



Complete Genome Sequence of *Streptomyces lunaelactis* MM109^T, Isolated from Cave Moonmilk Deposits

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ABSTRACT *Streptomyces lunaelactis* MM109^T is a ferroverdin A (anticholesterol) producer isolated from cave moonmilk deposits. The complete genome sequence of MM109^T was obtained by combining Oxford Nanopore MinION and Illumina HiSeq and MiSeq technologies, revealing an 8.4-Mb linear chromosome and two plasmids, pSLUN1 (127,264 bp, linear) and pSLUN2 (46,827 bp, circular).

Streptomyces lunaelactis MM109 is the first *Streptomyces* type strain originating from cave carbonate speleothems called moonmilk deposits (1). It was isolated in the cave Grotte des Collemboles (Comblain-au-Pont, Belgium) and produces the green-pigmented intracellular iron chelator ferroverdin A (1, 2). *S. lunaelactis* strains have been found in all analyzed moonmilk deposits in the studied cave (1, 3, 4), suggesting that MM109^T could serve as a representative species for investigating the adaptation of streptomycetes to highly oligotrophic and mineral environments. Obtaining the complete genome sequence of MM109^T is therefore required to (i) compare the genomic features between cave-dwelling and soil-dwelling streptomycetes, (ii) accurately mine its predisposition at participating in moonmilk genesis (5), and (iii) evaluate its potential at producing natural compounds and identify the ferroverdin A biosynthetic gene cluster.

The complete genome sequence of *S. lunaelactis* MM109^T was obtained by combining sequencing data from three different technologies/approaches, namely, one-dimension Oxford Nanopore MinION (Oxford Nanopore Technologies, UK) and HiSeq and MiSeq (Illumina, CA, USA). The genome was assembled in four steps. First, the Nanopore-generated reads were error corrected, trimmed, and assembled using the long-read assembler Canu version 1.5 (6), which produced three contigs summing to a total length of 8,439,008 bp. Second, the Illumina HiSeq and MiSeq reads were assembled using SPAdes version 3.10.1 (7) and polished in two rounds with the Pilon version 1.22 tool (8), producing 198 contigs with a total length of 8,554,609 bp (contigs ≥ 1 kbp). The polishing step resolved inconsistencies between the Illumina draft assembly and Illumina read mapping in an iterative fashion until self-consistency. The third step consisted of improving the initial Nanopore assembly in eight successive rounds of polishing with the Illumina reads using Pilon, bringing the total length to 8,589,330 bp with 7,827 coding sequences. Finally (step 4), the genome was manually curated to resolve residual misassemblies, indels, and base differences observed upon the mapping of the Illumina assembly contigs (from step 2) onto the polished Nanopore

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assembly (from step 3). Inspection of the average Illumina sequencing coverage of the three assembled contigs revealed that the two shorter contigs were likely to be low-copy-number plasmids (named pSLUN1 and pSLUN2), with coverages ($541\times$ and $608\times$) three-fold greater than that of the chromosome ($177\times$). The presence of repeated sequences at both ends of pSLUN2 suggested a circular plasmid, which was circularized using the Circlator software (9). The automatic annotation of the genome was performed with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) upon submission to GenBank.

The final assembly of the *S. lunaelactis* MM109^T genome revealed a single linear chromosome with a size of 8,396,100 bp, and two plasmids, the linear pSLUN1 (127,264 bp) and the circular pSLUN2 (46,827 bp). The genome displayed an overall G+C content of 69.75%. A total of 7,718 protein-coding genes were predicted, as well as 66 tRNAs and 6 rRNA operons. Genome mining allowed the identification from nucleotide positions 4637038 to 4656557 of a 17-membered biosynthetic cluster presenting 85% and 91% amino acid identity and similarity, respectively, with the *fev* cluster (GenBank accession number AB689797) of another ferroverdin A producer, *Streptomyces* sp. strain WK53-44.

Accession number(s). This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession numbers [CP026304](#) for the chromosome and [CP026305](#) and [CP026306](#) for the plasmids pSLUN1 and pSLUN2, respectively. Raw sequence data are also available from the NCBI Sequence Read Archive under accession numbers SRR6475285 to SRR6475287.

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REFERENCES

- Maciejewska M, Pessi IS, Arguelles-Arias A, Noifalisse P, Luis G, Ongena M, Barton H, Carnol M, Rigali S. 2015. *Streptomyces lunaelactis* sp. nov., a novel ferroverdin A-producing *Streptomyces* species isolated from a moonmilk speleothem. *Antonie Van Leeuwenhoek* 107:519–531. <https://doi.org/10.1007/s10482-014-0348-4>.
- Chain EB, Tonolo A, Carilli A. 1955. Ferroverdin, a green pigment containing iron produced by a streptomycete. *Nature* 176:645. <https://doi.org/10.1038/176645a0>.
- Maciejewska M, Adam D, Martinet L, Naômé A, Całusińska M, Delfosse P, Carnol M, Barton HA, Hayette M-P, Smargiasso N, De Pauw E, Hanikenne M, Baurain D, Rigali S. 2016. A phenotypic and genotypic analysis of the antimicrobial potential of cultivable *Streptomyces* isolated from cave moonmilk deposits. *Front Microbiol* 7:1455. <https://doi.org/10.3389/fmicb.2016.01455>.
- Adam D, Maciejewska M, Naômé A, Martinet L, Coppieters W, Karim L, Baurain D, Rigali S. 2018. Isolation, characterization, and antibacterial activity of hard-to-culture actinobacteria from cave moonmilk deposits. *Antibiot Basel Switz* 7:28. <https://doi.org/10.3390/antibiotics7020028>.
- Maciejewska M, Adam D, Naômé A, Martinet L, Tenconi E, Całusińska M, Delfosse P, Hanikenne M, Baurain D, Compère P, Carnol M, Barton HA, Rigali S. 2017. Assessment of the potential role of *Streptomyces* in cave moonmilk formation. *Front Microbiol* 8:1181. <https://doi.org/10.3389/fmicb.2017.01181>.
- Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive *k*-mer weighting and repeat separation. *Genome Res* 27:722–736. <https://doi.org/10.1101/gr.215087.116>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski 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 J Comput Mol Cell Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Walker BJ, Abeel T, Shea T, Priest M, Bouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963. <https://doi.org/10.1371/journal.pone.0112963>.
- Hunt M, De Silva N, Otto TD, Parkhill J, Keane JA, Harris SR. 2015. Circlator: automated circularization of genome assemblies using long sequencing reads. *Genome Biol* 16:294. <https://doi.org/10.1186/s13059-015-0849-0>.