

# Draft Genome Sequence of a Multidrug-Resistant Clinical Isolate of *Mycobacterium tuberculosis* Belonging to a Novel Spoligotype

Shamsudheen Karuthedath Vellarikkal,<sup>a</sup> Ajay Vir Singh,<sup>c</sup> Pravin Kumar Singh,<sup>c</sup> Parul Garg,<sup>c</sup> Viswa Mohan Katoch,<sup>c</sup> Kiran Katoch,<sup>c</sup> Open Source Drug Discovery Consortium,<sup>d</sup> D. S. Chauhan,<sup>c</sup> Sridhar Sivasubbu,<sup>a</sup> Vinod Scaria<sup>b</sup>

Genomics and Molecular Medicine, CSIR Institute of Genomics and Integrative Biology, Delhi, India<sup>a</sup>; GN Ramachandran Knowledge Center for Genome Informatics, CSIR Institute of Genomics and Integrative Biology, Delhi, India<sup>b</sup>; National JALMA Institute of Leprosy and other Mycobacterial Diseases, Tajganj, Agra, India<sup>c</sup>; CSIR Open Source Drug Discovery Unit, Anusandhan Bhavan, New Delhi, India<sup>d</sup>

**We describe the genome sequencing and analysis of a multidrug-resistant (MDR) clinical isolate of *Mycobacterium tuberculosis*, strain OSDD105 from India, belonging to a novel spoligotype.**

Received 14 October 2013 Accepted 14 October 2013 Published 21 November 2013

**Citation** Vellarikkal SK, Singh AV, Singh PK, Garg P, Katoch VM, Katoch K, Open Source Drug Discovery Consortium, Chauhan DS, Sivasubbu S, Scaria V. 2013. Draft genome sequence of a multidrug-resistant clinical isolate of *Mycobacterium tuberculosis* belonging to a novel spoligotype. *Genome Announc.* 1(6):e00965-13. doi:10.1128/genomeA.00965-13.

**Copyright** © 2013 Vellarikkal et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](http://creativecommons.org/licenses/by/3.0/).

Address correspondence to Vinod Scaria, vinods@igib.in, or Sridhar Sivasubbu, s.sivasubbu@igib.res.in.

Tuberculosis is caused by a closely related group of pathogenic species encompassing the *Mycobacterium tuberculosis* complex. The strains are generally characterized by their distinct spoligotype patterns and grouped into seven major lineages (1). Spoligotype patterns are assayed based on repeat loci in the genome and have been extensively studied in relation to strain lineage, geographical distribution, evolution, virulence, and drug sensitivity. Distinct spoligotypes, including novel spoligotypes, have been previously shown to be associated with specific resistance phenotypes (2–4). Genome sequences of representative members of major genogroups have been reported recently (5–9). The availability of genome sequences of novel spoligotypes would offer a novel opportunity to understand the genome architecture and diversity of these strains and would provide insights into their phenotypic properties. In the present article, we describe the draft genome sequence of a multidrug-resistant (MDR) clinical isolate of *Mycobacterium tuberculosis*, OSDD105, belonging to a novel spoligotype pattern (77773777620000), closely clustering with the Euro-American genogroup.

The clinical isolate OSDD105 was obtained from the strain repository maintained at the National JALMA Institute of Leprosy and other Mycobacterial Diseases, Agra, and was maintained as a part of the Open Source Drug Discovery Open Access Repository. Spoligotyping analysis was performed and drug sensitivity was evaluated per standard protocols (10–12). Analysis revealed that the strain belonged to a novel spoligotype closely clustering to the T2 spoligotype of the Euro-American lineage, with a Spotclust (13) probability of 0.99. Drug sensitivity analysis revealed the isolate to be resistant to streptomycin, rifampin, isoniazid, ethambutol, cycloserine, and pyrazinamide and sensitive to ofloxacin, kanamycin, ethionamide, amikacin, capreomycin, and *para*-aminosalicylate sodium (PAS). DNA was isolated per standard protocols. The raw sequence data were generated after library preparation on Ion Torrent PGM per protocols recommended by manufacturers. Draft genomes were assembled *de novo* using CLC Genomics Workbench 6. The assembly resulted in 181 contigs at

$N_{50}$  values of 40,206 bp and a total assembly of 4,267,020 bp. Further, automated gene prediction on the draft genomes was performed using the RAST server (14). Analysis revealed 4,760 genes, including 48 RNA genes.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession [AUXD000000000](http://www.ncbi.nlm.nih.gov/nuccore/AUXD000000000). The version described in this paper is version AUXD02000000.

## ACKNOWLEDGMENTS

We thank Swati Subodh (TCGA) and Nupur Mehrotra (Premas Biotech) for scientific discussions and help in maintaining the strains. We also acknowledge S. Ramachandran for valuable input.

The project was funded by CSIR India through the Open Source Drug Discovery Programme (HCP001). The sequencing facility is supported through an FAC002 and OLP1105 grant from CSIR, India, and the computational analysis was performed at the CSIR Center for *In Silico* Biology at CSIR-IGIB.

