



# Complete Genome Sequence of a Type Strain of *Mycobacterium abscessus* subsp. *bolletii*, a Member of the *Mycobacterium abscessus* Complex

Mitsunori Yoshida,<sup>a</sup> Hanako Fukano,<sup>a</sup> Yuji Miyamoto,<sup>a</sup> Keigo Shibayama,<sup>b</sup> Masato Suzuki,<sup>c</sup> Yoshihiko Hoshino<sup>a</sup>

<sup>a</sup>Department of Mycobacteriology, Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan

<sup>b</sup>Department of Bacteriology II, National Institute of Infectious Diseases, Tokyo, Japan

<sup>c</sup>Antimicrobial Resistance Research Center, National Institute of Infectious Diseases, Tokyo, Japan

**ABSTRACT** *Mycobacterium abscessus* subsp. *bolletii* is a rapidly growing mycobacterial organism for which the taxonomy is unclear. Here, we report the complete genome sequence of a *Mycobacterium abscessus* subsp. *bolletii* type strain. This sequence will provide essential information for future taxonomic and comparative genome studies of these mycobacteria.

The number of cases of nontuberculosis mycobacteria (NTM) is increasing, especially in developed countries. In our hospital-based survey of pulmonary NTM disease patients in Japan, the number of pulmonary *Mycobacterium abscessus* disease cases increased 5-fold relative to a survey conducted 7 years earlier (1). Moreover, the finding that the multidrug-resistant *M. abscessus* complex (MABC) is transmissible between patients with conditions such as cystic fibrosis provided a radical shift in thinking about MABC acquisition, which was previously thought to be environmental (2–4). Despite advances made in the genomics and documentation of clinical phenotypical differences among MABCs, discrepancies in conventional DNA-DNA hybridization (DDH) results led to debate about MABC taxonomic differentiation (3, 5–9).

Here, we report the complete genome sequence of *M. abscessus* subsp. *bolletii* BD<sup>T</sup> (=CIP108541<sup>T</sup>). The strain was grown in Middlebrook 7H9 medium, and DNA was extracted using a standard phenol-chloroform method. The genome sequence was determined using PacBio reads (112,992 reads) obtained with the RS II system (Pacific Biosciences, Menlo Park, CA, USA) (10–12). The reads were *de novo* assembled with Canu version 1.5 (13), and the assembled genome was circularized by manually trimming the repeated sequences. Illumina 2 × 300-bp paired-end reads (100,578,382 reads) were obtained with the MiSeq system (Illumina, San Diego, CA, USA) and mapped to the assembly using the Burrows-Wheeler aligner (14) for sequence and assembly error correction with Pilon (15). The DDBJ Fast Annotation and Submission Tool (DFAST) (<https://dfast.nig.ac.jp/>) was used for annotation (16). Average nucleotide identity (ANI), genome-to-genome distance (GGD), and genomic signature-delta distance (GS-DD) were calculated by JSpeciesWS, Genome-to-Genome Distance Calculator 2.1 (<http://ggdc.dsmz.de/ggdc.php>), and δ\*-differences (<http://www.cmbl.uga.edu/software/delta-differences.html>), respectively (17–19).

The length of the *M. abscessus* subsp. *bolletii* BD<sup>T</sup> genome is 5,080,450 bp (64.1% G+C content). Mycobacterium-related ANI was 96.95% to *M. abscessus* subsp. *abscessus* (complete genome of strain ATCC\_19977<sup>T</sup> [20]) and 96.73% to *M. abscessus* subsp. *massiliense* (complete genome of strain JCM\_15300<sup>T</sup> [7]). GGD-estimated DDH values between strains BD<sup>T</sup> and ATCC\_19977<sup>T</sup>, strains ATCC\_19977<sup>T</sup> and

Received 14 December 2017 Accepted 21 December 2017 Published 1 February 2018

**Citation** 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:e01530-17. <https://doi.org/10.1128/genomeA.01530-17>.

