



# Draft Genome Sequence of a *Mycobacterium* Strain Isolated from a Clinical Wound Sample

Eric M. Ransom,<sup>a,\*</sup>  Sanjam S. Sawhney,<sup>a,b</sup>  Gautam Dantas,<sup>a,b,c,d</sup>  Carey-Ann D. Burnham,<sup>a,c,e</sup>  Skye R. S. Fishbein<sup>a,b</sup>

<sup>a</sup>Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, Missouri, USA

<sup>b</sup>The Edison Family Center for Genome Sciences & Systems Biology, Washington University School of Medicine, St. Louis, Missouri, USA

<sup>c</sup>Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri, USA

<sup>d</sup>Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, Missouri, USA

<sup>e</sup>Departments of Pediatrics and Medicine, Washington University School of Medicine, St. Louis, Missouri, USA

**ABSTRACT** We report the draft genome sequence of an unusual *Mycobacterium* isolate recovered from a patient's arm tissue. The 4,025,753-bp draft genome exhibits a GC content of 71.02%, and a 16S rRNA gene analysis found that the closest relative was *Mycobacterium grossiae*.

**M**ycobacteria are aerobic, acid-fast, nonmotile, non-spore-forming bacilli, and some species, such as *Mycobacterium tuberculosis*, are important human pathogens. *Mycobacterium* other than tuberculosis (MOTTs), also called nontuberculous mycobacteria (NTMs), can also cause human disease. Recent changes to mycobacterial taxonomy include the subgenera *Mycolicibacterium*, *Mycolicibacter*, *Mycolicibacillus*, and *Mycobacteroides* (1); these contain over 180 species and are rapidly expanding.

A *Mycobacterium*-like isolate was recovered from arm tissue collected during surgical amputation following a motor vehicle accident. The isolate was detected after a 38-day incubation at 35°C in a Mycobacteria Growth Indicator Tube (MGIT) (Becton Dickinson). Clinical identification methods, including Bruker Biotyper and Vitek MS matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) systems, failed to provide a genus- or species-level identification (2). However, the isolate was acid fast and a scotochromogen (3). Subcultured growth on Middlebrook 7H10 agar in atmospheric air was observed at 30°C and 35°C after 3 days (rapid grower). The isolate was forwarded to the University of Texas at Tyler mycobacteriology laboratory for susceptibility testing and was pansusceptible at the lowest dilutions tested (Table 1) (4).

To characterize this isolate, it was cultured for whole-genome sequencing on Middlebrook 7H10 mycobacterial agar for 14 days at 30°C in O<sub>2</sub>. Colony growth was suspended in 1 mL of molecular biology-grade water. DNA was purified using the QIAamp BiOstic bacteremia DNA kit (Qiagen), followed by use of the Nextera XT library preparation kit (Illumina), and was sequenced using the NovaSeq 6000 sequencing system to acquire 2 × 150-bp paired-end reads. For all software used subsequently, default parameters were used unless otherwise specified. We generated 8,960,765 reads after quality filtering using Trimmomatic v0.36 (5). SPAdes v3.13.0 (6) was used for *de novo* assembly of a draft genome, and assembly quality was measured by QUAST (7). The draft genome was 4,025,753 bp, with a GC content of 71.02%, and consisted of 23 contigs, with an  $N_{50}$  value of 401,536 bp and coverage of 333×. Contigs of ≤500 bp were removed before assembly deposition. The genome was annotated with PGAP (8) and contained 3,946 coding sequences.

Using RNAmmer v1.2 (9), we isolated 16S rRNA gene sequences from the draft genome and 17 other actinomycete taxa for comparison, with pairwise identity determined using the EZBioCloud database (10). This analysis confirmed that the closest relative was *Mycobacterium grossiae*, with other species of the subgenus *Mycolicibacterium* having

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Address correspondence to Skye R. S. Fishbein, [skye.f@wustl.edu](mailto:skye.f@wustl.edu).

\*Present address: Eric M. Ransom, Department of Pathology, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, Ohio, USA.

