



Draft Genome Sequence of a *Clostridioides difficile* Sequence Type 97 Strain Belonging to Hypervirulent Clade 2

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ABSTRACT *Clostridioides difficile* hypervirulent clade 2 lineages are the major lineages responsible for the outbreaks in North America and Europe. However, the genome sequences of Japanese isolates are scarcely available. Herein, we report the draft genome sequence of *C. difficile* strain TMD0138 sequence type 97 (ST97), belonging to hypervirulent clade 2, isolated in Japan.

The morbidity and severity of *Clostridioides difficile* infections have increased since the 2000s in both health care facilities and communities (1). The epidemic hypervirulent strain known as ribotype 027 (RT027)/sequence type 1 (ST1), belonging to clade 2, is characterized by the production of binary toxins, increased production of toxins A and B owing to an 18-bp deletion in *tcdC*, hypersporulation, and high-level fluoroquinolone resistance (1–3). However, in comparison to North America and Europe, whole-genome sequence data for Japanese clade 2 lineages are extremely scarce, including the data published in our previous report (4). In this study, we report the draft genome of the community-acquired strain *C. difficile* TMD0138, belonging to hypervirulent clade 2, RT027/ST97, recovered from a 31-year-old male patient admitted to the Medical Hospital of Tokyo Medical and Dental University in 2001 (5).

C. difficile strain TMD0138 was grown in brain heart infusion (BHI) broth supplemented with 5 g/liter yeast extract and 0.1% (wt/vol) L-cysteine and incubated at 37°C under anaerobic conditions for 7 h. Genomic DNA was extracted using a NucleoSpin tissue kit (TaKaRa Bio) and then quantified with a QuantiFluor double-stranded DNA (dsDNA) system (Promega). A library was prepared from 50 ng of fragmented DNA using a KAPA HyperPrep kit (Kapa Biosystems) and then sequenced with the NextSeq platform (Illumina) to produce 2 × 151-bp paired-end reads. A total of 3,730,693 reads (average length, 150 nucleotides) were generated, following which quality control was performed on the raw reads using Sickle v. 1.33 (<https://github.com/najoshi/sickle>). The reads were assembled *de novo*, using SPAdes v. 3.10.1 (6), into 104 contigs, larger than 200 bp (largest scaffold, 483,781 bp; N_{50} , 196,318 bp; L_{50} , 8; average GC content, 28.44%), with an estimated genome size of 4,132,232 bp and an average coverage of >100×. Default parameters were used for all software.

In silico multilocus sequence typing (MLST) performed by MLST v. 2.0 (7) assigned strain TMD0138 to ST97, which belongs to clade 2. A previous study demonstrated that TMD0138 belongs to the same strain characterized as RT027 by PCR ribotyping (5). Comparative analyses of the pathogenicity locus (PaLoc) and Cdt locus (CdtLoc) sequences in TMD0138 were conducted by BLAST and ClustalW v. 2.1. The PaLoc sequence was homologous to reference genome 630, belonging to RT012/ST54 (GenBank accession number [NC_009089](https://.ncbi.nlm.nih.gov/nuccore/NC_009089); 100% query coverage and 96.57% identity), and strain R20291, belonging to RT027/ST1 (GenBank accession number [FN545816](https://ncbi.nlm.nih.gov/nuccore/FN545816); 100% query coverage and 99.83% identity). The negative regulator gene, *tcdC*, in

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TMD0138 was 99.41% identical to that in R20291, with 3 nucleotide substitutions, but lacked the single-nucleotide deletion at position 117. The CdtLoc sequence in TMD0138 showed 99.88% identity with that of R20291, and the binary toxin regulator gene, *cdtR*, in TMD0138 was completely conserved with that of R20291.

PHASTER v. 4.3X (8) identified two intact prophage regions (scores, >90), the *C. difficile* bacteriophages phiMMP03 and phiMMP01. The genome of TMD0138 also possessed an incomplete prophage region, and the most common phage was phiCDHM19 (score, 40).

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under BioProject number [PRJNA530817](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA530817) and accession number [WUUI00000000](https://www.ncbi.nlm.nih.gov/sra/SRR11241614). The raw data are available under SRA accession number [SRR11241614](https://www.ncbi.nlm.nih.gov/sra/SRR11241614).

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