

## AMERICAN SOCIETY FOR MICROBIOLOGY

## Draft Genome Sequence of *Mycobacterium virginiense* Strain GF75, Isolated from the Mud of a Swine Farm in Japan

Toshihiro Ito,<sup>a</sup> 📴 Fumito Maruyama,<sup>a</sup> Kotaro Sawai,<sup>b</sup> Keiko Nozaki,<sup>c</sup> Keiko Otsu,<sup>c</sup> 🖻 Kenji Ohya<sup>b,d</sup>

<sup>a</sup>Department of Microbiology, Graduate School of Medicine, Kyoto University, Kyoto, Japan <sup>b</sup>Laboratory of Veterinary Microbiology, Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan <sup>c</sup>Gifu Prefectural Chuo Livestock Hygiene Service Center, Gifu, Japan <sup>d</sup>United Graduate School of Veterinary Sciences, Gifu University, Gifu, Japan

**ABSTRACT** Mycobacterium virginiense, a newly described species of the Mycobacterium terrae complex, is a cause of tenosynovitis and osteomyelitis in the United States. Here, we report the 4,849,424-bp draft genome sequence of *M. virginiense* strain GF75, isolated from a mud sample taken from a Japanese swine farm.

**N**ontuberculous mycobacteria (NTM), including the *Mycobacterium terrae* complex, are environmentally transmitted pathogens that are relevant to human and animal health (1). A newly described species of the *M. terrae* complex, *M. virginiense*, was recently recognized as an infectious agent of clinical importance (2). *M. virginiense* was first described in 2016 on the basis of three independent clinical strains as the etiological agent of tenosynovitis and osteomyelitis in the United States (3, 4). The three isolates of *M. virginiense* in previous studies were acid fast and nonpigmented on Middlebrook 7H10 agar, slow growing, and resistant to various antibiotics, including rifampin and the quinolones (5). The distribution and incidence of NTM are known to vary temporally and geographically (6, 7). Hence, the characterization of NTM, such as *M. virginiense*, isolated from different sources and/or geographic areas will enable a better understanding of their ecology and epidemiology.

Here, we report the draft genome sequence of an *M. virginiense* strain isolated from a mud sample taken from a swine farm in the Tokai area of Japan, where an outbreak of swine mycobacteriosis occurred (data not shown). The mud sample was decontaminated overnight with an equal volume of 2% NaOH. The suspension was washed with phosphate-buffered saline (PBS) for neutralization and then inoculated on 2% Ogawa slant (Kyokuto Pharmaceutical, Tokyo, Japan) at 37°C for 4 weeks. Colonies were subcultured on Middlebrook 7H11 agar supplemented with 10% oleic acid-albumindextrose-catalase (OADC) enrichment (Becton, Dickinson, MD, USA). DNA was extracted using the PureLink genomic DNA extraction kit according to the manufacturer's instructions (Invitrogen, MA, USA), and paired-end libraries with an average insert size of 350 bp were prepared. These underwent  $2 \times 150$ -bp sequencing on a HiSeq X Ten sequencing platform (Illumina, San Diego, CA, USA) at the Beijing Genomics Institute (Shenzhen, China).

Raw reads were preprocessed using SOAPnuke (8) and assembled using the A5miseq pipeline (9). Contigs were binned and clustered on the basis of tetranucleotide frequency, G+C content, and differential coverage using BinSanity (10). Reads mapping to the resulting bin were extracted using Burrows-Wheeler Aligner (BWA) (11) and SAMtools (12). Reassembly and final scaffolding were performed using the A5 MiSeq pipeline (9). The resulting scaffold was refined to reduce contamination on the basis of tetranucleotide frequency, G+C content, and differential coverage using RefineM (13). CheckM (14) reported 100% completeness with 1.15% potential contamination for the 2018 Published 26 April 2018 Citation Ito T, Maruyama F, Sawai K, Nozaki K,

Otsu K, Ohya K. 2019. Draft genome sequence of *Mycobacterium virginiense* strain GF75, isolated from the mud of a swine farm in Japan. Genome Announc 6:e00362-18. https:// doi.org/10.1128/genomeA.00362-18.

Received 26 March 2018 Accepted 26 March

**Copyright** © 2018 Ito et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Kenji Ohya, kohya@gifu-u.ac.jp.

lto et al.

final draft genome sequence, which was then annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (15).

The combined length of the final draft genome sequence was 4,849,424 bp comprising 128 scaffolds with a 67.25% G+C content, a total of 4,714 coding sequences, and 54 predicted RNAs, including 4 rRNAs, 47 tRNAs, and 3 noncoding RNAs. Based on blastn analysis against the NCBI nucleotide database (downloaded on 16 February 2018), 65% and 34% of the draft genome sequence exhibited significant sequence similarity with *M. terrae* strain NCTC10856 (GenBank accession number LT906469) and *M. sinense* strain JDM601 (GenBank accession number CP002329), respectively.

**Accession number(s).** The draft genome sequence of *M. virginiense* strain GF75 has been deposited at DDBJ/ENA/GenBank under the accession number PUEV00000000.

