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The complete genome sequence of *Clostridium perfringens* MFDS1012647, isolated from a barbeque

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바비큐에서 분리된 *Clostridium perfringens* MFDS1012647의 유전체 서열 분석

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Clostridium perfringens is a bacterium that cause food poisoning outbreaks worldwide. This study presents the complete genome sequence of the *C. perfringens* strain MFDS1012647, isolated in 2018 from the barbeque of a cafeteria in Seoul, South Korea. The MFDS1012647 genome sequence contains 2,954,425 bp chromosomal DNA, a 61,476 bp plasmid, and a 28,917 bp plasmid, with 28%, 24.5%, and 26.5% GC contents, respectively. Gene prediction revealed that this strain possesses 2,668 coding sequences (CDSs), 94 tRNAs, and 30 rRNAs in the genome.

Keywords: *Clostridium perfringens*, barbeque, complete genome

Clostridium perfringens is a spore-forming, anaerobic, Gram-positive, rod-shaped bacterium belonging to the phylum *Firmicutes* (Shimizu *et al*., 2002; Johansson *et al*., 2006; Wong *et al*., 2014) that inhabits diverse environments such as soil, sewage, and animal intestines (Minton *et al*., 2016). Although *C. perfringens* does not invade healthy cells, it acts as a pathogen by producing enterotoxin, alpha-toxin and various enzymes (*nanH*, *nagH*, *nagJ*); it is a common cause of food poisoning outbreaks. In this study, we determined the complete genome sequence of *C. perfringens* collected in Seoul, South Korea, in 2018.

Clostridium perfringens MFDS1012647 was identified during a food poisoning investigation by the South Korean Ministry of Food and Drug Safety (MFDS). The MFDS1012647 was isolated from the barbeque of a cafeteria and detected in the patients suffering from food poisoning. The MFDS1012647 isolated was incubated anaerobically in tryptic soy agar medium at 37°C for 24 h. The total genomic DNA of MFDS1012647 was extracted using the Genomic DNA Prep Kit for Bacteria (Bioneer). The genomic DNA qualitatively and quantitatively was measured using a NanoDrop 2000 UV-visible spectrophotometer (Thermo Fisher Scientific) and a Qubit™ 3.0 Fluorometer (Invitrogen) with dsDNA HS Assay Kit, respectively. DNA libraries were constructed using a Nextera DNA Flex Library Prep Kit (Illumina). The concentration and quality of the libraries were determined using a Qubit and 2100 Bioanalyzer instrument (Agilent Technologies). Sequencing was performed using a MiSeq sequencing system (Illumina) on a paired-end library using the MiSeq Reagent Kit v3 (600

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Table 1. Genome features of *C. perfringens* MFDS1012647

Genomic feature	Value
Contig	3
Genome size (bp)	3,044,818
GC content (%)	28
CDSs	2,668
rRNAs (5S, 16S, 23S)	30 (10, 10, 10)
tRNAs	94

cycles). After sequencing, individual sequence reads were analyzed using FastQC-v.0.11.8. The illumina sequencing yielded 2.5 M reads, including 637 Mb, with 207.9× coverage. The sequencing quality values Q20 and Q30 were 98.33% and 94.93% respectively. For nanopore sequencing, the Oxford Nanopore Rapid Barcoding Kit (Oxford Nanopore Technologies) was used to construct the libraries. Nanopore sequencing data were basecalled on guppy_barcoder v6.0.1. The Nanopore sequencing yielded 101 K reads, including 177 Mb, with 51.8 x coverage (N50, 3 kb). The sequencing mean quality was 11.7.

Both Illumina and nanopore sequencing data were processed and *de novo* assembled with Unicycler v0.4.8, using the Pathosystems Resource Integration Center (PATRIC) v3.6.12 web server. The complete genome sequence of *C. perfringens* MFDS1012647 was 3,044,818 bp (GC content 28%). The entire genome contains 2,668 coding sequences, 94 tRNA genes, and 30 rRNA genes (Table 1). The MFDS1012647 genome consists of a circular chromosome of 2,954,425 bp (28% G + C content) and two plasmids (61,476 bp, 24.5% G + C content; 28,917 bp, 26.5% G + C content) (Fig. 1).

The genome was annotated on the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova *et al*., 2016; Haft *et al*., 2018; Li *et al*., 2021), with a total of 2,850 genes