## REFERENCES

1. Brudey K, Driscoll JR, Rigouts L, Prodinge WM, Gori A, Al-Hajj SA, Allix C, Aristimuño L, Arora J, Baumanis V, Binder L, Cafrune P, Cataldi A, Cheong S, Diel R, Ellermeier C, Evans JT, Fauville-Dufaux M, Ferdinand S, Garcia de Viedma D, Garzelli C, Gazzola L, Gomes HM, Guttierrez MC, Hawkey PM, van Helden PD, Kadiwal GV, Kreiswirth BN, Kremer K, Kubin M, Kulkarni SP, Liens B, Lillebaek T, Ho ML, Martin C, Martin C, Mokrousov I, Narvskaja O, Ngew YF, Naumann L, Niemann S, Parwati I, Rahim Z, Rasolofon-Razanamparany V, Rasolonavalona T, Rossetti ML, Rüsche-Gerdes S, Sajduda A, Samper S, Shemyakin IG, Singh UB, Somoskovi A, Skuce RA, van Soolingen D, Streicher EM, Suffys PN, Tortoli E, Tracevska T, Vincent V, Victor TC, Warren RM, Yap SF, Zaman K, Portaels F, Rastogi N, Sola C. 2006. *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. *BMC Microbiol.* 6:23.
2. Bazira J, Asiimwe BB, Joloba ML, Bwanga F, Matee MI. 2011. *Mycobacterium tuberculosis* spoligotypes and drug susceptibility pattern of isolates from tuberculosis patients in South-Western Uganda. *BMC Infect. Dis.* 11:81.
3. Yeboah-Manu D, Asante-Poku A, Bodmer T, Stucki D, Koram K,

- Bonsu F, Pluschke G, Gagneux S. 2011. Genotypic diversity and drug susceptibility patterns among *M. tuberculosis* complex isolates from South-Western Ghana. PLoS One 6:e21906. doi:10.1371/journal.pone.0021906.
4. Mokrousov I, Vyazovaya A, Otten T, Zhuravlev V, Pavlova E, Tarashkevich L, Krishevich V, Vishnevsky B, Narvskaya O. 2012. *Mycobacterium tuberculosis* population in northwestern Russia: an update from Russian-EU/Latvian border region. PLoS One 7:e41318. doi:10.1371/journal.pone.0041318.
  5. Miyoshi-Akiyama T, Matsumura K, Iwai H, Funatogawa K, Kirikae T. 2012. Complete annotated genome sequence of *Mycobacterium tuberculosis* Erdman. J. Bacteriol. 194:2770.
  6. Narayanan S, Deshpande U. 2013. Whole-genome sequences of four clinical isolates of *Mycobacterium tuberculosis* from Tamil Nadu, south India. Genome Announc. 1(3):e00186-13. doi:10.1128/genomeA.00186-13.
  7. Karuthedath Vellarikkal S, Patowary A, Singh M, Periwal V, Singh AV, Singh PK, Garg P, Mohan Katoch V, Katoch K, Jangir PK, Sharma R, Open Source Drug Discovery Consortium, Chauhan DS, Scaria V, Sivasubbu S. 2013. Draft genome sequence of a clinical isolate of multidrug-resistant *Mycobacterium tuberculosis* East African Indian strain OSDD271. Genome Announc. 1(4):e00541-13. doi:10.1128/genomeA.00541-13.
  8. Karuthedath Vellarikkal S, Vir Singh V, Kumar Singh P, Garg P, Mohan Katoch V, Katoch K, Open Source Drug Discovery Consortium, Chauhan DS, Scaria V, Sivasubbu S. 2013. Draft genome sequence of an extensively drug-resistant *Mycobacterium tuberculosis* clinical isolate of the Ural strain OSDD493. Genome Announc. 1(6):e00928-13. doi:10.1128/genomeA.00928-13.
  9. Zhang Y, Chen C, Liu J, Deng H, Pan A, Zhang L, Zhao X, Huang M, Lu B, Dong H, Du P, Chen W, Wan K. 2011. Complete genome sequences of *Mycobacterium tuberculosis* strains CCDC5079 and CCDC5080, which belong to the Beijing family. J. Bacteriol. 193: 5591–5592.
  10. Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, Bunschoten A, Molhuizen H, Shaw R, Goyal M, van Embden J. 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J. Clin. Microbiol. 35: 907–914.
  11. National Committee for Clinical Laboratory Standards. 2002. Susceptibility testing of mycobacteria, *Nocardia*, and other aerobic actinomycetes, 2nd ed. Tentative standard M24T2. National Committee for Clinical Laboratory Standards, Wayne, PA.
  12. Canetti G, Fox W, Khomeiko A, Mahler HT, Menon NK, Mitchison DA, Rist N, Smelev NA. 1969. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. Bull. World Health Organ. 41:21–43.
  13. Vitol I, Driscoll J, Kreiswirth B, Kurepina N, Bennett KP. 2006. Identifying *Mycobacterium tuberculosis* complex strain families using spoligo-types. Infect. Genet. Evol. 6:491–504.
  14. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:1471–2164.