**Copyright** © 2018 Yoshida 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.

JCM\_15300, and strains JCM\_15300 and BD<sup>T</sup> were 87.0%, 86.0%, and 88.1%, respectively. The GS-DD was 23 for these 3 comparisons. These values support the proposed taxonomic position of *M. abscessus* subsp. *bolletii* (3). The number of predicted protein-coding sequences in the genome ( $n = 4,976$ ) is nearly equivalent to those of *M. abscessus* subsp. *abscessus* ( $n = 4,920$ ) and *M. abscessus* subsp. *massiliense* ( $n = 4,857$ ) (7, 20). The numbers of rRNA operons ( $n = 3$ ) and tRNA genes ( $n = 49$ ) are similar or equivalent to those of close relatives. Although a draft genome sequence for *Mycobacterium abscessus* subsp. *bolletii* BD<sup>T</sup> was previously determined (21), the number of predicted genes differed significantly from that obtained here. We also identified a 97.1-kb prophage that was longer than that seen in a previous study (63 kb) (21). The complete genome sequence of *M. abscessus* subsp. *bolletii* BD<sup>T</sup> represents essential data for future taxonomic and comparative genome studies.

**Accession number(s).** The chromosome sequence was deposited in DDBJ/ENA/GenBank under accession no. [AP018436](#).

## ACKNOWLEDGMENTS

This work was supported by a grant from the Japan Agency for Medical Research and Development/Japan International Cooperation Agency to Y. Hoshino and by a Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science (JSPS) to Y. Hoshino.

