

Draft Genome Sequence of *Mycobacterium tuberculosis* Strain SNMICRO 2047-20, Isolated from Intraocular Infection

Mani Vimalin Jeyalatha,^a Umashankar Vetrivel,^{b,c} Rajagopalan Harinee,^d Dhanurekha Lakshmiathy,^d Suganeswari Ganesan,^e Jyotirmay Biswas,^f  Appakkudal R. Anand^d

^aL & T Microbiology Research Centre, Vision Research Foundation, Sankara Nethralaya, Chennai, India

^bICMR-National Institute of Traditional Medicine, Indian Council of Medical Research, Belagavi, India

^cVirology and Biotechnology/Bioinformatics Division, ICMR-National Institute for Research in Tuberculosis, Chennai, India

^dL & T Microbiology Research Centre, Medical Research Foundation, Sankara Nethralaya, Chennai, India

^eShri Bhagwan Mahavir Vitreoretinal Services, Medical Research Foundation, Sankara Nethralaya, Chennai, India

^fDepartment of Uveitis and Ocular Pathology, Medical Research Foundation, Sankara Nethralaya, Chennai, India

ABSTRACT Here, we communicate the draft genome sequence of an ocular *Mycobacterium tuberculosis* strain (SNMICRO 2047-20) that was isolated from the vitreous fluid of a patient diagnosed with endophthalmitis. The genome sequence was 4,391,538 bp long with 3,898 protein-encoding genes and clustered to the East African-Indian lineage.

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* and continues to be a major public health concern (1, 2). Ocular tuberculosis (TB) is an extrapulmonary form that is challenging to diagnose due to a wide spectrum of presentations. Here, we report the coding draft genome sequences of an *M. tuberculosis* strain isolated from the eye. This study was approved by the institutional ethics committee of the Medical and Vision Research Foundation (854-20209-P). The *M. tuberculosis* strain (SNMICRO 2047-20) was grown in Middlebrook 7H9 medium using a Bactec MGIT 320 system (Becton, Dickinson) and isolated from the vitreous fluid of a 44-year female who was clinically suspected to have endogenous endophthalmitis, after 14 days of incubation. The strain was found to be sensitive to all first-line drugs tested, namely, streptomycin, isoniazid, rifampin, ethambutol, and pyrazinamide. For whole-genome sequencing (WGS), the genomic DNA was extracted using a QIAampDNA mini kit (Qiagen, Hilden, Germany). The WGS libraries were prepared using a Kapa High Throughput library preparation kit (Roche, CA), loaded onto a cBot2 system for cluster generation, and sequenced using an Illumina HiSeqXten sequencer.

The generated paired-end reads were trimmed to remove the low-quality reads and adapters using the Trimgalore 0.6.3 tool (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). The filtered reads (mean length, 150 bp) were aligned and mapped to the *Mycobacterium tuberculosis* H37Rv reference genome (accession number NC_000962.3) using the SNIPPY 4.5.0 pipeline (<https://github.com/tseemann/snippy>) with default optimal parameters, single nucleotide polymorphism (SNP) calling, and consensus generation. Furthermore, the mapped reads were also analyzed using the Tb profiler 3.0.8 tool with default settings to infer the lineage and drug sensitivity profile. Also, the consensus-derived genomic sequences were annotated using the RAST server (3), and the coding sequence distribution was plotted using Circos software (4).

The Illumina HiSeq platform generated 992,862,146 bp of paired-end reads which on adapter trimming yielded 974,882,690 bp of high-quality reads, and the SNIPPY run generated 46 contigs with a total length of 4,391,538 bp (N_{50} , 179,591 bp) (Table 1). The sequencing depth was found to be 221.8× with 99.4% genome coverage. On PGAP annotation of the 46 contigs (<http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html>), 3,898 protein-coding genes, 51 RNA-coding genes, and 3 clustered regularly interspaced short palindromic repeat

Editor Julie C. Dunning Hotopp, University of Maryland School of Medicine

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Address correspondence to Appakkudal R. Anand, aranand@gmail.com.

The authors declare no conflict of interest.

