

Draft Genome Sequence of the Nontoxigenic *Clostridium difficile* Strain CD37

Michael S. M. Brouwer, Elaine Allan, Peter Mullany, and Adam P. Roberts

UCL Eastman Dental Institute, London, United Kingdom

Here we report the draft genome sequence of *Clostridium difficile* strain CD37, the first nontoxigenic strain sequenced. Every sequenced strain of *Clostridium difficile* has been shown to contain multiple different mobile genetic elements. The draft genome sequence of strain CD37 reveals the presence of two putative conjugative transposons.

Clostridium difficile is an anaerobic, spore-forming, Gram-positive bacterium that is an opportunistic pathogen of humans and other mammals (2). The main virulence factors are the toxins A and B and the less-well-characterized binary toxin (18). Interestingly, nontoxigenic *C. difficile* strains (which lack the genes for toxins A and B and the binary toxin) are relatively common, although little is known about their biology (18). *C. difficile* has previously been shown to contain various mobile genetic elements, such as conjugative transposons (5, 6, 17, 19, 20), mobilizable transposons (11), prophages (12), and other elements, such as the IStron (4) and the *C. difficile* skin (*skin*^{Cd}) element (13).

Genome shotgun sequencing of strain CD37, ribotype 009 (5), was performed using an Illumina GAI-X. A total of 7,515,781 72-bp paired-end reads resulted in ~130-fold coverage of the genome in 245 contigs. Initial genome assembly was carried out using the Velvet software suite (21). The assembled genome was compared and mapped against the annotated 630 reference genome (GenBank accession no. AM180355) using xBase (9). Gene prediction was performed using Glimmer (10), tRNA genes were searched for with tRNAScan-SE (16), rRNA genes were searched for with RNAMmer (15), and Protein BLAST (1) was run using the translated coding sequences as a query against the 630 reference sequence. The best result for each BLAST search was imported as the gene annotation. A comparison file with concatenated reference and sequence files was produced using MUMMER (14). Analysis of the genome was performed using ACT (8).

Based on an alignment with strain 630 as the reference sequence, CD37 contains putative conjugative transposons with high nucleotide sequence similarity to CTn4 and CTn7 of strain 630 (~96% and ~98%, respectively). These mobile genetic elements are consistently found in *C. difficile* genomes (5, 6, 20). To date, all *C. difficile* genome sequences contain a *skin*^{Cd} element inserted within *sigK*, an RNA polymerase sigma factor shown to be essential for sporulation in *C. difficile*. However, *skin*^{Cd} is absent in the CD37 sequence, confirming previous PCR data (13).

In strain CD37, the PaLoc (encoding toxin A and B) is absent; in its place is 115 bp of noncoding DNA. This has also been observed in the nontoxigenic strain 7322 (3). Carter et al. (7) previously reported a 68-bp linker in place of the binary toxin locus in strain CD37. Our data show that within this 68 bp there is in fact only 16 bp of unique sequence in strain CD37 compared to that in strain 630. The fact that none of the main toxin genes are present in strain CD37 means that it will be a useful resource for workers wishing to investigate the effect of putative virulence factors without the complication of the toxins, which may mask other effects.

Nucleotide sequence accession numbers. This Whole Genome Shotgun project has been deposited in DDBJ/EMBL/GenBank under accession number AHJJ00000000. The version described in this paper is the first version, AHJJ01000000.

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Address correspondence to Adam P. Roberts, adam.roberts@ucl.ac.uk.

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