The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## REFERENCES

1. Namkoong H, Kurashima A, Morimoto K, Hoshino Y, Hasegawa N, Ato M, Mitarai S. 2016. Epidemiology of pulmonary nontuberculous mycobacterial disease, Japan. *Emerg Infect Dis* 22:1116–1117. <https://doi.org/10.3201/eid2206.151086>.
2. Bryant JM, Grogono DM, Rodriguez-Rincon D, Everall I, Brown KP, Moreno P, Verma D, Hill E, Drijkoningen J, Gilligan P, Esther CR, Noone PG, Giddings O, Bell SC, Thomson R, Wainwright CE, Coulter C, Pandey S, Wood ME, Stockwell RE, Ramsay KA, Sherrard LJ, Kidd TJ, Jabbour N, Johnson GR, Knibbs LD, Morawska L, Sly PD, Jones A, Bilton D, Laurenson I, Ruddy M, Bourke S, Bowler IC, Chapman SJ, Clayton A, Cullen M, Daniels T, Dempsey O, Denton M, Desai M, Drew RJ, Edenborough F, Evans J, Folb J, Humphrey H, Isalska B, Jensen-Fangel S, Jonsson B, Jones AM, et al. 2016. Emergence and spread of a human-transmissible multidrug-resistant nontuberculous mycobacterium. *Science* 354: 751–757. <https://doi.org/10.1126/science.aaf8156>.
3. Hoshino Y, Suzuki K. 2015. Differential diagnostic assays for discriminating mycobacteria, especially for nontuberculous mycobacteria: what does the future hold? *Future Microbiol* 10:205–216. <https://doi.org/10.2217/fmb.14.120>.
4. Thomson R, Tolson C, Carter R, Coulter C, Huygens F, Hargreaves M. 2013. Isolation of nontuberculous mycobacteria (NTM) from household water and shower aerosols in patients with pulmonary disease caused by NTM. *J Clin Microbiol* 51:3006–3011. <https://doi.org/10.1128/JCM.00899-13>.
5. Adekambi T, Sassi M, van Ingen J, Drancourt M. 2017. Reinstating *Mycobacterium massiliense* and *Mycobacterium bolletii* as species of the *Mycobacterium abscessus* complex. *Int J Syst Evol Microbiol* 67: 2726–2730. <https://doi.org/10.1099/ijsem.0.002011>.
6. Leao SC, Tortoli E, Euzéby JP, Garcia MJ. 2011. Proposal that *Mycobacterium massiliense* and *Mycobacterium bolletii* be united and reclassified as *Mycobacterium abscessus* subsp. *bolletii* comb. nov., designation of *Mycobacterium abscessus* subsp. *abscessus* subsp. nov. and emended description of *Mycobacterium abscessus*. *Int J Syst Evol Microbiol* 61: 2311–2313. <https://doi.org/10.1099/ijts.0.023770-0>.
7. Sekizuka T, Kai M, Nakamura K, Nakata N, Kazumi Y, Maeda S, Makino M, Hoshino Y, Kuroda M. 2014. Complete genome sequence and comparative genomic analysis of *Mycobacterium massiliense* JCM 15300 in the *Mycobacterium abscessus* group reveal a conserved genomic island MmGI-1 related to putative lipid metabolism. *PLoS One* 9:e114848. <https://doi.org/10.1371/journal.pone.0114848>.
8. Tortoli E, Kohl TA, Brown-Elliott BA, Trovato A, Cardoso-Leão S, Garcia MJ, Vasireddy S, Turenne CY, Griffith DE, Philley JV, Niemann S, Wallace RJ, Cirillo DM. 2017. *Mycobacterium abscessus*, a taxonomic puzzle. *Int J Syst Evol Microbiol*. <https://doi.org/10.1099/ijsem.0.002457>.
9. Tortoli E, Kohl TA, Brown-Elliott BA, Trovato A, Leão SC, Garcia MJ, Vasireddy S, Turenne CY, Griffith DE, Philley JV, Baldan R, Campana S, Cariati L, Colombo C, Tacchetti G, Teri A, Niemann S, Wallace RJ, Jr, Cirillo DM. 2016. Emended description of *Mycobacterium abscessus*, *Mycobacterium abscessus* subsp. *abscessus* and *Mycobacterium abscessus* subsp. *bolletii* and designation of *Mycobacterium abscessus* subsp. *massiliense* comb. Nov. *Int J Syst Evol Microbiol* 66:4471–4479. <https://doi.org/10.1099/ijsem.0.001376>.
10. Fukano H, Yoshida M, Katayama Y, Omatsu T, Mizutani T, Kurata O, Wada S, Hoshino Y. 2017. Complete genome sequence of *Mycobacterium stephanolepidis*. *Genome Announc* 5:e00810-17. <https://doi.org/10.1128/genomeA.00810-17>.
11. 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>.
12. 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:e01050-16. <https://doi.org/10.1128/genomeA.01050-16>.
13. 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>.
14. 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>.
15. 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>.
16. Yoshida M, Izumiya S, Fukano H, Sugiyama K, Suzuki M, Shibayama K, Hoshino Y. 2017. Draft genome sequence of *Mycobacterium* sp. strain shizuoka-1, a novel mycobacterium isolated from groundwater of a bathing facility in Shizuoka, Japan. *Genome Announc* 5:e01309-17. <https://doi.org/10.1128/genomeA.01309-17>.
17. 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>.
18. Auch AF, von Jan M, Klenk HP, Göker M. 2010. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. *Stand Genomic Sci* 2:117–134. <https://doi.org/10.4056/sigs.531120>.
19. Karlin S, Mrázek J, Campbell AM. 1997. Compositional biases of bacterial genomes and evolutionary implications. *J Bacteriol* 179:3899–3913. <https://doi.org/10.1128/jb.179.12.3899-3913.1997>.
20. Ripoll F, Pasek S, Schenowitz C, Dossat C, Barbe V, Rottman M, Macheras E, Heym B, Herrmann JL, Daffé M, Brosch R, Risler JL, Gaillard JL. 2009. Non mycobacterial virulence genes in the genome of the emerging pathogen *Mycobacterium abscessus*. *PLoS One* 4:e5660. <https://doi.org/10.1371/journal.pone.0005660>.
21. Choi GE, Cho YJ, Koh WJ, Chun J, Cho SN, Shin SJ. 2012. Draft genome sequence of *Mycobacterium abscessus* subsp. *bolletii* BD<sup>T</sup>. *J Bacteriol* 194:2756–2757. <https://doi.org/10.1128/JB.00354-12>.