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GENOME SEQUENCES

Draft Genome Sequences of Two Bison-Type and Two Sheep-Type Strains of *Mycobacterium avium* subsp. *paratuberculosis*

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ABSTRACT Genome sequences of two type B and two type S strains of *Mycobacterium avium* subsp. *paratuberculosis* are presented. These strains were isolated in the United States from sheep, bison, and cattle suffering from Johne's disease. These genomes will increase our understanding of the minor differences that exist among strains of this genetically stable subspecies.

Mycobacterium avium subsp. *paratuberculosis* is the causative agent of Johne's disease, which affects ruminants. This subspecies of *M. avium* is divided into two primary lineages, designated type C and type S strains (1). These lineages are justified by several molecular genotyping methods (2–5). The type C lineage contains a subgroup of type B strains, which have been isolated from both cattle and bison (6, 7). Although the type B strains are circulating in Canada and the United States (8, 9), they are predominant in Korea (7) and India (10, 11). Type B strains can be distinguished from type C and type S by a novel GTG repeat in the MAP_RS04120 homolog, which encodes MoaD, a molybdenum biosynthesis protein (7). The two type B strains sequenced in this study contain 5 GTG repeats typical of type B, while the type S strains contain 3 GTG repeats. The type S strains commonly isolated from sheep can be further divided into sublineage types I and III (1). Both type S strains in this study contain single nucleotide polymorphisms (SNPs) in *gyrAB*, characteristic of the type III genotype (4), and were isolated in the United States. Thus, no type I strains of *M. avium* subsp. *paratuberculosis* have been identified in the United States as yet, although they are present in Australia, New Zealand, and the United Kingdom (1).

The type S strains were obtained from a breeding ram in Iowa (isolate 08-8281) and from an Icelandic sheep in Colorado (isolate 11-1786). The type B strains were obtained from a Holstein cow in Idaho (10-4404) and a bison in Colorado (10-5975).

These isolates were obtained from milk or feces using the NADC culture method on Herold's egg yolk medium (12). Isolated colonies were subcultured in Middlebrook 7H9 broth (BD Biosciences, San Jose, CA) medium supplemented as described previously (13). Cultures at an optical density at 540 nm (OD_{540}) of 0.65 were harvested by centrifugation, and genomic DNA was extracted using an enzymatic digestion method described previously (13) followed by loading the extracts onto Qiagen 500/G columns. The 500/G columns were processed according to instructions from the manufacturer. Purified genomic DNA concentrations were determined using a Qubit v3.0 fluorometer (Thermo Fisher Scientific, Inc., Ottawa, Canada) and analyzed by gel electrophoresis.

Purified genomic DNA from each isolate was subjected to whole-genome shotgun sequencing using the Ion Torrent PGM sequencing platform with 200-bp read chemistry (Life Technologies). Library preparation was performed using the Ion Plus fragment library kit (Life Technologies) according to the manufacturer's specifications. The Bioanalyzer 2100 (Agilent Technologies) was used with the high-sensitivity DNA chip to determine the quality, size, and concentration of the libraries. The sequencing reads were input into the MIRA assembler without trimming or filtering. A *de novo* assembly of each genome sequence was obtained using MIRA assembly software version 3.2.0, which yielded a range of contigs

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TABLE 1 Sequencing statistics of the sheep and bison type isolates of *M. avium* subsp. *paratuberculosis*

	Data for isolate:			
Metric	**10-4404**	**10-5975**	**08-8281**	**11-1786**
Strain type	Type B	Type B	Type S (III)[b]	Type S (III)[b]
Host species	Holstein cow	Bison	Ram	Icelandic sheep
Total reads	3,649,020	885,627	2,685,999	1,210,301
Avg read length (bp)	141.8	163.4	139.5	160.5
No. of contigs	546	1,256	465	1,308
N_{50} value (bp)	14,874	6,015	20,166	5,944
Genome coverage (×)	81.98	16.60	58.16	22.26
Total length (bp)	5,662,802	4,752,633	5,316,504	4,819,346
G+C content (%)	69.2	69.1	69.1	69.0
No. of genes	4,621	4,633	4,664	4,706
No. of CDS[c]	3,860	4,057	3,330	4,173
No. of pseudogenes	706	524	1,281	479
No. of rRNAs	4	3	3	3
No. of tRNAs	47	45	46	47
BioProject no.	PRJNA216936	PRJNA216934	PRJNA216937	PRJNA216930
SRA[a] accession no.	SRX10714410	SRX10704210	SRX10715497	SRX10716683
GenBank accession no.	AYNR00000000	AYNW00000000	AYLX00000000	AYOA00000000

[a] SRA, Sequence Read Archive.
[b] Type III sublineage.
[c] CDS, coding sequences.

among the four strains (Table 1). The default parameters were used for all software tools. Sequencing metrics are listed in Table 1 for each genome. All sequences were annotated using the NCBI Prokaryotic Genome Automatic Annotation Pipeline version 2.2 (14).

Data availability. The sequences were deposited in NCBI's GenBank database under the BioProject and sequence accession numbers listed in Table 1.

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