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GENOME SEQUENCES

Complete Genome Sequence of *Mycobacterium avium* subsp. *paratuberculosis* Strain 42-13-1, Isolated in Japan

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ABSTRACT Here, we report the complete genome sequence of *Mycobacterium avium* subsp. *paratuberculosis* strain 42-13-1, isolated from cattle presenting with chronic diarrhea caused by Johne's disease in Japan, which was assembled via long- and short-read hybrid assembly.

Mycobacterium avium subsp. *paratuberculosis* is the causative agent of Johne's disease with chronic diarrhea in ruminants. Although *M. avium* subsp. *paratuberculosis* has been isolated worldwide (1) and genome sequences have been deposited in databases (2), a genome sequence for a Japanese variant was unavailable prior to this report.

Here, we report the complete genome sequence of *M. avium* subsp. *paratuberculosis* strain 42-13-1, which was isolated from a 3-year-old male Holstein with chronic diarrhea in Ibaraki prefecture (36°03′N, 11°140′E) in 2010. *M. avium*-specific antibodies were detected in the serum from the cattle using an enzyme-linked immunosorbent assay kit (Johne Screening-Pourquier; Kyoto Biken Laboratories, Japan), and *M. avium* subsp. *paratuberculosis*-specific DNA was detected via direct quantitative PCR (3) of a fecal sample from which the strain was isolated after a 2-month incubation at 37°C in ambient air on Middlebrook 7H10 agar-based slants completed with the same ingredients as described previously (3). After cloning a single colony, an isolate designated 42-13-1 was determined to be *M. avium* subsp. *paratuberculosis* via PCR for IS*900* detection (3) and serves as a reference strain for diagnosing Johne's disease in Japan (4, 5).

For the genomic DNA extraction, 42-13-1 was cultured using the same method as that used for bacterial isolation. Genomic DNA was extracted from a streak of 42-13-1 on the agar slant using a Johne-Pure-Spin kit (FASMAC, Japan) according to the manufacturer's instructions with the following modifications: 1 mg/ml RNase (Nippongene, Japan) was added to the lysis buffer for cell disruption using MicroSmash MS-100 (Tomy Seiko, Japan) with 8 s of agitation.

Sequencing was performed by Macrogen Japan Corporation using the PacBio RS II platform (Pacific Biosciences, USA) and the NovaSeq 6000 platform (150-bp paired-end reads) (Illumina, USA). For PacBio sequencing, DNA was fragmented to 20 kbp using a g-TUBE (Covaris, USA), and a DNA library was constructed using a SMRTbell template prep kit (Pacific Biosciences). For NovaSeq sequencing, DNA was fragmentated to 350 bp using a Covaris LE220 ultrasonicator (Covaris), and a DNA library was constructed using a TruSeq DNA PCR-free library prep kit (Illumina). The fragment size and concentration were measured using an Agilent 2100 bioanalyzer (Agilent, USA) with a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA).

PacBio sequencing yielded 107,498 preprocessed reads (1.40 Gbp), and Illumina sequencing yielded 39,172,852 preprocessed reads (5.92 Gbp). The mean length and N_{50} value of the PacBio subreads were 8,888 bp and 12,078 bp, respectively. The PacBio subreads were assembled using Canu version 1.0.6 (6). Default parameters were used for all

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software unless otherwise specified. Illumina reads were trimmed using Platanus_trim (http://platanus.bio.titech.ac.jp/pltanus_trim) and reassembled with Canu-assembled contigs using SPAdes version 3.13.1 using "careful" and "trusted-contigs" (7). Accuracy of the scaffold sequence was confirmed by sequence mapping using Illumina reads with the Burrows-Wheeler Aligner (BWA) version 0.6.2 (8) and SAMtools version 0.1.19 (9). Although three indistinct insertions/deletions of 19, 24, and 45 bp were identified by comparison between sequences of the Canu-constructed contig and the hybrid contig using a BLAST search of *in silico* Molecular Cloning Genomics Edition (IMC-GE) software version 7.32 (In Silico Biology, Japan), these were confirmed by PCR and Sanger sequencing. The length of the complete genome of *M. avium* subsp. *paratuberculosis* strain 42-13-1 was 4,832,738 nucleotides (nt), and the GC content was 69.3%. The average coverages of the PacBio and Illumina reads were 289.7× and 1,167.5×, respectively. Genome annotation was performed with the NCBI Prokaryotic Genome Annotation Pipeline version 5.0 (10) and consisted of 4,321 coding sequences, 1 rRNA, and 47 tRNAs.

Data availability. The complete genome sequence of *M. avium* subsp. *paratuberculosis* strain 42-13-1 is available under accession number CP066812. The raw sequence is available under Sequence Read Archive accession number DRA011321. The BioProject accession number is PRJDB10997, and the BioSample accession number is SAMD00268082.

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