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GENOME SEQUENCES

Complete Genome Sequence of a Type III Ovine Strain of *Mycobacterium avium* subsp. *paratuberculosis*

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ABSTRACT The complete genome sequence of a type III strain of *Mycobacterium avium* subsp. *paratuberculosis* was determined. The genome size for this pathogen of sheep is 4,895,755 bp with no plasmid DNA. The chromosome contains 19 copies of the hallmark IS*900* element, which is routinely used to identify this subspecies.

Mycobacterium avium subsp. *paratuberculosis* is the causative agent of Johne's disease, which affects ruminant animals, most notably cattle and sheep (1). This subspecies is divided into two primary lineages, namely, bovine strains (also known as type II) and ovine strains, which are further subdivided into types I and III (2).

Rationale for sequencing. There are currently a dozen complete genome sequences for the type II strains (3–7) and one complete genome of the type I strain (8). Conversely, there are only two draft genome sequences available for the type III strain of *M. avium* subsp. *paratuberculosis* (9, 10) and no finished genome sequences. The draft genome sequence for one of these type III strains (S397) could not be completed because long repeats in the sequence prevented closure (9). Now, with improved long-read sequencing technology, the S397 genome assembly was closed and reported here.

Provenance of the isolate and culture conditions. The isolate was cultured in Middlebrook 7H9 broth (BD Biosciences, San Jose, CA) medium supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC; 2% glucose, 5% bovine serum albumin factor V, and 0.85% NaCl), 0.05% Tween 80, and 2 μg/ml of mycobactin J at 37°C (11). The *M. avium* subsp. *paratuberculosis* ovine S397 strain was obtained from a Suffolk breed in Iowa. It was isolated from the distal ileum at necropsy in 2004 by our group at the National Animal Disease Center. The isolate was genotyped using the IS*1311* restriction endonuclease, which yielded the 2-band pattern typical of ovine strains (12). It was further tested and confirmed for type III-specific single nucleotide polymorphisms (SNPs) in the *gyrB* gene (2).

Genome sequencing. Genomic DNA was extracted using a series of hydrolytic enzymes to degrade the proteins and lipids prior to loading onto a Qiagen 500/G genomic tip. This procedure was described in detail previously (13). Extracted DNA was quantified using a Qubit v3.0 fluorometer (Life Technologies, Thermo Fisher Scientific, Inc., Ottawa, Canada). A hybrid assembly was obtained using Illumina MiSeq (97× coverage) and Nanopore GridION X5 systems (Oxford Nanopore Technologies [ONT]; 74× coverage) at the National Animal Disease Center and Iowa State University. The library prep for Illumina sequencing was done with a Nextera XT kit (14) and run on a 500-cycle chemistry split into 2 × 250-bp lanes with no deviations and no shearing of DNA. Illumina MiSeq paired-end reads, without further processing, were used for error correction of the long-read assembly.

For ONT sequencing, extracted DNA was first concentrated with 0.8× AmPure beads (Beckman Coulter, Indianapolis, IN) and then processed by the Short Read

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Eliminator XS kit (Circulomics, Inc., Baltimore, MD, USA) for size selection. The SQK-LSK109 kit was used for library preparation following the 1D genomic DNA by ligation protocol (15) and loaded into an R9.4.1 flow cell (FLO-MIN106). Raw sequence bases were called in real time using Guppy v3.6.0 (ONT), which was also used for barcode trimming. Default parameters were used for all software unless otherwise specified. Unicycler v0.4.4 (16) was used for hybrid *de novo* assembly of the genome as well as for trimming overlapping ends and rotating to the start of the *dnaA* gene. Canu v2.1 (17) long-read-only assemblies were error corrected using Pilon v1.23 (18), with options "—fix bases—mindepth 5" and the Illumina reads. Iterative Pilon runs were performed until no more corrections were made. The genome was trimmed of any overlaps and rotated to start at the *dnaA* gene, after which Pilon and the Illumina reads were again used iteratively until no more corrections were made.

The genome was *de novo* assembled into a single circular chromosome of 4,895,755 bp with a GC content of 69.3%. A total of 4,603 genes, of which 4,346 encode proteins, were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (19).

Data availability. The sequence was deposited in the NCBI public sequence database under the accession number CP053749 (BioProject number PRJNA66075). The raw reads are also available at SRR13195562 (Illumina) and SRR13195563 (Nanopore).

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During the review of the manuscript, another *M. avium* subsp. *paratuberculosis* type III strain sequence was published (20).

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