



Draft Genome Sequences of *Mycolicibacter senuensis* Isolate GF74 and *Mycobacterium colombiense* Isolates GF28 and GF76 from a Swine Farm in Japan

Toshihiro Ito,^a Kotaro Sawai,^b Mikihiko Kawai,^a Keiko Nozaki,^c Keiko Otsu,^c Hideto Fukushi,^{b,d} ^(D)Kenji Ohya,^{b,d} ^(D)Fumito Maruyama^{a,e}

^aDepartment of Microbiology, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan ^bLaboratory of Veterinary Microbiology, Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan ^cGifu Prefectural Chuo Livestock Hygiene Service Center, Gifu, Japan ^dUnited Graduate School of Veterinary Sciences, Gifu University, Gifu, Japan ^eScientific and Technological Bioresource Nucleus, Universidad de La Frontera, Temuco, Chile

ABSTRACT Several nontuberculous mycobacteria (NTM) occasionally infect humans and animals. Here, we report the draft genome sequences of *Mycolicibacter senuensis* isolate GF74 (4,792,997 bp) and *Mycobacterium colombiense* isolates GF28 and GF76 (5,473,554 bp and 5,426,852 bp, respectively) isolated from a swine farm in Japan. These sequences provide further information on NTM research.

Nontuberculous mycobacteria (NTM), encompassing mycobacteria other than *Mycobacterium tuberculosis* complex and *Mycobacterium leprae*, include more than 170 species. NTM usually inhabit the natural environment, but most are considered opportunistic pathogens of humans and animals (1). Based on comprehensive phylogenomic analyses, it has been proposed that the single genus *Mycobacterium* be divided into five distinct monophyletic clades, as follows: an emended genus *Mycobacterium* ("Tuberculosis-Simiae" clade) and four novel genera, *Mycolicibacterium* gen. nov. ("Fortuitum-Vaccae" clade), *Mycolicibacter* gen. nov. ("Terrae" clade), *Mycolicibacillus* gen. nov. ("Triviale" clade), and *Mycobacteroides* gen. nov. ("Abscessus-Chelonae" clade) (2).

Recent comprehensive genomic studies have increased our knowledge on the genetic features and classification of NTM (2–4). However, more information is needed about NTM, including genome sequences, which are indispensable for understanding ecology and etiology and for developing reliable diagnostic tools. Here, we report the draft genome sequences of *Mycolicibacter senuensis* (basonym: *Mycobacterium senuense*) isolate GF74 and *Mycobacterium colombiense* isolates GF28 and GF76 from soil in Japan. *Mycolicibacter senuensis*, first isolated from a Korean patient with a symptomatic pulmonary infection, belongs to the Terrae clade (5). *Mycobacterium colombiense*, initially isolated from HIV-positive patients in Colombia, is a member of the *Mycobacterium avium* complex (6).

All isolates were obtained from mud at a swine farm in the Tokai area of Japan, as described previously (7). Briefly, the mud samples were decontaminated with equal volumes of 2% NaOH and then inoculated onto a 2% Ogawa slant (Kyokuto Pharmaceutical, Tokyo, Japan) at 37°C for up to 4 weeks. Each single colony on the slant was subcultured on Middlebrook 7H11 agar supplemented with 10% oleic acid-albumindextrose-catalase (OADC) enrichment (Becton, Dickinson, MD, USA). The species of the isolates were identified by analyzing 16S rRNA, *hsp65*, and *rpoB* genes (8, 9). DNA was extracted using a PureLink genomic DNA extraction kit, according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA), and paired-end libraries with an average insert size of 350 bp were prepared from each 3 μ g of genomic DNA. These

Received 14 July 2018 Accepted 14 August 2018 Published 13 September 2018

Citation Ito T, Sawai K, Kawai M, Nozaki K, Otsu K, Fukushi H, Ohya K, Maruyama F. 2018. Draft genome sequences of *Mycolicibacter senuensis* isolate GF74 and *Mycobacterium colombiense* isolates GF28 and GF76 from a swine farm in Japan. Microbiol Resour Announc 7:e00936-18. https://doi.org/10.1128/MRA.00936-18.

Editor Jason Stajich, University of California, Riverside

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.

		Genome	No. of	G+C	No. of	No. of	No. of	GenBank
Species	Isolate	size (bp)	scaffolds ^a	content (%)	CDSs ^b	rRNAs	tRNAs	accession no.
Mycolicibacter senuensis	GF74	4,792,997	304	67.95	4,809	3	42	QMEX0000000
Mycobacterium colombiense	GF28	5,473,554	146	67.69	5,238	3	51	QMEV0000000
Mycobacterium colombiense	GF76	5,426,852	216	67.64	5,249	3	47	QMEU0000000

TABLE 1 Summary information for the draft genome sequences of nontuberculous mycobacterial isolates obtained from mud at a swine farm

^aNumbers of scaffolds >500 bp are shown.

^bCDSs, coding sequences.

