AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Draft Genome Sequence of *Mycobacterium simiae*, a Potential Pathogen Isolated from the Normal Human Oral Cavity

Varsha Chauhan,[a] Kamal Shrivastava,[a] Sakshi Anand,[a] Chanchal Kumar,[a] Anupriya Singh,[a] Mandira Varma-Basil[a]

[a]Department of Microbiology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India

ABSTRACT We report the draft genome sequence of *Mycobacterium simiae*, a slowly growing nontuberculous mycobacterium (NTM) isolated from a mouthwash sample of a healthy person. This genome of 6,603,693 bp exhibited a 66.13% GC content and 6,391 genes with 6,257 coding sequences, 3 rRNAs, and 78 tRNAs.

Mycobacterium simiae is a slow-growing, photochromogenic, nontuberculous mycobacterium (NTM), phylogenetically related to 18 other species included in the *M. simiae* complex group, including *M. triplex*, *M. genavense*, *M. heidelbergense*, *M. lentiflavum*, *M. sherrisii*, *M. parmense*, *M. stomatepiae*, and *M. florentinum* (1, 2). *M. simiae* was first isolated in 1965 from rhesus monkey (3). This organism is ubiquitous and is found in environmental sources, including soil and water (4, 5). *M. simiae* also frequently colonizes the lung and is not always considered a potential pathogen in immunocompetent individuals (2, 6). However, several studies have reported disseminated multidrug-resistant *M. simiae* infection in both immunocompetent (7–11) and immunocompromised patients (12, 13). *M. simiae* infection has been associated with infection in lungs (8), parotid glands (7), the skeletal and genitourinary systems (9), and blood and bone marrow (12).

Here, we report the draft genome sequence of *M. simiae* isolated from the mouthwash sample of a healthy person from Delhi, India. The study was approved by the institutional ethical committee (IEC number VPCI/DIR/PS/IEC/2018). The mouthwash sample was decontaminated using 4% NaOH to prevent the growth of normal oral commensals. The sample was inoculated on Löwenstein-Jensen (LJ) medium and incubated at 37°C for 3 to 4 weeks (14). The isolated organism was confirmed as acid fast by Ziehl-Neelsen staining and further identified as an NTM by duplex PCR assay (15). The isolated NTM was identified as *M. simiae* by Hain Lifescience's GenoType *Mycobacterium* CM/AS and by sequencing of the internal transcribed spacer (ITS) region. Whole-genome sequencing further confirmed the organism as *M. simiae*.

Genomic DNA from *M. simiae* was isolated using the cetyltrimethylammonium bromide (CTAB) DNA extraction method (16). Paired-end libraries were prepared using a NEBNext Ultra DNA library preparation kit. The sequencing was carried out on the Illumina Hi-Seq platform to generate 2 × 150-bp sequence reads at 100× sequencing depth. A total of 3,351,716 paired-end reads with an average length of 150 bp were recovered. The paired-end reads were quality controlled using FastQC v0.11.8 (17), filtered and trimmed by fastp v0.201 (18), and assembled with Shovill v1.0.4 (19). Default parameters were used for all software. A total of 3,256,162 paired-ends reads were obtained after filtering and trimming. Shovill assembled the reads in 123 contigs for a total assembly size of 6,603,693 bp with an N_{50} value of 189,271 bp. Annotation was carried out with Prokka v1.14.5 (20), which gave 6,259 coding sequences. The genome also carries 3 rRNAs and 78 tRNAs. The GC content of the genome is 66.13%. The average nucleotide identity (ANI) was calculated with DFAST taxonomic check (21). *Mycobacterium sherrisii* ATCC BAA-832 and *Mycobacterium triplex* DSM 44626 were the closest aligned sequenced genomes, with approximately 90% and 83.91% nucleotide

Citation Chauhan V, Shrivastava K, Anand S, Kumar C, Singh A, Varma-Basil M. 2020. Draft genome sequence of *Mycobacterium simiae*, a potential pathogen isolated from the normal human oral cavity. Microbiol Resour Announc 9:e01185-20. https://doi.org/10.1128/MRA.01185-20.

Editor Steven R. Gill, University of Rochester School of Medicine and Dentistry

Address correspondence to Mandira Varma-Basil, mandirav@rediffmail.com.

Received 14 October 2020
Accepted 16 October 2020
Published 12 November 2020

identity, respectively. *M. triplex* is also a member of the *M. simiae* complex and known as a potential pathogenic species for human beings (1, 4).

The genome sequence of *M. simiae* presented here is from an isolate obtained from a mouthwash sample, representing essential data for future phylogenetic and comparative genome studies; thus, it will be useful for better understanding the *M. simiae* complex.

Data availability. The whole-genome sequence of *M. simiae* was deposited at GenBank under the BioProject accession number PRJNA647829 (SRA accession number SRR12285193).

ACKNOWLEDGMENTS

This work was funded by the Indian Council of Medical Research (ICMR/5/8/5/22/2019 ECD-I). K.S. (5/3/8/6/ITR-F/2018-ITR), C.K., and A.S. (ICMR/5/8/5/22/2019 ECD-I) acknowledge the ICMR for providing fellowships.

