Complete Genome Sequence of *Mycobacterium fortuitum* subsp. *fortuitum* JCM 6387, a Type Strain of Human-Pathogenic Mycobacteria Showing Inducible Macrolide Resistance

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ABSTRACT   *Mycobacterium fortuitum* subsp. *fortuitum* is a rapidly growing mycobacterial species for which pathogenic features are unclear. Here, we report the complete genome sequence of the *Mycobacterium fortuitum* subsp. *fortuitum* type strain. This sequence will provide essential information for future comparative genome studies of this *mycobacterium*.

*M*ycobacterium fortuitum* subsp. *fortuitum* is a rapidly growing nontuberculous mycobacterium (NTM) and a member of *M. fortuitum* group (1–3). It is noteworthy that *M. fortuitum* subsp. *fortuitum* possesses an erythromycin resistance methylase (*erm*) gene [designated *erm*(39)] that induces macrolide resistance. In contrast, NTM in the same group, such as *M. peregrinum* and *M. senegalense*, do not have this gene function (4, 5). Here, we report the complete genome sequence of *M. fortuitum* subsp. *fortuitum* type strain JCM 6387.

M. fortuitum subsp. *fortuitum* strain JCM 6387^T (=ATCC 6841, =CIP 104534, =DSM 46621) was inoculated on 2% Ogawa media (Kyokuto, Tokyo, Japan) and incubated at 30°C for 5 days. Genomic DNA was extracted by a standard phenol-chloroform method (6, 7). Long-read data were obtained with the MinION platform (Oxford Nanopore Technologies, Oxford, UK) as follows. Approximately 1 μg of the genomic DNA was used for library preparation with a ligation sequencing kit (Q20+) (SQK-Q20EA). The library was sequenced using an R10.3 flow cell (FLO-MIN111). Raw sequence data were base-called using Guppy version 5.0.16 with the base-calling model for Q20+ chemistry (https://github.com/nanoporetech/rerio). Using NanoFilt software (8), we trimmed the first 75 bp of each read and filtered the trimmed reads with quality scores of less than 12 or shorter than 1,000 bp. The remaining reads (458,025 reads and a read length N_{50} of 12,020 bp) were *de novo* assembled into one contig (6,485,838 bp) with the "suggestCircular" flag, using Canu version 2.2 (9) with following parameters: corOutCoverage, 1,000; ContigFilter, 50 10,000 1.0 0.5 50; and genomeSize, 6m. The nonredundant sequence of the contig (bp 44836 to 6450843) was extracted by SeqKit (10). Using the same DNA, Illumina paired-end (2 × 150-bp) reads were obtained with the MiniSeq system (Illumina, San Diego, CA). The DNA library was prepared using the Nextera XT DNA library kit. After checking the quality of raw reads (775,520 reads) using FastQC version 0.11.9 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), these reads were mapped to the assembly using the BWA aligner version 0.7.17 (11) for sequence and assembly error correction with Pilon version 1.2.4 (12). Using DFAST version 1.4.0 with default setting (13–15), the polished assembly (6,406,072 bp, 66.2% G+C content, and 426-fold coverage) was subjected to taxonomic checks based on average nucleotide identity (ANI) values, gene annotation, and rotation to start with the *dnaA* gene.

The ANI value between JCM 6387^T and a reported draft genome sequence of JCM 6387 (GCA_000295855.1) was 99.93%. Also, the ANI values for *M. fortuitum* subsp. *acetamidolyticum* JCM6368 (GCA_001570465.1), *M. peregrinum* DSM 43271 (GCA_

002102345.1), *M. conceptionense* CCUG 50187 (GCA_002102065.1), and *M. boenickei* JCM 15653 (GCA_010731295.1), which are the mycobacterial species phylogenetically closest to *M. fortuitum* subsp. *fortuitum* (16), were 98.75%, 88.13%, 87.11%, and 87.05%, respectively. The numbers of predicted coding sequences, rRNA operons, and tRNAs in the genome were 6,171, 6, and 65, respectively. We confirmed that JCM 6387^T harbors the *erm*(39) gene (located between bp 1817406 and 1818146 of the chromosome). The complete genome sequence of JCM 6387^T comprises important data for future comparative genome studies.

Data availability. The genome sequence and annotations of *M. fortuitum* were deposited at DDBJ/EMBL/GenBank under the accession number AP025518. Raw sequence data for strain JCM 6387^T were deposited under DRA accession number DRA013323.

