



Draft Genome Sequence of *Mycobacterium montefiorense* Isolated from Japanese Black Salamander (*Hynobius nigrescens*)

Hanako Fukano,^a Mitsunori Yoshida,^a Akane Shimizu,^b Hajime Iwao,^c Yukie Katayama,^d Tsutomu Omatsu,^d Tetsuya Mizutani,^d Osamu Kurata,^b Shinpei Wada,^b [©] Yoshihiko Hoshino^a

^aDepartment of Mycobacteriology, Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan

^bThe Laboratory of Aquatic Medicine, Nippon Veterinary and Life Science University, Tokyo, Japan

^cNiigata City Aquarium, Niigata, Japan

^dResearch and Education Center for Prevention of Global Infectious Diseases of Animals, Tokyo University of Agriculture and Technology, Tokyo, Japan

ABSTRACT Mycobacterium montefiorense is a member of the Mycobacterium simiae complex, the largest group of nontuberculous mycobacteria. Here, we report the genome sequence of *M. montefiorense* isolate BS, isolated from diseased Japanese black salamander (*Hynobius nigrescens*) reared in an aquarium in Japan. This is the first reported case of an *M. montefiorense* infection in an amphibian.

Nontuberculous mycobacteria (NTMs) are ubiquitous in the environment and exist even in water (1). Some of the species can cause outbreaks of NTM infectious disease in humans or fish (2–4). *Mycobacterium montefiorense* is a member of the *M. simiae* complex, which is the largest group of nontuberculous mycobacterial species (5). In 2003, this species was reported as a new nontuberculous mycobacterium (NTM) species closely related to *M. triplex*, a human-pathogenic species (6). It was first isolated from a disease outbreak in two species of moray eels, green moray (*Gymnothorax funebris*) and spotted moray (*G. moringa*), caught near the Florida Keys in 1991 to 1992 and being reared in synthetic seawater for an exhibit (7). This captive population of moray eels died from this NTM infection while showing ulcerative granulomatous dermatitis and fasciitis (7).

From 2010 to 2014, an outbreak of infections caused by *M. montefiorense* was recorded in some salamander species, namely, Japanese black salamander (*Hynobius nigrescens*) and Hakuba salamander (*H. hidamontanus*) reared in an aquarium. Some of the diseased salamanders showed ulcerative lesions on the skin, but the most prominent features were granulomatous lesions observed in the liver with numerous acid-fast bacteria present. We isolated this causative agent from the liver of a dead Japanese black salamander and named the strain BS.

We hereby report the draft genome sequence of *M. montefiorense* strain BS isolated from Japanese black salamander. This is the first case of an *M. montefiorense* infection of amphibians.

The strain was inoculated on Middlebrook 7H10 agar supplemented with 10% oleic acid-albumin-dextrose-catalase enrichment and incubated at 25°C for about 1 to 2 months. Genomic DNA was extracted by a phenol-chloroform method.

Libraries were constructed using the Nextera XT DNA library preparation kit (Illumina, Inc., San Diego, CA, USA). The genome sequence was obtained by using Illumina 300-bp \times 2 paired-end reads (741,188,100 reads) (Illumina, Inc.). The paired-end reads were assembled with Platanus version 1.1 into 50 contigs. Automated annotation was

Received 16 April 2018 Accepted 18 April 2018 Published 24 May 2018

Citation Fukano H, Yoshida M, Shimizu A, Iwao H, Katayama Y, Omatsu T, Mizutani T, Kurata O, Wada S, Hoshino Y. 2018. Draft genome sequence of *Mycobacterium montefiorense* isolated from Japanese black salamander (*Hynobius nigrescens*). Genome Announc 6:e00448-18. https://doi.org/10.1128/genomeA.00448-18.

Copyright © 2018 Fukano et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Yoshihiko Hoshino, yhoshino@nih.go.jp.

carried out with the DDBJ FAST Annotation and Submission Tool (DFAST) (https://dfast .nig.ac.jp). Average nucleotide identity (ANI) was calculated with JSpeciesWS version 1.2.1 (8, 9).