REFERENCES

1. Sassi M, Robert C, Raoult D, Drancourt M. 2013. Non-contiguous genome sequence of *Mycobacterium simiae* strain DSM 44165 T. Stand Genomic Sci 8:306–317. https://doi.org/10.4056/sigs.3707349.
2. Hamieh A, Tayyar R, Tabaja H, E L Zein S, Bou Khalil P, Kara N, Kanafani ZA, Kanj N, Bou Akl I, Araj G, Berjaoui G, Kanj SS. 2018. Emergence of *Mycobacterium simiae*: a retrospective study from a tertiary care center in Lebanon. PLoS One 13:e0195390. https://doi.org/10.1371/journal.pone.0195390.
3. Karassova V, Weissfeiler J, Krasznay E. 1965. Occurrence of atypical mycobacteria in *Macacus rhesus*. Acta Microbiol Acad Sci Hung 12:275–282.
4. Makovcova J, Slany M, Babak V, Slana I, Kralik P. 2014. The water environment as a source of potentially pathogenic mycobacteria. J Water Health 12:254–263. https://doi.org/10.2166/wh.2013.102.
5. Mishra PS, Narang P, Narang R, Goswami B, Mendiratta DK. 2018. Spatio-temporal study of environmental nontuberculous mycobacteria isolated from Wardha district in Central India. Antonie Van Leeuwenhoek 111:73–87. https://doi.org/10.1007/s10482-017-0927-2.
6. Steffani-Vallejo JL, Brunck ME, Acosta-Cruz EY, Montiel R, Barona-Gómez F. 2018. Genomic insights into *Mycobacterium simiae* human colonization. Stand Genomic Sci 13:1. https://doi.org/10.1186/s40793-017-0291-x.
7. Hankins D, Kelly M, Vijayan V. 2013. *Mycobacterium simiae* infection of the parotid gland in an immunocompetent child. J Pediatric Infect Dis Soc 2:394–396. https://doi.org/10.1093/jpids/pis098.
8. Jeong SH, Kim SY, Lee H, Ham JS, Hwang KB, Hwang S, Shin SH, Chung MJ, Lee SH, Shin SJ, Koh WJ. 2015. Nontuberculous mycobacterial lung disease caused by *mycobacterium simiae*: the first reported case in South Korea. Tuberc Respir Dis 78:432–435. https://doi.org/10.4046/trd.2015.78.4.432.
9. Javeri H, Vélez-Mejía C, Cadena J. 2018. Disseminated *Mycobacterium simiae* infection in a non-immunosuppressed patient in the USA. ID Cases 11:58–60. https://doi.org/10.1016/j.idcr.2017.11.010.
10. Patel NC, Minifee PK, Dishop MK, Munoz FM. 2007. *Mycobacterium simiae* cervical lymphadenitis. Pediatr Infect Dis J 26:362–363. https://doi.org/10.1097/01.inf.0000258614.98241.4e.
11. Cruz AT, Goytia VK, Starke JR. 2007. *Mycobacterium simiae* complex infection in an immunocompetent child. J Clin Microbiol 45:2745–2746. https://doi.org/10.1128/JCM.00359-07.
12. Al-Abdely HM, Revankar SG, Graybill JR. 2000. Disseminated *Mycobacterium simiae* infection in patients with AIDS. J Infect 41:143–147. https://doi.org/10.1053/jinf.2000.0700.
13. Salas NM, Byrd TF. 2020. Granulomatous interstitial nephritis in the setting of disseminated *Mycobacterium simiae:* a rare presentation of immune reconstitution inflammatory syndrome. Int J STD AIDS 31:911–913. https://doi.org/10.1177/0956462420926881.
14. Kent PT, Kubica GP. 1985. Public health mycobacteriology: a guide for the level III laboratory. Centers for Disease Control and Prevention, Atlanta, GA.
15. Shrivastava K, Garima K, Narang A, Bhattacharyya K, Vishnoi E, Singh RK, Chaudhry A, Prasad R, Bose M, Varma-Basil M. 2017. Rv1458c: a new diagnostic marker for identification of *Mycobacterium tuberculosis* complex in a novel duplex PCR assay. J Med Microbiol 66:371–376. https://doi.org/10.1099/jmm.0.000440.
16. Van Soolingen DI, Hermans PW, De Haas PE, Soll DR, Van Embden JD. 1991. Occurrence and stability of insertion sequences in *Mycobacterium tuberculosis* complex strains: evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. J Clin Microbiol 29:2578–2586. https://doi.org/10.1128/JCM.29.11.2578-2586.1991.
17. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.
18. Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890. https://doi.org/10.1093/bioinformatics/bty560.
19. Seeman T. 2018. Shovill: faster SPAdes assembly of Illumina reads (v0.9.0). https://github.com/tseemann/shovill.
20. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
21. Tanizawa Y, Fujisawa T, Nakamura Y. 2018. DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. Bioinformatics 34:1037–1039. https://doi.org/10.1093/bioinformatics/btx713.