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Resource Announcements

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. *liflandii*.

*M*ycobacterium 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
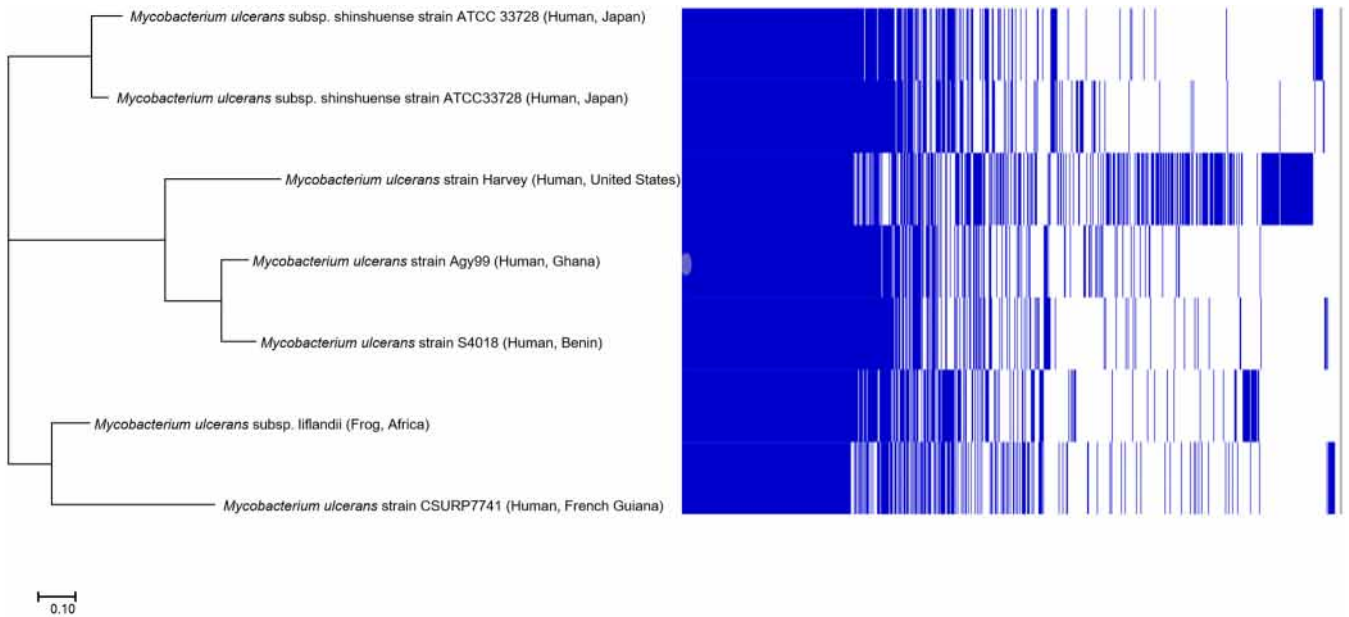
FIG 1 Pangenome tree incorporating *Mycobacterium ulcerans* strain CSURP7741 and all available complete *M. ulcerans* genome sequences in the NCBI database (as of January 2019).

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 IS*2404* (184 bp, 8× depth normalized with a monocopy *rpoB* gene) and IS*2606* (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 (12) were, respectively, 99.49% and 94.8% with *M. ulcerans* subsp. *liflandii* 128FXT, 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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