The genome of *M. montefiorense* BS is 5,744,567 bp in length, with a 65.1% G+C content. In total, 5,384 protein-coding sequences, 3 rRNAs, and 53 tRNAs were predicted. The ANI values versus *M. simiae* ATCC 25275^T (GenBank accession numbers CBMJ020000001 to CBMJ020000359) and *M. triplex* DSM 44626^T (GenBank accession number NZ_CCAU000000000) were 85.32% and 81.97%, respectively.

This sequence is the first genome sequence of *M. montefiorense* isolated from an amphibian, and it will provide significant epidemiological information of NTMs in an aquatic environment.

Accession number(s). The scaffold sequences and annotations of *M. montefiorense* BS were deposited at DDBJ/EMBL/GenBank under the accession number BFCH00000000.

ACKNOWLEDGMENTS

We thank Sachiko Harada at the Niigata Aquarium for providing us with reared salamanders from which we isolated strain BS.

This work was in part supported by a grant from the Japan Agency for Medical Research and Development/Japan International Cooperation Agency (AMED) to Y. Hoshino (jp18fk0108064, jp18fk0108075, and jp18jm0510004), by a Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science (JSPS) to Y. Hoshino (jp18K08312), by a Grant-in-Aid for Young Scientists (B) to M. Yoshida (jp17K16066), and by a Grant-in-Aid for Early Career Scientists to H. Fukano (jp18K15966). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

- Falkinham JO. 2009. Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment. J Appl Microbiol 107:356–367. https://doi.org/10.1111/j.1365-2672.2009.04161.x.
- Fukano H, Wada S, Kurata O, Mizuno K, Nakanaga K, Hoshino Y. 2015. Nontuberculous mycobacteriosis in farmed thread-sail filefish *Stephanolepis cirrhifer*. Fish Pathol 50:68–74. https://doi.org/10.3147/jsfp.50.68.
- Fukano H, Wada S, Kurata O, Katayama K, Fujiwara N, Hoshino Y. 2017. *Mycobacterium stephanolepidis* sp. nov., a rapidly growing species related to mycobacterium chelonae, isolated from marine teleost fish, *Stephanolepis cirrhifer*. Int J Syst Evol Microbiol 67:2811–2817. https://doi.org/10 .1099/ijsem.0.002028.
- Feng Y, Xu H, Wang H, Zhang C, Zong W, Wu Q. 2011. Outbreak of a cutaneous *Mycobacterium marinum* infection in Jiangsu Haian, China. Diagn Microbiol Infect Dis 71:267–272. https://doi.org/10.1016/j.diagmicrobio.2011 .07.003.
- Fusco da Costa AR, Fedrizzi T, Lopes ML, Pecorari M, Oliveira da Costa WL, Giacobazzi E, da Costa Bahia JR, De Sanctis V, Batista Lima KV, Bertorelli R, Grottola A, Fabio A, Mariottini A, Ferretti P, Di Leva F, Fregni Serpini G, Tagliazucchi S, Rumpianesi F, Jousson O, Segata N, Tortoli E. 2015. Char-

acterization of 17 strains belonging to the *Mycobacterium simiae* complex and description of *Mycobacterium paraense* sp. nov. Int J Syst Evol Microbiol 65:656–662. https://doi.org/10.1099/ijs.0.068395-0.

- Levi MH, Bartell J, Gandolfo L, Smole SC, Costa SF, Weiss LM, Johnson LK, Osterhout G, Herbst LH. 2003. Characterization of *Mycobacterium montefiorense* sp. nov., a novel pathogenic mycobacterium from moray eels that is related to *Mycobacterium triplex*. J Clin Microbiol 41:2147–2152.
- Herbst LH, Costa SF, Weiss LM, Johnson LK, Bartell J, Davis R, Walsh M, Levi M. 2001. Granulomatous skin lesions in moray eels caused by a novel *Mycobacterium* species related to *Mycobacterium triplex*. Infect Immun 69:4639–4646. https://doi.org/10.1128/IAI.69.7.4639-4646.2001.
- Richter M, Rosselló-Móra R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A 106:19126–19131. https://doi.org/10.1073/pnas.0906412106.
- Yoshida M, Miyamoto Y, Ogura Y, Hayashi T, Hoshino Y. 2017. Complete chromosome sequence of a mycolactone-producing mycobacterium, *Mycobacterium pseudoshottsii*. Genome Announc 5:e01363-17. https://doi .org/10.1128/genomeA.01363-17.