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Complete Genome Sequence of *Mycobacterium marinum* ATCC 927^T, Obtained Using Nanopore and Illumina Sequencing Technologies

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ABSTRACT *Mycobacterium marinum* is a slowly growing, broad-host-range mycobacterial species. Here, we report the complete genome sequence of a *Mycobacterium marinum* type strain that was isolated from tubercles of diseased fish. This sequence will provide essential information for future taxonomic and comparative genome studies of its relatives.

Mycobacterium marinum is a major nontuberculosis mycobacterium, which was first isolated from diseased fish (1) and later shown to be a human pathogen (2). The pathological features of the granuloma are similar to those caused by *M. tuberculosis*, and the two share many orthologous genes, which is why *M. marinum* has been used as a surrogate model for tuberculosis (3–6). Another important aspect is that *M. marinum* is a close relative of *M. ulcerans*, which produces a macrolide toxin called mycolactone and results in Buruli ulcer (7, 8). Genomic studies indicated that *M. ulcerans* was diverged from a *M. marinum* progenitor (9–11). In addition, *M. marinum*-related mycolactone-producing mycobacteria (MPMs) were also isolated from frogs and fish (12–15). Hence, detailed genomic information of *M. marinum* will be helpful for understanding evolutionary pathways of these MPMs. Here, we present the complete genome sequence of the first isolate of *M. marinum*, registered as ATCC 927^T.

The strain was grown in Middlebrook 7H9 medium, and DNA was extracted by a phenol-chloroform method. Sequence reads (100,513 reads) were obtained with the MinION nanopore sequencer (Oxford Nanopore Technologies, Oxford, UK). Genomic DNA sequencing was performed with the Nanopore SQK-RAD003 rapid sequencing kit in accordance with the manufacturer's protocol. The library was loaded on a SpotON Mk I (R9.4) flow cell and sequenced using MinKNOW version 1.7.14, and raw sequence data (FAST5 format) were base-called using Albacore Sequencing Pipeline version 2.0.2 software. The reads were *de novo* assembled into two contigs (6,456,544 bp and 173,286 bp) with Canu version 1.5 (15, 16, 17), and the assembled genome was circularized by manually trimming the repeated sequences. Illumina paired-end (2 × 150-bp) reads (266,781,451 reads) were obtained with the MiniSeq system (Illumina, San Diego, CA, USA) and mapped to the assembly using the Burrows-Wheeler aligner (15, 17, 18) for sequence and assembly error correction with Pilon (15, 17, 19). The resulting sequences (chromosome and one plasmid) were annotated using DFAST-core (20). Orthologous gene clusters were identified using Cd-hit (21). Average nucleotide identity (ANI) was calculated by JSpeciesWS (15, 17, 22).

The chromosome of *M. marinum* ATCC 927 is 6,451,936 bp (65.7% G+C content). The average nucleotide identities to two reported genomes of *M. marinum* were 98.2% (strain M) and 99.48% (strain E11). The number of predicted coding sequences (CDSs)

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in the genome (n = 5,906) was more than the number of CDSs for strain M (n = 5,593) and strain E11 (n = 5,383). The numbers of rRNA operons (n = 6) and tRNAs (n = 51) were equivalent to those of strain E11 but more than those of strain M. We also found a phage-like plasmid (127,216 bp, named pMMRN), whose size is different from that of the plasmid pRAW in strain E11 (114,229 bp) and the plasmid pMM23 (23,317 bp) in strain M. Plasmid pMMRN contains 123 CDSs, whereas 97 and 29 CDSs were present in pRAW and pMM23, respectively. We identified 4,330 orthologous gene clusters among the three strains, whereas 1,223, 714, and 287 gene clusters were specific to ATCC 927, M, and E11, respectively. The complete genome sequence of *M. marinum* ATCC 927^T comprises essential data for future taxonomic and comparative genome studies.

Accession number(s). The chromosome and plasmid sequences reported here were deposited in DDBJ/ENA/GenBank under the accession no. AP018496 and AP018497, respectively.

