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GENOME SEQUENCES

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

Karen B. Register,[a] Darrell O. Bayles,[b] Hao Ma,[a] M. Claire Windeyer,[c] Jose Perez-Casal,[d] Ana L. Bras,[c]* Muhammad Suleman,[d]* Murray Woodbury,[e] Murray D. Jelinski,[e] David P. Alt[b]

[a]Ruminant Diseases and Immunology Research Unit, USDA, Agricultural Research Service, National Animal Disease Center, Ames, Iowa, USA
[b]Infectious Bacterial Diseases Research Unit, USDA, Agricultural Research Service, National Animal Disease Center, Ames, Iowa, USA
[c]Department of Production Animal Health, University of Calgary, Calgary, AB, Canada
[d]Vaccine and Infectious Disease Organization-International Vaccine Centre, University of Saskatchewan, Saskatoon, SK, Canada
[e]Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

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% CO_2 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

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Address correspondence to Karen B. Register, karen.register@usda.gov.

* Present address: Ana L. Bras, Feedlot Health Management Services, Ltd., Okotoks, Alberta, Canada; Muhammad Suleman, University of Veterinary and Animal Sciences, Lahore, Pakistan.

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TABLE 1 Strain and genome information of *M. bovis* isolates in this report

Isolate name	Geographic origin (province)	Anatomic site of origin	Yr of origin	Length (bp)	No. of reads from:		Genome coverage (×)	G+C content (%)	Total no. of CDSs[a]	No. of pseudogenes	No. of RNA genes	SRA accession no. for:		GenBank accession no.
					PacBio	Illumina						PacBio reads	Illumina reads	
NADC18	Alberta	Lung of aborted fetus	2012	1,119,681	81,403	875,877	579	29.1	914	76	43	SRX8236869	SRX8236886	CP022589
NADC54	Saskatchewan	Lung	2012	1,143,450	84, 172	977,092	209	29.0	929	74	43	SRX8236870	SRX8236887	CP022590
NADC55	Manitoba	Lung	2012	1,159,440	68,072	1,375,282	948	29.0	940	81	43	SRX8236871	SRX8236888	CP022591
NADC56	Manitoba	Lung	2012	1,085,052	116,898	1,177,141	267	29.1	884	66	43	SRX8236872	SRX8236889	CP022592
NADC57[b]	Alberta	Joint	2013	978,015	100,885	1,120,174	287	29.3	806	50	43	SRX8236873	SRX8236890	CP022593
NADC62[b]	Alberta	Joint	2013	1,157,131	72,345	1,097,492	545	29.1	951	90	43	SRX8236877	SRX8236894	CP022595
NADC58	Saskatchewan	Lung	2013	1,098,413	51,044	901,441	483	29.1	893	72	43	SRX8236874	SRX8236891	CP022594
NADC59	Alberta	Lung	2014	1,183,547	47,071	956,285	878	29.0	992	131	43	SRX8236875	SRX8236892	CP042939
NADC61	Manitoba	Lung	2012	1,119,569	92,537	1,219,230	726	29.1	909	72	43	SRX8236876	SRX8236893	CP022599
NADC67[b]	Alberta	Joint	2013	1,113,699	29,650	855,320	305	29.1	902	83	43	SRX8236878	SRX8236895	CP022596
NADC68[b]	Alberta	Lung	2013	1,107,634	31,736	962,980	323	29.0	902	103	41	SRX8236879	SRX8236867	CP022597
NADC70	Alberta	Pericardium	2014	1,103,068	45,377	1,084,481	403	29.0	904	99	41	SRX8236880	SRX8236868	CP022598
MJ1	Alberta	Joint	2014	1,024,574	103,538	816,930	1,272	29.4	843	84	43	SRX8236864	SRX8236881	CP042938
MJ2	Alberta	Lung	2014	970,516	83,560	840,623	795	29.3	801	56	43	SRX8236865	SRX8236882	CP022586
MJ3	Alberta	Lung	2014	1,027,226	80,405	903,132	423	29.3	835	56	43	SRX8236885	SRX8236883	CP022587
MJ4	Alberta	Lung	2014	1,053,367	102,039	941,902	768	29.3	854	84	43	SRX8236866	SRX8236884	CP022588

[a] Includes pseudogenes. CDSs, coding DNA sequences.
[b] Isolate obtained from a single animal.

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/).

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