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ARTICLE

Complete Genome Sequence of *Enterococcus faecalis* CAUM157 Isolated from Raw Cow's Milk

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Abstract

Enterococcus faecalis CAUM157, isolated from raw cow's milk, is a Gram-positive, facultatively anaerobic, and non-spore-forming bacterium capable of inhabiting a wide range of environmental niches. *E. faecalis* CAUM157 was observed to produce a two-peptide bacteriocin that had a wide range of activity against several pathogens, including *Listeria monocytogenes*, *Staphylococcus aureus*, and periodontitis-causing bacteria. The whole genome of *E. faecalis* CAUM157 was sequenced using the PacBio RS II platform, revealing a genome size of 2,972,812 bp with a G+C ratio of 37.44%, assembled into two contigs. Annotation analysis revealed 2,830 coding sequences, 12 rRNAs, and 61 tRNAs. Further, *in silico* analysis of the genome identified a single bacteriocin gene cluster.

Keywords

Enterococcus faecalis, whole genome, bacteriocin, raw milk

Antimicrobial drug resistance and the rapid emergence of resistant bacterial species continue to be a growing concern as it threatens the effectivity of traditional antimicrobial compounds against its target pathogens [1,2]. This evolutionary arms race demands the identification and development of novel antimicrobial compounds that have the potential to substitute antibiotic use and other similar applications. Bacteriocins, which are ribosomal-synthesized peptides produced by bacteria to inhibit the growth of closely related strains [2,3], are potential alternatives to traditional antibiotics [4]. *Enterococcus faecalis* CAUM157, a Gram-positive, facultatively anaerobic, non-spore-forming cocci [5], was observed to produce an antimicrobial protein with potent activity against a wide range of bacteria including periodontal disease-causing pathogens, *Prevotella intermedia* and *Fusobacterium nucleatum* [6], and *Listeria monocytogenes*, and *Staphylococcus aureus* (unpublished data). Whole-genome sequencing of strain CAUM157 was performed to further investigate the molecular characteristics of the bacteriocin gene structure.

E. faecalis CAUM157 was isolated from raw cow's milk obtained from a local farm in Anseong, Korea. Strain CAUM157 was routinely grown in de Man, Rogosa, and Sharpe (MRS, Difco Laboratory, USA) broth supplemented with 1% L-cysteine at 37℃. The genomic DNA was extracted from 12 h cultures using QIAamp PowerFecal DNA Kit (Qiagen, Germany) according to the manufacturer's instructions. Genomic DNA of strain CAUM157 was sent to ChunLab (Korea) and sequenced using the Pacific Biosciences (PacBio, USA) RSII Single Molecule Real-Time (SMRT) platform with 20 kb SMRTbell™ template library. The PacBio reads were assembled *de novo* using the PacBio SMAR Analysis ver. 2.3.0 program. Genome annotation was performed with the Rapid Annotation using Subsystem Technology (RAST) using default parameters [7] and CLgenomics™

ver. 1.55 software. Transfer RNAs (tRNAs) were identified using tRNAscan-SE ver. 1.3.1 [8]. Ribosomal RNAs (rRNAs) and non-coding RNAs were identified using INFERNAL ver. 1.1.3 software with Rfam 12.0 database [9]. Functional annotation of protein-coding sequences (CDSs) was performed using the PRODIGAL ver. 2.6.2 software [10] and compared to protein databases (SwissProt, KEGG, SEED, EggNOG) using USEARCH ver. 8.1 [11]. The complete genome of *E. faecalis* CAUM157 (Fig. 1) has a length of 2,972,812 bp with a G+C content of 37.44% assembled into 2 contigs with an N_{50} value of 2,913,602 bp. The genome contains 2,830 coding genes, 12 rRNAs, and 61 tRNAs (Table 1).

Putative bacteriocin-encoding gene clusters were determined *in silico* using the BAGEL4 software tool [12] which revealed 1 area of interest (AOI) in contig CM157.00001, corresponding to the bacteriocin genes. Two open reading frames (ORFs) encoding the core peptides of bacteriocin MR10A (E value, 2×10^{-27}) and MR10B (E value, 1×10^{-27}) were detected. The amino acid sequences of the two core peptides deduced from the putative bacteriocin gene share a similar sequence and lack the YGNGVXC motif characteristic of the leader sequence, which suggests that the bacteriocin is of class IIB – leaderless two-peptide bacteriocin [13]. Additionally, the amino acid sequence exhibited a high degree of similarity with a previously described bacteriocin (MR10A and MR10B) from *E. faecalis* [14]. The corresponding sequences are homologous with the plasmid-encoded enterocins L50A and L50B described in *E. faecium* L50 [15] except for a conservative change (Glu 38 to Asp) in MR10A and two residue change (Thr

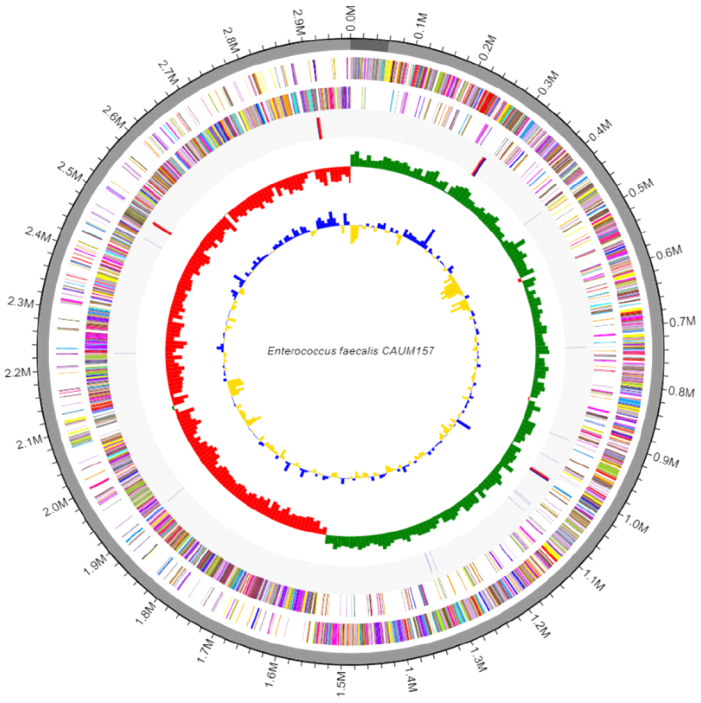

Fig. 1. Circular genome map of *Enteroccocus faecalis* CAUM157. Circles represent the following characteristics from the outermost circle to the center: (1) contig information, (2) coding sequences on forward strand, (3) coding sequences on reverse strand, (4) transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), (5) GC skew, and (6) GC ratio.

Table 1. Genome features of *Enterococcus faecalis* CAUM157

Attribute	Value
Genome size (bp)	2,972,812
GC content (%)	37.4
No. of contigs	2
Total genes	2,903
Protein-coding gene	2,830
tRNA	61
rRNA	12
Plasmids	0
GenBank accession no.	JACSYK000000000

9 to Ala, and Leu 15 to Phe) in MR10B [16]. Furthermore, genes encoding for self-immunity and ABC-transport system (efflux RND transporter, ABC transporter ATP-binding protein, and ABC transporter permease) were detected downstream of the putative bacteriocin genes.

Nucleotide Sequence Accession Number

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JACSYK000000000. The version described in this paper is version JACSYK010000000.

Conflict of Interest

The authors declare no potential conflict of interest.

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