REFERENCES

1. Brown-Elliott BA, Wallace RJ. 2002. Clinical and taxonomic status of pathogenic nonpigmented or late-pigmenting rapidly growing mycobacteria. Clin Microbiol Rev 15:716–746. https://doi.org/10.1128/CMR.15.4.716-746.2002.
2. Schinsky MF, Morey RE, Steigerwalt AG, Douglas MP, Wilson RW, Floyd MM, Butler WR, Daneshvar MI, Brown-Elliott BA, Wallace RJ, McNeil MM, Brenner DJ, Brown JM. 2004. Taxonomic variation in the Mycobacterium fortuitum third biovariant complex: description of Mycobacterium boenickei sp. nov., Mycobacterium houstonense sp. nov., Mycobacterium neworleansense sp. nov. and Mycobacterium brisbanense sp. nov. and recognition of Mycobacterium porcinum from human clinical isolates. Int J Syst Evol Microbiol 54:1653–1667. https://doi.org/10.1099/ijs.0.02743-0.
3. Wallace RJ, Brown-Elliott BA, Brown J, Steigerwalt AG, Hall L, Woods G, Cloud J, Mann L, Wilson R, Crist C, Jost KC, Byrer DE, Tang J, Cooper J, Stamenova E, Campbell B, Wolfe J, Turenne C. 2005. Polyphasic characterization reveals that the human pathogen Mycobacterium peregrinum type II belongs to the bovine pathogen species Mycobacterium senegalense. J Clin Microbiol 43:5925–5935. https://doi.org/10.1128/JCM.43.12.5925-5935.2005.
4. Nash KA, Zhang Y, Brown-Elliott BA, Wallace RJ. 2005. Molecular basis of intrinsic macrolide resistance in clinical isolates of Mycobacterium fortuitum. J Antimicrob Chemother 55:170–177. https://doi.org/10.1093/jac/dkh523.
5. Nash KA, Andini N, Zhang Y, Brown-Elliott BA, Wallace RJ. 2006. Intrinsic macrolide resistance in rapidly growing mycobacteria. Antimicrob Agents Chemother 50:3476–3478. https://doi.org/10.1128/AAC.00402-06.
6. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (ed). 2002. Short protocols in molecular biology, 5th ed. Wiley & Sons, Inc, New York, NY.
7. Somerville W, Thibert L, Schwartzman K, Behr MA. 2005. Extraction of Mycobacterium tuberculosis DNA: a question of containment. J Clin Microbiol 43:2996–2997. https://doi.org/10.1128/JCM.43.6.2996-2997.2005.
8. De Coster W, D'Hert S, Schultz DT, Cruts M, Van Broeckhoven C. 2018. NanoPack: visualizing and processing long-read sequencing data. Bioinformatics 34:2666–2669. https://doi.org/10.1093/bioinformatics/bty149.
9. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res 27:722–736. https://doi.org/10.1101/gr.215087.116.
10. Shen W, Le S, Li Y, Hu F. 2016. SeqKit: a cross-platform and ultrafast toolkit for FASTA/Q file manipulation. PLoS One 11:e0163962. https://doi.org/10.1371/journal.pone.0163962.
11. Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760. https://doi.org/10.1093/bioinformatics/btp324.
12. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal.pone.0112963.
13. Ciufo S, Kannan S, Sharma S, Badretdin A, Clark K, Turner S, Brover S, Schoch CL, Kimchi A, DiCuccio M. 2018. Using average nucleotide identity to improve taxonomic assignments in prokaryotic genomes at the NCBI. Int J Syst Evol Microbiol 68:2386–2392. https://doi.org/10.1099/ijsem.0.002809.
14. Tanizawa Y, Fujisawa T, Nakamura Y. 2018. DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. Bioinformatics 34:1037–1039. https://doi.org/10.1093/bioinformatics/btx713.
15. Tanizawa Y, Fujisawa T, Kaminuma E, Nakamura Y, Arita M. 2016. DFAST and DAGA: web-based integrated genome annotation tools and resources. Biosci Microbiota Food Health 35:173–184. https://doi.org/10.12938/bmfh.16-003.
16. Tortoli E, Meehan CJ, Grottola A, Fregni SG, Fabio A, Trovato A, Pecorari M, Cirillo DM. 2019. Genome-based taxonomic revision detects a number of synonymous taxa in the genus Mycobacterium. Infect Genet Evol 75:103983. https://doi.org/10.1016/j.meegid.2019.103983.