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REFERENCE

1. Aronson JD. 1926. Spontaneous tuberculosis in salt water fish. J Infect Dis 39:315–320. https://doi.org/10.1093/infdis/39.4.315.
2. Linell F, Norden A. 1954. *Mycobacterium balnei*, a new acid-fast bacillus occurring in swimming pools and capable of producing skin lesions in humans. Acta Tuberc Scand Suppl 33:1–84.
3. Stinear TP, Seemann T, Harrison PF, Jenkin GA, Davies JK, Johnson PD, Abdellah Z, Arrowsmith C, Chillingworth T, Churcher C, Clarke K, Cronin A, Davis P, Goodhead I, Holroyd N, Jagels K, Lord A, Moule S, Mungall K, Norbertczak H, Quail MA, Rabbinowitsch E, Walker D, White B, Whitehead S, Small PL, Brosch R, Ramakrishnan L, Fischbach MA, Parkhill J, Cole ST. 2008. Insights from the complete genome sequence of *Mycobacterium marinum* on the evolution of *Mycobacterium tuberculosis*. Genome Res 18:729–741. https://doi.org/10.1101/gr.075069.107.
4. Carlsson F, Kim J, Dumitru C, Barck KH, Carano RA, Sun M, Diehl L, Brown EJ. 2010. Host-detrimental role of Esx-1-mediated inflammasome activation in mycobacterial infection. PLoS Pathog 6:e1000895. https://doi.org/10.1371/journal.ppat.1000895.
5. Davis JM, Ramakrishnan L. 2009. The role of the granuloma in expansion and dissemination of early tuberculous infection. Cell 136:37–49. https://doi.org/10.1016/j.cell.2008.11.014.
6. Tobin DM, Ramakrishnan L. 2008. Comparative pathogenesis of *Mycobacterium marinum* and *Mycobacterium tuberculosis*. Cell Microbiol 10:1027–1039. https://doi.org/10.1111/j.1462-5822.2008.01133.x.
7. Zingue D, Bouam A, Tian RBD, Drancourt M. 2018. Buruli ulcer, a prototype for ecosystem-related infection, caused by *Mycobacterium ulcerans*. Clin Microbiol Rev 31:e00045-17. https://doi.org/10.1128/CMR.00045-17.
8. Yoshida M, Nakanaga K, Ogura Y, Toyoda A, Ooka T, Kazumi Y, Mitarai S, Ishii N, Hayashi T, Hoshino Y. 2016. Complete genome sequence of *Mycobacterium ulcerans* subsp. *shinshuense*. Genome Announc 4(5):e01050-16. https://doi.org/10.1128/genomeA.01050-16.
9. Stinear TP, Jenkin GA, Johnson PD, Davies JK. 2000. Comparative genetic analysis of *Mycobacterium ulcerans* and *Mycobacterium marinum* reveals evidence of recent divergence. J Bacteriol 182:6322–6330. https://doi.org/10.1128/JB.182.22.6322-6330.2000.
10. Stinear TP, Seemann T, Pidot S, Frigui W, Reysset G, Garnier T, Meurice G, Simon D, Bouchier C, Ma L, Tichit M, Porter JL, Ryan J, Johnson PDR, Davies JK, Jenkin GA, Small PLC, Jones LM, Tekaia F, Laval F, Daffé M, Parkhill J, Cole ST. 2007. Reductive evolution and niche adaptation inferred from the genome of *Mycobacterium ulcerans*, the causative agent of Buruli ulcer. Genome Res 17:192–200. https://doi.org/10.1101/gr.5942807.
11. Yip MJ, Porter JL, Fyfe JA, Lavender CJ, Portaels F, Rhodes M, Kator H, Colorni A, Jenkin GA, Stinear T. 2007. Evolution of *Mycobacterium ulcerans* and other mycolactone-producing mycobacteria from a common *Mycobacterium marinum* progenitor. J Bacteriol 189:2021–2029. https://doi.org/10.1128/JB.01442-06.
12. Mve-Obiang A, Lee RE, Umstot ES, Trott KA, Grammer TC, Parker JM, Ranger BS, Grainger R, Mahrous EA, Small PL. 2005. A newly discovered mycobacterial pathogen isolated from laboratory colonies of *Xenopus* species with lethal infections produces a novel form of mycolactone, the *Mycobacterium ulcerans* macrolide toxin. Infect Immun 73:3307–3312. https://doi.org/10.1128/IAI.73.6.3307-3312.2005.
13. Rhodes MW, Kator H, Kotob S, van Berkum P, Kaattari I, Vogelbein W, Floyd MM, Butler WR, Quinn FD, Ottinger C, Shotts E. 2001. A unique *Mycobacterium* species isolated from an epizootic of striped bass (*Morone saxatilis*). Emerg Infect Dis 7:896–899. https://doi.org/10.3201/eid0705.017523.
14. Rhodes MW, Kator H, McNabb A, Deshayes C, Reyrat JM, Brown EBA, Wallace R, Jr., Trott KA, Parker JM, Lifland B, Osterhout G, Kaattari I, Reece K, Vogelbein W, Ottinger CA. 2005. *Mycobacterium pseudoshottsii* sp. nov., a slowly growing chromogenic species isolated from Chesapeake Bay striped bass (*Morone saxatilis*). Int J Syst Evol Microbiol 55:1139–1147. https://doi.org/10.1099/ijs.0.63343-0.
15. Yoshida M, Miyamoto Y, Ogura Y, Hayashi T, Hoshino Y. 2017. Complete chromosome sequence of a mycolactone-producing Mycobacterium, Mycobacterium pseudoshottsii. Genome Announc 5(48):e01363-17. https://doi.org/10.1128/genomeA.01363-17.
16. 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.
17. Yoshida M, Fukano H, Miyamoto Y, Shibayama K, Suzuki M, Hoshino Y. 2018. Complete genome sequence of a type strain of *Mycobacterium*

abscessus subsp. *bolletii*, a member of the *Mycobacterium abscessus* complex. Genome Announc 6(5):e01530-17. https://doi.org/10.1128/genomeA.01530-17.
18. 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.
19. 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.
20. 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.
21. Li W, Godzik A. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22:1658–1659. https://doi.org/10.1093/bioinformatics/btl158.
22. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. 2016. JSpeciesWS: a Web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 32:929–931. https://doi.org/10.1093/bioinformatics/btv681.