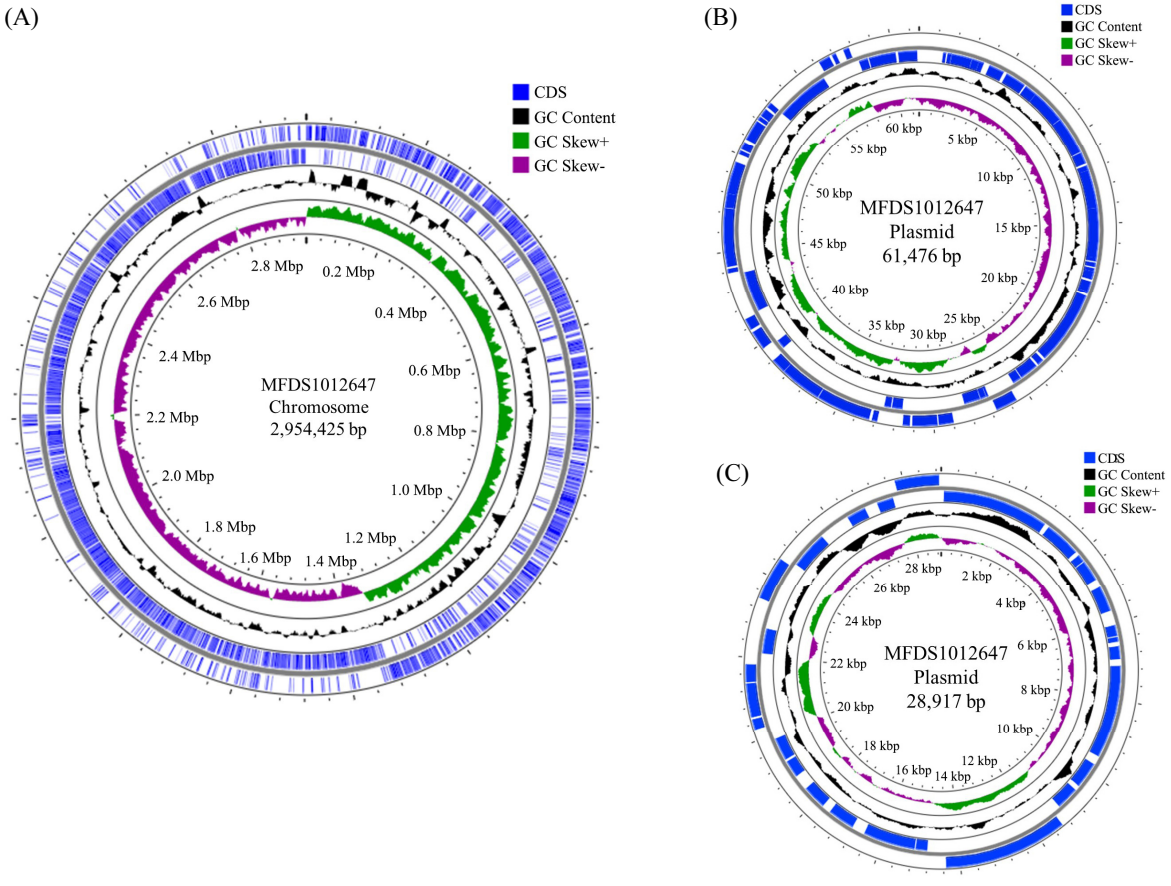

Fig. 1. The complete genome map of MFDS1012647. The circular genome visualization of the bacterium was performed using the Proksee (https://proksee.ca/) web server. The second inner circle with the green and purple histogram shows the GC skew +/− whereas the fourth inner circle with the black histogram indicates the GC content. (A) The chromosome genome map of MFDS1012647. (B) The first plasmid of MFDS1012647 (pMFDS1012647-1) genome map. (C) The second plasmid of MFDS1012647 ((pMFDS1012647-2) genome map.

were predicted. Prediction of the virulence-associated genes was conducted using the Virulence Factor Database (Liu *et al*., 2019), PATRIC_VF (Snyder *et al*., 2007), and the Comprehensive Antibiotic Resistance Database (McArthur *et al*., 2013) on the PATRIC web server. The virulence associated genes predicted are enterotoxin (*cpe*), alpha-toxin (*plc*), and exo-alpha-sialidase (*nanH*), hyaluronidase (mu-toxin) (*nagH* and *nagJ*). Additionally, the bacterium had putative antibiotic resistance genes (*tetA*(P) and *tetB*(P)). The complete *C. perfringens* genome reported here will be useful for future investigations regarding the understanding of foodborne pathogens, and provides the genetic basis for a more detailed analysis of virulence factors.

Nucleotide sequence accession number(s)

Nucleotide sequence accession numbers. The complete genome sequence of *Clostridium perfringens* MFDS1012647 has been deposited at the NCBI GenBank database under the accession numbers CP106926 (chromosome, MFDS1012647), CP106927 (the first plasmid, pMFDS1012647-1) and CP106928 (the second plasmid, pMFDS1012647-2). The strain has been deposited in the Korean Culture Collection for foodborne Pathogens under the strain number MFDS1012647.

적 요

클로스트리디움 퍼프린젠스는 전 세계적으로 식중독을 일으키는 세균 중 하나이다. 본 연구에서는 2018년 서울의 대학교 기숙사 식당에서 일어난 식중독 사고의 원인식품으로 추정되는 바비큐 구이로부터 분리된 *Clostridium perfringens* (MFDS 1012647)의 유전체 분석을 수행하였다. *Clostridium perfringens* MFDS1012647는 한 개의 chromosome (2,954,425 bp)과 두 개의 plasmid (61,476 bp; 28,917 bp)로 구성되어 있었고, 각각의 G + C contents는 28%, 24.5%, 26.5%로 확인되었다. 또한, 유전체에서 2,668개의 단백질 코딩 유전자(CDSs), 94개의 tRNA, 30개의 rRNA를 확인하였다.

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Conflict of Interest

There are no conflicts of interest to declare.

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