


# Complete Genome Sequence of the *Mycobacterium immunogenum* Type Strain CCUG 47286

Daniel Jaén-Luchoro,<sup>a</sup> Carolina Seguí,<sup>a</sup> Francisco Aliaga-Lozano,<sup>a,b</sup> Francisco Salvà-Serra,<sup>a,c,d</sup> Antonio Busquets,<sup>a</sup> Margarita Gomila,<sup>a</sup> Antonio Ramírez,<sup>e</sup> Mikel Ruiz,<sup>c</sup>  Edward R. B. Moore,<sup>c,d</sup> Jorge Lalucat,<sup>a</sup>  Antoni Bennisar-Figueras<sup>a</sup>

Microbiologia, Departament de Biologia, Universitat de les Illes Balears, Palma de Mallorca, Illes Balears, Spain<sup>a</sup>; Microbiologia, Clínica Rotger, Palma de Mallorca, Illes Balears, Spain<sup>b</sup>; Culture Collection University of Gothenburg (CCUG), Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden<sup>c</sup>; Department of Infectious Diseases, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden<sup>d</sup>; Servicio de Microbiología, Hospital Universitari Son Espases, Palma de Mallorca, Illes Balears, Spain<sup>e</sup>

**Here, we report the complete genome sequence of *Mycobacterium immunogenum* type strain CCUG 47286, a nontuberculous mycobacterium. The whole genome has 5,573,781 bp and covers as many as 5,484 predicted genes. This genome contributes to the task of closing the still-existing gap of genomes of rapidly growing mycobacterial type strains.**

Received 7 April 2016 Accepted 12 April 2016 Published 26 May 2016

**Citation** Jaén-Luchoro D, Seguí C, Aliaga-Lozano F, Salvà-Serra F, Busquets A, Gomila M, Ramírez A, Ruiz M, Moore ERB, Lalucat J, Bennisar-Figueras A. 2016. Complete genome sequence of the *Mycobacterium immunogenum* type strain CCUG 47286. *Genome Announc* 4(3):e00401-16. doi:10.1128/genomeA.00401-16.

**Copyright** © 2016 Jaén-Luchoro et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Antoni Bennisar-Figueras, [toni.bennisar@uib.es](mailto:toni.bennisar@uib.es).

Nosocomial infections with nontuberculous mycobacteria (NTM) have acquired an important role due to the inherent clinical problems caused by several of its members. Among these bacteria, *Mycobacterium immunogenum* is a nonpigmented and rapidly growing mycobacterium (RGM). Strain CCUG 47286<sup>T</sup> (= CIP 106684<sup>T</sup> = MC 779<sup>T</sup>), isolated from waterborne contamination of a bronchoscope washer in St. Louis, MO, USA, was described as a new species closely related to *Mycobacterium abscessus* and *Mycobacterium chelonae* (1).

Several strains identified as *M. immunogenum* stand out for their ubiquity and implications in nosocomial infections (2), including pseudo-outbreaks in bronchoalveolar lavage procedures (3), skin infections (4), mesotherapy treatment infections (5), and hypersensitivity pneumonitis (6). In 2006, the first keratitis outbreak associated with *M. immunogenum* was reported (3). Although NTM are not commonly associated with disseminated infections, in 2012, the first reported case was published of a patient with septic shock derived from a disseminated infection of *M. immunogenum* (2). Additionally, its high resistance to antibiotics (1) and capability to grow and disseminate through water system suppliers (7) make *M. immunogenum* an important opportunistic pathogen of the NTM group.

Bulk genomic DNA (gDNA) was prepared from *M. immunogenum* CCUG 47286<sup>T</sup> using a protocol optimized for mycobacteria, combining the Wizard genomic DNA purification kit (Promega, Spain) and mechanical disruption with Disruptor Genie (Scientific Industries, Inc., USA). The gDNA was used for paired-end (PE) library preparation with the Illumina genomic Nextera XT kit, followed by sequencing on a HiSeq 2500 platform. The genome was also sequenced with a Pacific Biosciences (PacBio) RSII single-molecule real-time (SMRT) platform. PacBio 10-kb SMRTbell libraries were prepared, according to the protocols of the manufacturer.

A hybrid Illumina-PacBio *de novo* assembly protocol was applied to obtain a highly continuous genome. Briefly, high-quality

Illumina reads were obtained by filtering and enhancement with BMap version 35.34 (<http://sourceforge.net/projects/bbmap>). The ABySS software, version 1.5.1 (8), was used for an initial *de novo* assembly. The initial contigs and 61,476 PacBio reads (average read length, 2,677 bp), after being processed and filtered using the SMRT Analysis pipeline version 2.2, were aligned with BLASR (9). This alignment was used to estimate the orientation, arrangement, and distances between contigs (10). The gap regions were partially closed by iteratively passing 2,863,242 PE (100-bp) Illumina reads (11). A BLAST database was generated from contigs previously assembled using Velvet version 1.1.04 (12) and high-quality 100-bp PE Illumina reads, at 50× coverage. This searchable database was used to confirm and close the remaining gaps and resolve conflicting assembled regions. The fully closed genome had a final coverage of 125×.

