 | 29.4 | 843 | 84 | SRX82368642 | SRX8236881 CP042938 |
| MJ2 | Alberta | Lung | 2014 | 970,516 | 83,560 | 840,623 | 795 | 29.3 | 801 | 56 | SRX82368653 | SRX8236882 CP022586 |
| MJ3 | Alberta | Lung | 2014 | 1,027,226 | 80,405 | 903,132 | 423 | 29.3 | 835 | 56 | SRX82368658 | SRX8236883 CP022587 |
| MJ4 | Alberta | Lung | 2014 | 1,053,367 | 102,039 | 941,902 | 768 | 29.3 | 854 | 84 | SRX82368666 | SRX8236884 CP022588 |

*Includes pseudogenes. CDSs, coding DNA sequences.

*Consecutive shaded rows indicate three pairs of isolates each obtained from a single animal. All of the other isolates were sourced from different animals.

Yale Center for Genome Analysis (New Haven, CT). Following fragmentation and end repair of genomic DNA, BluePippin size selection was used to enrich for 10- to 20-kb fragments. Libraries were sequenced using a single SMRTcell per isolate and P6-C4 chemistry on a PacBio RS II instrument. Illumina sequencing was carried out on the MiSeq system using the MiSeq v2 reagent kit to generate 2 × 150-bp paired-end reads. Libraries were prepared with the Nextera XT DNA library prep kit (Illumina), as detailed in the reference guide.

Genomes were assembled from PacBio reads using Canu (13), v. 1.5 or v. 1.8, or PacBio SMRT analysis v. 2.3.0, using the default assembly options. All assemblies were error corrected and polished by iteratively running v. 1.18 or v. 1.22 of Pilon (14) with Illumina reads, using the “fix bases” option, until no additional corrections were made. Genomes were oriented to start at the dnaA gene and trimmed to remove overlapping sequences. Genomes were annotated by NCBI using the Prokaryotic Genome Annotation Pipeline v. 4.2 (15). Details pertaining to each isolate and the corresponding assembly are provided in Table 1.

**Data availability.** Genome assemblies have been deposited in GenBank under BioProject accession number PRJNA378768, with the isolate-specific accession numbers indicated in Table 1. Raw sequence reads have been deposited in SRA and may be obtained by searching for the BioProject accession number using the SRA Run Selector (https://www.ncbi.nlm.nih.gov/Traces/study/).

**ACKNOWLEDGMENTS**

We gratefully acknowledge the excellent technical assistance of William Boatwright. A portion of this work was funded by the Alberta Livestock & Meat Agency, Ltd. The mention of trade names, proprietary products, or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable.

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