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GENOME SEQUENCES

# Draft Genome Sequences of Eight *Mycobacterium montefiorense* Strains Isolated from Salamanders in Captivity

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**ABSTRACT** *Mycobacterium montefiorense* is a nontuberculous mycobacterium that causes infections in fish and salamanders. Here, we report annotated draft genome sequences of eight strains that were isolated in 2014 and 2018 from salamanders reared in an aquarium in Japan.

*Mycobacterium montefiorense* is a nontuberculous mycobacterium (NTM) that causes mycobacteriosis in fish and salamanders (1–3). *Mycobacterium montefiorense* has also been isolated from soil and pond water (4). We sequenced the genomes of eight *M. montefiorense* strains that were isolated in 2014 and 2018 from salamanders reared in Niigata City Aquarium (Niigata, Japan).

Several dead salamanders in the aquarium were collected (Table 1) and routinely dissected. The liver tissues were sampled, homogenized, and decontaminated with 1 mL of *N*-acetyl-L-cysteine-sodium citrate-NaOH for no more than 15 min. After neutralization with 6 mL of phosphate buffer (pH 6.8), the samples were centrifuged; the pellets obtained were then inoculated on Middlebrook 7H10 agar supplemented with 10% BBL Middlebrook oleic acid-albumin-dextrose-catalase (OADC) enrichment (Becton, Dickinson and Company, USA) and on 2% Ogawa egg slants (Kyokuto Pharmaceutical Industrial Co., Ltd., Japan). Isolates obtained were identified as *M. montefiorense* based on the Runyon classification system (5) and phylogenetic analysis of the 401-bp 65-kDa heat shock protein gene (*hsp65*) with the Tb11/Tb12 primer set (6).

Frozen stocks (−80°C in 20% glycerol) of *M. montefiorense* strains were streaked on 2% Ogawa slants, and single colonies were grown at 25°C for approximately 4 weeks. The collected colonies were boiled at 95°C for 15 min, frozen at −20°C overnight, and disrupted twice (4,500 rpm for 1 min) with approximately 0.5-mm-diameter zirconia/silica beads (BioSpec Products, Inc., USA) using a Micro Smash MS-100 disrupter (Tomy Digital Biology Co., Ltd., Japan). Genomic DNA was extracted using the NucleoSpin Plant II kit (Macherey-Nagel GmbH & Co. KG, Germany) in accordance with the manufacturer's instructions.

Sequencing libraries were prepared using the Nextera XT DNA library preparation kit (Illumina, USA) and sequenced on the Illumina HiSeq X platform (2 × 150 bp). The quality of the raw reads was assessed with FastQC v0.11.9 (7). The sequence reads were trimmed for quality using fastp v0.20.1 (8) and assembled *de novo* using Platanus_B v1.1.0 (9). We also assembled the genome of the type strain (*M. montefiorense* ATCC BAA-256) using the raw data (accession number DRR361296) from the National Center for Biotechnology Information (NCBI) GenBank database using the same method as described above. Automated annotation was conducted with the DNA Data Bank of Japan (DDBJ) Fast Annotation and Submission Tool (DFAST) (https://dfast.ddbj.nig.ac.jp). The annotated assemblies for eight strains and the type strain were deposited in the DDBJ. All genomic statistics are given in Table 1. Default parameters were used for all software unless otherwise noted.

Average nucleotide identity (ANI) analysis was conducted to determine relationships

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**TABLE 1** Strain information and assembly statistics

| Strain | Isolate source | Year of isolation | No. of raw reads | Genome size (bp) | No. of contigs | $N_{50}$ (bp) | Coverage (×) | Total no. of CDSs[a] | G+C content (%) | SRA accession no. | Contig accession no. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NJB14191 | Hakuba salamander (*Hynobius hidamontanus*) | 2014 | 3,646,896 | 5,744,673 | 123 | 171,591 | 85 | 5,348 | 65.1 | DRR357474 | BQYA00000000 |
| NJB14192 | Hakuba salamander (*H. hidamontanus*) | 2014 | 1,841,220 | 5,776,754 | 234 | 157,068 | 43 | 5,366 | 65.1 | DRR357475 | BQYB00000000 |
| NJB14194 | Hakuba salamander (*H. hidamontanus*) | 2014 | 1,946,204 | 5,753,077 | 155 | 169,431 | 46 | 5,382 | 65.1 | DRR357476 | BQYC00000000 |
| NJB14195 | Hakuba salamander (*H. hidamontanus*) | 2014 | 2,424,346 | 5,749,641 | 116 | 201,183 | 58 | 5,381 | 65.1 | DRR357477 | BQYD00000000 |
| NJB14197 | Hakuba salamander (*H. hidamontanus*) | 2014 | 3,047,344 | 5,745,062 | 108 | 201,635 | 72 | 5,364 | 65.1 | DRR357478 | BQYE00000000 |
| NJB18182 | Tohoku hynobiid salamander (*Hynobius lichenatus*) | 2018 | 2,308,180 | 5,764,439 | 210 | 136,848 | 52 | 5,352 | 65.1 | DRR357479 | BQYF00000000 |
| NJB18183 | Tohoku hynobiid salamander (*H. lichenatus*) | 2018 | 2,811,918 | 5,749,386 | 127 | 207,711 | 64 | 5,376 | 65.1 | DRR357480 | BQYG00000000 |
| NJB18185 | Tohoku hynobiid salamander (*H. lichenatus*) | 2018 | 4,174,608 | 5,739,217 | 88 | 240,008 | 99 | 5,381 | 65.1 | DRR357481 | BQYH00000000 |
| ATCC BAA-256 | Green moray (*Gymnothorax funebris*) | NC[b] | 8,308,120 | 5,226,877 | 735 | 16,108 | 396 | 4,149 | 65.2 | DRR361296 | BSAJ00000000 |

[a] CDSs, coding sequences.
[b] NC, not clear.