## ACKNOWLEDGMENTS

This study was supported by the Japan Agency for Medical Research and Development (AMED) through the AMED grant (17fk0108116h0401), the Japan Racing Association (JRA) through the JRA Livestock Industry Promotion Project (28-239), the Ito Foundation through Research Grants for Meat and Meat Products (H28-130), a Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research (B) (26304039), and the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT), for the Joint Research Program of the Research Center for Zoonosis Control, Hokkaido University.

We thank Sarah Williams from the Edanz Group for editing a draft of this article.

## REFERENCES

- Tortoli E. 2014. Microbiological features and clinical relevance of new species of the genus *Mycobacterium*. Clin Microbiol Rev 27:727–752. https://doi.org/10.1128/CMR.00035-14.
- Janda JM. 2017. Taxonomic update on proposed nomenclature and classification changes for bacteria of medical importance, 2016. Diagn Microbiol Infect Dis 88:100–105. https://doi.org/10.1016/j.diagmicrobio .2017.02.003.
- Vasireddy R, Vasireddy S, Brown-Elliott BA, Wengenack NL, Eke UA, Benwill JL, Turenne C, Wallace RJ. 2016. *Mycobacterium arupense*, *Mycobacterium heraklionense*, and a newly proposed species, "*Mycobacterium virginiense*" sp. nov., but not *Mycobacterium nonchromogenicum*, as species of the *Mycobacterium terrae* complex causing tenosynovitis and osteomyelitis. J Clin Microbiol 54:1340–1351. https://doi.org/10.1128/ JCM.00198-16.
- Ridderhof JC, Wallace RJ, Jr, Kilburn JO, Butler WR, Warren NG, Tsukamura M, Steele LC, Wong ES. 1991. Chronic tenosynovitis of the hand due to *Mycobacterium nonchromogenicum*: use of high-performance liquid chromatography for identification of isolates. Rev Infect Dis 13: 857–864. https://doi.org/10.1093/clinids/13.5.857.
- Vasireddy R, Vasireddy S, Brown-Elliott BA, Wengenack NL, Eke UA, Benwill JL, Turenne C, Wallace RJJ. 2017. Correction for Vasireddy et al., Mycobacterium arupense, Mycobacterium heraklionense, and a newly proposed species, "Mycobacterium virginiense" sp. nov., but not Mycobacterium nonchromogenicum, as species of the Mycobacterium terrae complex causing tenosynovitis and osteomyelitis. J Clin Microbiol 55: 985–985. https://doi.org/10.1128/JCM.02290-16.
- 6. Hoefsloot W, van Ingen J, Andrejak C, Ängeby K, Bauriaud R, Bemer P, Beylis N, Boeree MJ, Cacho J, Chihota V, Chimara E, Churchyard G, Cias R, Daza R, Daley CL, Dekhuijzen PNR, Domingo D, Drobniewski F, Esteban J, Fauville-Dufaux M, Folkvardsen DB, Gibbons N, Gómez-Mampaso E, Gonzalez R, Hoffmann H, Hsueh PR, Indra A, Jagielski T, Jamieson F, Jankovic M, Jong E, Keane J, Koh WJ, Lange B, Leao S, Macedo R, Mannsåker T, Marras TK, Maugein J, Milburn HJ, Mlinkó T, Morcillo N, Morimoto K, Papaventsis D, Palenque E, Paez-Peña M, Piersimoni C, Polanová M, Rastogi N, Richter E, et al. 2013. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: an

NTM-NET collaborative study. Eur Respir J 42:1604–1613. https://doi.org/ 10.1183/09031936.00149212.

- Adjemian J, Olivier KN, Seitz AE, Falkinham JO, Holland SM, Prevots DR. 2012. Spatial clusters of nontuberculous mycobacterial lung disease in the United States. Am J Respir Crit Care Med 186:553–558. https://doi .org/10.1164/rccm.201205-0913OC.
- Chen Y, Chen Y, Shi C, Huang Z, Zhang Y, Li S, Li Y, Ye J, Yu C, Li Z, Zhang X, Wang J, Yang H, Fang L, Chen Q. 2018. SOAPnuke: a MapReduce acceleration supported software for integrated quality control and preprocessing of high-throughput sequencing data. Gigascience 7:1–6. https://doi.org/10.1093/gigascience/gix120.
- Coil D, Jospin G, Darling AE. 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics 31:587–589. https://doi.org/10.1093/bioinformatics/btu661.
- Graham ED, Heidelberg JF, Tully BJ. 2017. BinSanity: unsupervised clustering of environmental microbial assemblies using coverage and affinity propagation. PeerJ 5:e3035. https://doi.org/10.7717/peerj.3035.
- 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.
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079. https://doi.org/10.1093/ bioinformatics/btp352.
- Parks DH, Rinke C, Chuvochina M, Chaumeil P-A, Woodcroft BJ, Evans PN, Hugenholtz P, Tyson GW. 2017. Recovery of nearly 8,000 metagenomeassembled genomes substantially expands the tree of life. Nat Microbiol 2:1533–1542. https://doi.org/10.1038/s41564-017-0012-7.
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055. https:// doi.org/10.1101/gr.186072.114.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44: 6614–6624. https://doi.org/10.1093/nar/gkw569.