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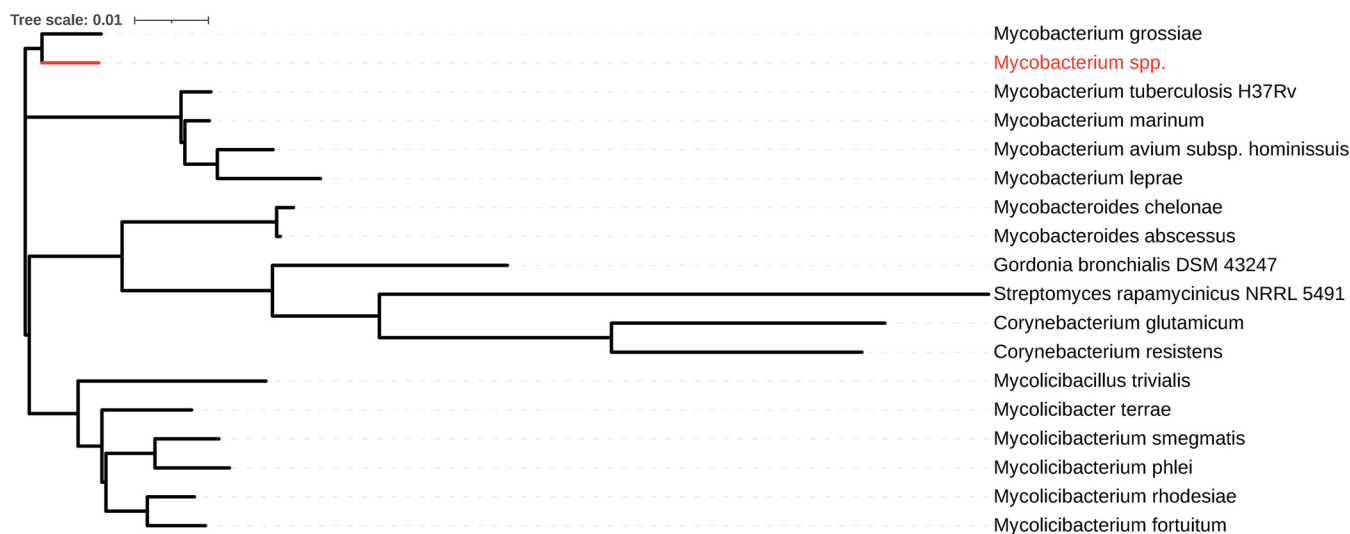
**TABLE 1** Antimicrobial susceptibility results for mycobacterial isolate

Antimicrobial agent	MIC ( $\mu\text{g}/\text{mL}$ )	CLSI interpretation <sup>a</sup>
Clarithromycin	$\leq 0.06$	S
Rifabutin	$\leq 0.25$	S
Moxifloxacin	$\leq 0.12$	S
Rifampin	$\leq 0.25$	S
Trimethoprim-sulfamethoxazole	$\leq 0.12/2.38$	S
Amikacin	$\leq 1$	S
Linezolid	$\leq 1$	S
Ciprofloxacin	$\leq 0.12$	S
Doxycycline	$\leq 0.12$	S
Bedaquiline	$\leq 0.001$	S
Clofazimine	$\leq 0.008$	S

<sup>a</sup> S, susceptible. Susceptibility testing and interpretative categories were according to CLSI document M24 (4).

>98% identity. We aligned the corresponding gene sequences using MUSCLE and constructed an approximate maximum likelihood tree using FastTree (11, 12). Phylogenetic analysis of 16S rRNA gene alignment revealed that this *Mycobacterium* genomospecies and *M. grossiae* formed a clade distinct from other well-known representative species of the four mycobacterial subgenera (Fig. 1). Previous phylogenetic analyses of NTMs clarified that *M. grossiae* grouped with the fast-growing and primarily environmental mycobacterial subgenus *Mycolicibacterium* (1), although *M. grossiae* remains unclassified by subgenus. Given the phenotypic characteristics of this *Mycobacterium* genomospecies and its genotypic attributes, it may represent a missing link between the *Mycobacterium* and *Mycolicibacterium* subgenera.

This study was performed with institutional review board (IRB) approval from Washington University in St. Louis (IRB approval number 202204102).