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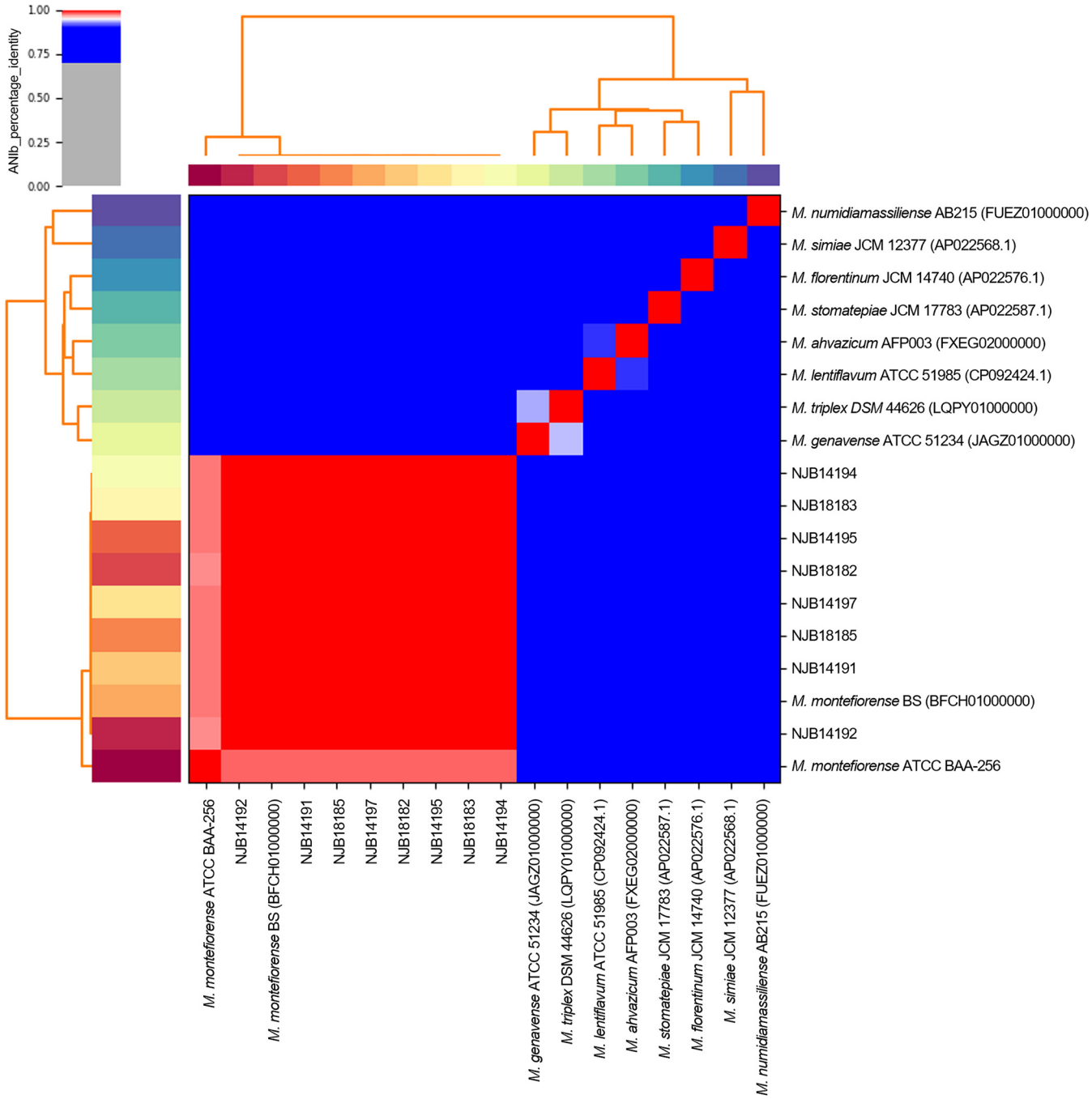


**FIG 1** Heatmap of ANI values for 18 *Mycobacterium* strains. The heatmap was generated using pyani v0.2.11 and a BLAST-based approach (ANIb).

among *Mycobacterium* species. ANI values were determined for the whole-genome sequences using pyani v0.2.11 and a BLAST-based approach (ANIb) (10). The ANI heatmap is shown in Fig. 1, and the ANI values for comparisons between the eight strains and *M. montefiorense* ATCC BAA-256 were >97.2%.

We report the draft genome sequences of eight *M. montefiorense* strains that were isolated from salamanders in captivity. These sequences will improve our understanding of the pathogenicity and evolution of this mycobacterial species.

**Data availability.** The genome sequencing and assembly projects have been deposited in the DDBJ under BioProject accession number PRJDB13312. See Table 1 for the DDBJ Sequence Read Archive (DRA) and DDBJ accession numbers.

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