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Whole-Genome Sequence of Mycobacterium ulcerans CSURP7741, a French Guianan Clinical Isolate

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ABSTRACT

Combined Nanopore and Illumina whole-genome sequencing of a French Guianan Mycobacterium ulcerans (Buruli ulcer agent) clinical isolate yielded a 5.12-Mbp genome with a 65.5% GC content, 5,215 protein-coding genes, and 51 predicted RNA genes. This publicly available M. ulcerans whole-genome sequence from a strain isolated in South America is closely related to M. ulcerans subsp. lieldingii.

Mycopacterium ulcerans is an environmental mycobacterium responsible for Buruli ulcer (1), a neglected infection currently reported in 34 tropical countries (2), including Mexico (3), Peru (4), Brazil (5), and French Guiana (6). While the whole-genome sequence of one South American isolate from French Guiana has been reported (Mu_1G897, isolated in 1988) (7), the sequence is still not available.

Total DNA was extracted from several colonies of a 6-week-old subculture on Coletsos culture medium of M. ulcerans CSURP7741 using the InstaGene matrix (Bio-Rad, Marnes-la-Coquette, France) following the manufacturer’s instructions. The M. ulcerans CSURP7741 strain was initially isolated in solid Löwenstein-Jensen medium at 30°C after 8 weeks of incubation from a cutaneous biopsy specimen of the left lower limb of a 65-year-old man at the Cayenne Hospital in 2017. Total DNA (0.2 μg/μl) was sequenced using a MiSeq platform (Illumina, Inc., San Diego, CA, USA). DNA was fragmented and amplified by 12 cycles of PCR. After purification on AMPure XP beads (Beckman Coulter, Inc., Fullerton, CA, USA), the libraries were normalized and pooled for sequencing on a MiSeq instrument. Seven runs of paired-end sequencing and automated cluster generation with dual-indexed 2 × 251-bp reads were performed. The total information of 8.2 Gb was obtained from a 1,207,000/mm² cluster density, and 89.3% of the cluster passed the quality control filters (10,507.2 passed filtered reads). Reads were quality checked using FastQC and trimmed using Trimmomatic version 0.36.6 (8) (SRA number ERR3335404). In parallel, MinION technology (Oxford Nanopore, Oxford, UK) was performed on one-dimensional (1D) genomic DNA sequencing using an SQK-LSK108 kit. Library AMPure XP beads (Beckman Coulter, Inc.) were constructed from 1.4 μg genomic DNA with an end-repair step and quantified using a Qubit assay (Life Technologies, Carlsbad, CA, USA). Then, 74.28 ng was loaded onto the flow cell, and 1,359 pores were activated and analyzed online using the WIMP workflow. A total of 59,875 reads were generated after a 23-minute run; 53,206 reads analyzed by the software EPI2ME yielded 130.6 Mb, an average 2.18-kb length, and a maximum read length of 68.2 kb (SRA number ERR3336325). Adding MinION reads to MiSeq reads yielded 367 contigs assembled using SPAdes software version 3.5.0 (9) with a 5,267,061-bp genome and a 65.5% GC content (ERS3388536). Contigs of under 800 bp
were removed after BLASTn analysis against the NCBI database (identified as possible contaminants). Annotation using Prokka version 1.12 (10) yielded 5,266 predicted genes, 5,215 protein-coding genes, and 51 RNA genes comprising 47 tRNAs, 3 rRNA operons, and 1 transfer-messenger RNA (tmRNA). In addition, MiSeq and MinION reads were mapped with the most closely related Mycobacterium liflandii 128FXT plasmid, pMUM002 (GenBank accession number EU271968), using CLC Genomics Workbench version 7 to yield one 190,582-bp plasmid (62.6% GC content) encoding genes for mycolactone synthesis (mlsA1 [20 kb], mlsA2 [4 kb], and mlsB [16 kb]). Mapping detected IS2404 (184 bp, 8× depth normalized with a monocopy rpoB gene) and IS2606 (1,404 bp, 2× normalized depth). Genomic similarities estimated using the OrthoANI software tool version 0.93.1 (11) and in silico DNA-DNA hybridization estimated using the Genome-to-Genome Distance Calculator (GGDC) version 2.0 online tool were, respectively, 99.49% and 94.8% with M. ulcerans subsp. liflandii, 99.21% and 92.9% with M. ulcerans subsp. shinshuense (NZ_AP017624), 99.09% and 91.6% with M. ulcerans Agy99 (NC_008611), 99.06% and 90.9% with M. ulcerans strain Harvey (JAOL01000097), 99.08% and 91.8% with M. ulcerans strain S4018 (NZ_MDUB01000418), and 98.24% and 83% with Mycobacterium marinum E11 (NZ_HG917972). These analyses yielded an M. ulcerans pan-genome of 11,766 total genes, 3,054 conserved genes, 3,711 genes common to several species, and 5,001 species-specific genes (Fig. 1). These observations confirm clustering of South American strains with globally distributed fish isolates and M. ulcerans subsp. liflandii, which was responsible for an outbreak of a lethal infection in the African clawed frog, Xenopus tropicalis (7, 13). Moreover, the genetic similarities between these two isolates may orient further research on the reservoirs of M. ulcerans in French Guiana, focusing, for instance, on amphibians and, more generally, on a wide variety of freshwater species.

**Data availability.** The Mycobacterium ulcerans CSURP7741 genome sequence has been deposited at NCBI under the BioSample accession number ERS3388536. MiSeq reads have been deposited under the SRA accession number ERR3335404, and MinION reads have been deposited under the SRA accession number ERR3336325.

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REFERENCES