The *M. immunogenum* genome has 5,573,781 bp and 64.3% G+C content. Two complete ribosomal operons and 61 tRNAs were detected after annotation with the Prokaryotic Genome Annotation Pipeline (PGAP) of the NCBI (13). After RAST analysis (14), from the predicted 5,484 coding sequences (CDSs), 1,825 (34%) were included in 407 subsystems. Among them, 109 CDSs were related to subsystems associated with virulence, disease, and defense; 33 were related to resistance to antibiotics and toxic compounds; and 79 were related to invasion and intracellular resistance processes. Additionally, two potential toxin-antitoxin systems were detected.

**Nucleotide sequence accession number.** The complete genome sequence of *M. immunogenum* type strain CCUG 47286 has been deposited in DDBJ/ENA/GenBank under the accession no. [CP011530](https://doi.org/10.1128/genomeA.00401-16). The version described in this paper is the first version.

## ACKNOWLEDGMENTS

This work was supported by the Spanish MINECO through projects CGL2012-39604 (with FEDER cofunding). M.G. was supported by a post-doctoral contract from the Conselleria d' Educació, Cultura i Universitats

del Govern de les Illes Balears and the European Social Fund. F.S.-S. was supported by a stipend for Basic and Advanced Research from the CCUG, through the Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg.

## FUNDING INFORMATION

This work, including the efforts of Antonio Bennisar-Figueras, was funded by Ministerio de Economía y Competitividad (MINECO) (CGL2012-39604).

## REFERENCES

1. Wilson RW, Steingrube VA, Böttger EC, Springer B, Brown-Elliott BA, Vincent V, Jost KC, Zhang Y, Garcia MJ, Chiu SH, Onyi GO, Rossmoore H, Nash DR, Wallace RJ. 2001. *Mycobacterium immunogenum* sp. nov., a novel species related to *Mycobacterium abscessus* and associated with clinical disease, pseudo-outbreaks and contaminated metalworking fluids: an international cooperative study on mycobacterial taxonomy. *Int J Syst Evol Microbiol* 51:1751–1764. <http://dx.doi.org/10.1099/00207713-51-5-1751>
2. Biggs HM, Chudgar SM, Pfeiffer CD, Rice KR, Zaas AK, Wolfe CR. 2012. Disseminated *Mycobacterium immunogenum* infection presenting with septic shock and skin lesions in a renal transplant recipient. *Transpl Infect Dis* 14:415–421. <http://dx.doi.org/10.1111/j.1399-3062.2012.00730.x>
3. Sampaio JLM, Junior DN, de Freitas D, Höfling-Lima AL, Miyashiro K, Alberto FL, Leão SC. 2006. An outbreak of keratitis caused by *Mycobacterium immunogenum*. *J Clin Microbiol* 44:3201–3207. <http://dx.doi.org/10.1128/JCM.00656-06>
4. Shedd AD, Edhegard KD, Lugo-Somolinos A. 2010. *Mycobacterium immunogenum* skin infections: two different presentations. *Int J Dermatol* 49:941–944. <http://dx.doi.org/10.1111/j.1365-4632.2009.04363.x>
5. del-Castillo M, Palmero DJ, Lopez B, Paul R, Ritacco V, Bonvehi P, Clara L, Ambroggi M, Barrera L, Vay C. 2009. Mesotherapy-associated outbreak caused by *Mycobacterium immunogenum*. *Emerg Infect Dis* 15:357–359. <http://dx.doi.org/10.3201/eid1502.081125>
6. Wallace RJ, Jr, Zhang Y, Wilson RW, Mann L, Rossmoore H. 2002. Presence of a single genotype of the newly described species *Mycobacterium immunogenum* in industrial metalworking fluids associated with hypersensitivity pneumonitis. *Appl Environ Microbiol* 68:5580–5584. <http://dx.doi.org/10.1128/AEM.68.11.5580-5584.2002>
7. De Groote MA, Huitt G. 2006. Infections due to rapidly growing mycobacteria. *Clin Infect Dis* 42:1756–1763. <http://dx.doi.org/10.1086/504381>
8. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJM, Birol I. 2009. ABySS: a parallel assembler for short read sequence data. *Genome Res* 19:1117–1123. <http://dx.doi.org/10.1101/gr.089532.108>
9. Chaisson MJ, Tesler G. 2012. Mapping single molecule sequencing reads using basic local alignment with successive refinement (BLASR): application and theory. *BMC Bioinformatics* 13:238. <http://dx.doi.org/10.1186/1471-2105-13-238>
10. Boetzer M, Pirovano W. 2014. SSPACE-LongRead: scaffolding bacterial draft genomes using long read sequence information. *BMC Bioinformatics* 15:211. <http://dx.doi.org/10.1186/1471-2105-15-211>
11. Boetzer M, Pirovano W. 2012. Toward almost closed genomes with Gap-Filler. *Genome Biol* 13:R56. <http://dx.doi.org/10.1186/gb-2012-13-6-r56>
12. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>
13. Tatusova T, Ciuffo S, Federhen S, Fedorov B, McVeigh R, O'Neill K, Tolstoy I, Zaslavsky L. 2015. Update on RefSeq microbial genomes resources. *Nucleic Acids Res* 43:D599–D605. <http://dx.doi.org/10.1093/nar/gku1062>
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* 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>