Received 2 August 2022

Accepted 28 November 2022

Published 21 December 2022

TABLE 1 Genomic features of *Mycobacterium tuberculosis* strain SNMICRO 2047-20

Parameter	Data
Lineage	3
Specimen	Vitreous aspirate
Reference-guided assembly no.	NC_000962.3
NCBI accession no.	JAHTBJ000000000
Genome size	4,391,538
No. of contigs	46
Total no. of genes	4,100
No. of protein-encoding genes	3,898
No. of CRISPR arrays	3
No. of ribosomal RNAs	3
No. of tRNAs	45
No. of noncoding RNAs	3
No. of pseudogenes	151
G+C content (%)	65.6
Genome coverage (×)	221.8
Total no. of phages	3

(CRISPR) arrays were predicted to span the draft genome sequence. TB profiler reported the classical regions of difference (RD) 750 deletion in this strain, and it was predicted to cluster with the East African-Indian family belonging to CAS lineage 3.

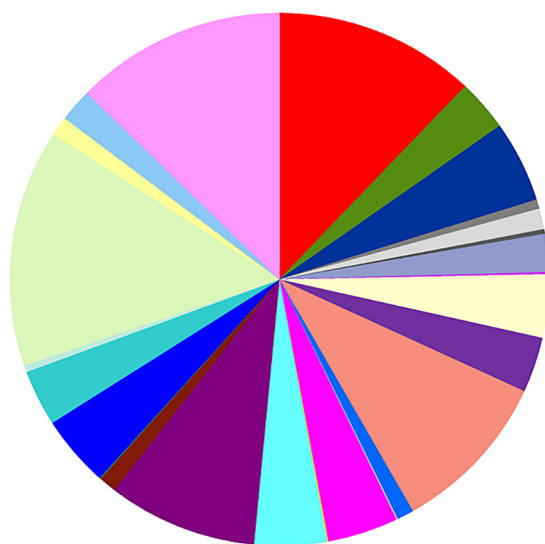
The RAST analysis revealed 405 subsystems (Fig. 1). A majority of the genes were involved in the synthesis of amino acids and derivatives ($n = 356$); carbohydrates (313); cofactors, vitamins, prosthetic groups, and pigments (299); protein metabolism (243); and virulence, disease, and defense (122). Notably, the strain also possesses all five T7 secretory systems (ESX 1 to 5). The draft genome sequence will provide important insights into factors involved in the evolution and adaptation of *M. tuberculosis* to establish infections at extrapulmonary sites, such as the eye.

Data availability. The draft genome sequence data of *M. tuberculosis* SNMICRO 2047-20 was submitted to NCBI GenBank under BioProject accession number [PRJNA742522](#), GenBank accession number [JAHTBJ000000000](#), and SRA accession number [SRR19090886](#).

System Coverage



Subsystem Category Distribution



Subsystem Feature Counts

- Cofactors, Vitamins, Prosthetic Groups, pigments (299)
- Cell Wall and Capsule (77)
- Virulence, Disease and Defense (122)
- Potassium metabolism (14)
- Photosynthesis (0)
- Miscellaneous (31)
- Phages, Prophages, Transposable elements, Plasmids (7)
- Membrane Transport (57)
- Iron acquisition and metabolism (3)
- RNA Metabolism (95)
- Nucleosides and Nucleotides (83)
- Protein Metabolism (244)
- Cell Division and Cell Cycle (25)
- Motility and Chemotaxis (2)
- Regulation and Cell signaling (105)
- Secondary Metabolism (2)
- DNA Metabolism (107)
- Fatty Acids, Lipids and Isoprenoids (222)
- Nitrogen Metabolism (26)
- Dormancy and Sporulation (2)
- Respiration (107)
- Stress Response (84)
- Metabolism of Aromatic Compounds (10)
- Amino Acids and Derivatives (356)
- Sulfur Metabolism (29)
- Phosphorus Metabolism (49)
- Carbohydrates (313)

FIG 1 RAST annotation showing subsystem distribution of *Mycobacterium tuberculosis* strain SNMICRO 2047-20; a total of 405 subsystems were classified, of which 122 subsystems were confined to virulence and disease.

ACKNOWLEDGMENTS

The support received from Indian Council of Medical Research (ICMR), New Delhi, India, is duly acknowledged.

We declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

This work was supported by ICMR (grant number OMI/22/2020-ECD-1).

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