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). Quality trimming and adapter trimming were conducted using Cutadapt (https://github.com/marcelm/cutadapt/) via TrimGalore! (https://github.com/FelixKrueger/TrimGalore). Mismatch correction of reads and assembly were carried out using SPAdes (10), and the assembly was polished using Pilon (11), with the aid of Unicycler (12). CheckM was used to estimate genome completeness (13). Draft genomes were then annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (14). The combined lengths of the final draft genomes, G+C contents, and the numbers of scaffolds, coding sequences (CDSs), rRNAs, and tRNAs are shown in Table 1. ANItools analysis (15) revealed that *Mycolicibacter senuensis* GF74 and *Mycobacterium colombiense* GF28 and GF76 showed 93.12% identity to *Mycobacterium* sp. strain JDM601, 86.73% identity to *Mycobacterium indicus* pranii, and 86.18% identity to *Mycobacterium intracellulare* MOTT, respectively.

Data availability. The draft genome sequences of *Mycolicibacter senuensis* GF74 and *Mycobacterium colombiense* GF28 and GF76 have been deposited in DDBJ/ EMBL/GenBank under the accession numbers QMEX00000000, QMEV00000000, and QMEU00000000, respectively (Table 1).

ACKNOWLEDGMENTS

This study was supported by the Japan Agency for Medical Research and Development (AMED) through grant 17fk0108116h0401, the Japan Racing Association (JRA) through the JRA livestock industry promotion project (grants H28-29_239 and H29-30_7), the Ito Foundation through Research grants for Meat and Meat Products (grants H28-130 and H30-60), the Japan Society for the Promotion of Science (JSPS) KAKENHI (grants JP26304039 and JP18K19674), and the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT), for Joint Research Program of the Research Center for Zoonosis Control, Hokkaido University.

Computational resources were partly provided by the Data Integration and Analysis Facility, National Institute for Basic Biology.

T. Ito, K. Ohya, and F. Maruyama designed the research. K. Sawai, K. Nozaki, K. Otsu, H. Fukushi, and K. Ohya conceived the experiments. T. Ito, M. Kawai, K. Ohya, and F. Maruyama analyzed the data. T. Ito, K. Ohya, and F. Maruyama wrote the manuscript.

We declare no conflicts of interest.

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.
- Gupta RS, Lo B, Son J. 2018. Phylogenomics and comparative genomic studies robustly support division of the genus *Mycobacterium* into an emended genus *Mycobacterium* and four novel genera. Front Microbiol 9:67. https://doi.org/10.3389/fmicb.2018.00067.
- Fedrizzi T, Meehan CJ, Grottola A, Giacobazzi E, Fregni Serpini G, Tagliazucchi S, Fabio A, Bettua C, Bertorelli R, De Sanctis V, Rumpianesi F, Pecorari M, Jousson O, Tortoli E, Segata N. 2017. Genomic characterization of nontuberculous mycobacteria. Sci Rep 7:45258. https://doi.org/ 10.1038/srep45258.
- Tortoli E, Fedrizzi T, Meehan CJ, Trovato A, Grottola A, Giacobazzi E, Serpini GF, Tagliazucchi S, Fabio A, Bettua C, Bertorelli R, Frascaro F, De Sanctis V, Pecorari M, Jousson O, Segata N, Cirillo DM. 2017. The new phylogeny of the genus *Mycobacterium*: the old and the news. Infect Genet Evol 56:19–25. https://doi.org/10.1016/j.meegid.2017.10.013.
- Mun H-S, Park J-H, Kim H, Yu H-K, Park Y-G, Cha C-Y, Kook Y-H, Kim B-J. 2008. Mycobacterium senuense sp. nov., a slowly growing, non-chromogenic species closely related to the Mycobacterium terrae complex. Int J Syst Evol Microbiol 58:641–646. https://doi.org/10.1099/ijs.0.65374-0.
- Murcia MI, Tortoli E, Menendez MC, Palenque E, Garcia MJ. 2006. Mycobacterium colombiense sp. nov., a novel member of the Mycobacterium avium complex and description of MAC-X as a new ITS genetic variant.

Int J Syst Evol Microbiol 56:2049–2054. https://doi.org/10.1099/ijs.0 .64190-0.

- Ito T, Maruyama F, Sawai K, Nozaki K, Otsu K, Ohya K. 2018. 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.
- Adékambi T, Colson P, Drancourt M. 2003. *rpoB*-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. J Clin Microbiol 41:5699–5708. https://doi.org/10.1128/JCM.41.12.5699-5708.2003.
- McNabb A, Eisler D, Adie K, Amos M, Rodrigues M, Stephens G, Black WA, Isaac-Renton J. 2004. Assessment of partial sequencing of the 65kilodalton heat shock protein gene (*hsp65*) for routine identification of *Mycobacterium* species isolated from clinical sources. J Clin Microbiol 42:3000–3011. https://doi.org/10.1128/JCM.42.7.3000-3011.2004.
- Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, Prjibelski AD, Pyshkin A, Sirotkin A, Sirotkin Y, Stepanauskas R, Clingenpeel SR, Woyke T, McLean JS, Lasken R, Tesler G, Alekseyev MA, Pevzner PA. 2013. Assembling single-cell genomes and minimetagenomes from chimeric MDA products. J Comput Biol 20:714–737. https://doi.org/10.1089/cmb.2013.0084.

- 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.
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595.
- 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.
- Han N, Qiang Y, Zhang W. 2016. ANItools Web: a Web tool for fast genome comparison within multiple bacterial strains. Database 2016: baw084. https://doi.org/10.1093/database/baw084.