**FIG 1** Phylogenetic tree based on 16S rRNA gene alignment of representative species ( $n = 17$ ) from closely related *Actinobacterium* genera outside *Mycobacterium* and from each mycobacterial subgenus. The red text and node indicate the location of the isolate presented. The alignment was constructed using MUSCLE, and the approximate maximum likelihood tree was constructed with FastTree using the generalized time-reversible model. Default parameters were used for FastTree and MUSCLE. The following genomes served as sources for 16S rRNA gene sequences used in the alignment and tree: *Mycobacterium leprae* MRHRU-235-G (GenBank accession number [NZ\\_CP029543.1](#)), *Mycolicibacterium smegmatis* NCTC8159 (GenBank accession number [NZ\\_LN831039.1](#)), *Mycolicibacterium rhodesiae* DSM 44223 (GenBank accession number [NZ\\_MVIH01000038.1](#)), *Mycobacterium grossiae* DSM 104744 (GenBank accession number [NZ\\_CP043474.1](#)), *Mycolicibacterium phlei* NCTC8156 (GenBank accession number [NZ\\_UGQI01000001.1](#)), *Mycolicibacter terrae* NCTC10856 (GenBank accession number [NZ\\_LT906469.1](#)), *Mycobacterium marinum* MMA1 (GenBank accession number [NZ\\_CP058277.1](#)), *Streptomyces rapamycinicus* NRRL 5491 (GenBank accession number [NZ\\_QYCY01000001.1](#)), *Mycolicibacillus trivialis* DSM 44153 (GenBank accession number [NZ\\_LQPZ01000055.1](#)), *Corynebacterium glutamicum* T6-13 N\_25 (GenBank accession number [NZ\\_LOQW01000011.1](#)), *Mycobacterium avium* subsp. *hominissuis* OCU464 (GenBank accession number [NZ\\_CP009360.4](#)), *Mycobacteroides chelonae* CCUG 47445 (GenBank accession number [NZ\\_CP007220.1](#)), *Mycobacteroides abscessus* FLAC013 (GenBank accession number [NZ\\_CP014955.1](#)), *Mycolicibacterium fortuitum* CT6 (GenBank accession number [NZ\\_CP011269.1](#)), *Corynebacterium resistens* DSM 45100 (GenBank accession number [NC\\_015673.1](#)), *Gordonia bronchialis* DSM 43247 (GenBank accession number [NC\\_013441.1](#)), and *Mycobacterium tuberculosis* H37Rv (GenBank accession number [KK339370.1](#)).

**Data availability.** This whole-genome shotgun project for *Mycobacterium* sp. strain MYCO198283 has been deposited in GenBank under DDBJ/ENA/GenBank accession number [JAJQJI000000000](https://www.ncbi.nlm.nih.gov/nuccore/JAJQJI000000000), BioProject accession number [PRJNA759261](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA759261), BioSample accession number [SAMN21161762](https://www.ncbi.nlm.nih.gov/biosample/SAMN21161762), and SRA accession number [SRS11245207](https://www.ncbi.nlm.nih.gov/sra/SRS11245207).

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## REFERENCES

- 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>.
- Wilén CB, McMullen AR, Burnham CA. 2015. Comparison of sample preparation methods, instrumentation platforms, and contemporary commercial databases for identification of clinically relevant mycobacteria by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 53:2308–2315. <https://doi.org/10.1128/JCM.00567-15>.
- Runyon EH. 1959. Anonymous mycobacteria in pulmonary disease. *Med Clin North Am* 43:273–290. [https://doi.org/10.1016/S0025-7125\(16\)34193-1](https://doi.org/10.1016/S0025-7125(16)34193-1).
- Clinical and Laboratory Standards Institute. 2018. Susceptibility testing of mycobacteria, *Nocardia* spp., and other aerobic actinomycetes. CLSI document M24. Clinical and Laboratory Standards Institute, Wayne, PA.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19: 455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Zhao Y, Wu J, Yang J, Sun S, Xiao J, Yu J. 2012. PGAP: pan-genomes analysis pipeline. *Bioinformatics* 28:416–418. <https://doi.org/10.1093/bioinformatics/btr655>.
- Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 35:3100–3108. <https://doi.org/10.1093/nar/gkm160>.
- Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, Seo H, Chun J. 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 67:1613–1617. <https://doi.org/10.1099/ijsem.0.001755>.
- Edgar RC. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5:113. <https://doi.org/10.1186/1471-2105-5-113>.
- Price MN, Dehal PS, Arkin AP. 2010. FastTree 2: approximately maximum-likelihood trees for large alignments. *PLoS One* 5:e9490. <https://doi.org/10.1371/journal.pone.0009490>.