

Complete genome sequence of multidrug-resistant *Enterococcus faecalis* strain CF3A-2134 isolated from feces of a pig

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돼지 분변으로부터 분리된 다제내성 *Enterococcus faecalis* CF3A-2134의 유전체 염기서열

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Multidrug-resistant *Enterococcus faecalis* strain CF3A-2134 belonging to sequence type 620 was isolated from feces of a pig which was resistant to ciprofloxacin, gentamicin, streptomycin, tetracycline, quinupristin-dalfopristin, and chloramphenicol. The complete genome of strain CF3A-2134 consisted of a 2,884,629 bp circular chromosome and one 92,100 bp circular plasmid with 37.5% of G + C content. Annotation detected 2,936 protein coding sequences, 12 rRNA genes, and 65 tRNA genes.

Keywords: *Enterococcus faecalis*, feces, multidrug-resistant, pig

Enterococci are commensal bacteria inhabiting the intestines of many mammals and various environments, including soil, water, and plants (Guzman Prieto *et al.*, 2016). Approximately 25% of the genes in enterococci are composed of mobile genetic elements, allowing them to acquire various antimicrobial resistance genes (Werner *et al.*, 2013). Antimicrobial resistance is one of the critical factors threatening public health (Prestinaci *et al.*, 2015). *Enterococcus faecalis* has emerged as the third leading cause of nosocomial infections in recent decades (García-Solache and Rice, 2019).

A rectal swab collected from the 35-day-old piglet at a pig farm in Chungbuk province was streaked onto enterococcosel agar (BD Bioscience) and incubated at 37°C for 48 h. After cultivation, suspected enterococcal colonies were sub-cultured onto blood agar plates (Synergy Innovation). In order to identify *E. faecalis*, nucleotide sequence of 16S rRNA gene was determined from obtained colony (Ryu *et al.*, 2013). Comparison of the sequence on GenBank database exhibited 100% identity to one of *E. faecalis* strain J-5-A (GenBank accession No. CP123624.1). The strain was named *E. faecalis* CF3A-2134 and identified as sequence type 620 using the PubMLST database (<https://pubmlst.org/>) based on seven house-keeping gene sequences (*gdh*, *gyd*, *pstS*, *gki*, *aroE*, *xpt*, and *yqiL*) (Ruiz-Garbajosa *et al.*, 2006). Antibiotic susceptibility was determined with disk diffusion and broth microdilution methods following Clinical and Laboratory Standards Institute guideline (CLSI, 2017). The strain CF3A-2134 was resistant against ciprofloxacin, gentamicin, streptomycin, tetracycline, quinupristin-dalfopristin, and chloramphenicol but sensitive to ampicillin, penicillin, nitrofurantoin, vancomycin, teicoplanin, linezolid, and tigecycline.

The total genomic DNA was extracted using the LaboPass™ Bacteria Mini DNA purification kit (Cosmogentech). Whole genome sequencing was performed using the iSeq 100 (Illumina)

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The GenBank/EMBL/DDBJ accession numbers for the complete genome sequence of strain CF3A-2134 are CP136403 and CP136404. The strain was deposited in KCTC under deposition number BP1917276.

and the MinION (Oxford Nanopore Technologies) sequencers. For the iSeq platform, the library was prepared using the Illumina DNA prep kit (Illumina) and Nextera DNA CD indexes (Illumina). For the MinION platform, the library preparation was performed using the ligation sequencing kit (SQK-LSK-109, Oxford Nanopore Technologies), native bar-coding expansion (EXP-NBD-104, Oxford Nanopore Technologies), and the NEBNext companion module (New England Biolabs). The MinION sequencing was performed using the flow cell (R9.4.1, Oxford Nanopore Technologies). Adapter or primers sequences and low-quality reads were removed from the iSeq sequencing raw data using Trimmomatic v.0.39 (Bolger *et al.*, 2014). Of MinION sequencing raw data, low-quality reads and adapter sequences were trimmed with Filtlong v.0.2.0 and Porechop v.0.2.4, respectively. A *de novo* genome assembly was performed with a Unicycler v.0.4.9b (Wick *et al.*, 2017). The genome of the CF3A-2134 strain was annotated and the antimicrobial resistance genes were identified using the bacterial and viral bioinformatics resource center web server (v.3.31.12) (Olson *et al.*, 2023). The completeness of the genome by CheckM v1.2.2 was evaluated as 100%. The genome consisted of one circular chromosome (2,884,629 bp) and one circular plasmid (92,100 bp) with a G + C content of 37.5% (Fig. 1).

Table 1. Genomic features of *Enterococcus faecalis* strain CF3A-2134

Genomic features	Chromosome	Plasmid
GenBank accession no.	CP136403	CP136404
Genome size (bp)	2,884,629	92,100
G + C content (%)	37.6	34.6
No. of total CDSs	2,819	117
No. of rRNA (5S, 16S, 23S)	4, 4, 4	0, 0, 0
No. of tRNA	65	0

CDS, protein coding sequence.

Annotation identified 2,936 protein coding sequences (CDSs), 12 rRNA genes, and 65 tRNA genes as shown in Table 1. Various antimicrobial resistance genes were found in the plasmid like as gentamicin (*aac6'-le-aph2''-Ia*), streptomycin (*aad[6]*, *ant[6]-Ia* and *ant[9]-Ia*), streptothricin (*sat-4*), kanamycin (*aph[3']-IIIa*), quinupristin-dalfopristin (*IsaE*), tetracyclines (*tetL* and *tetM*), erythromycin (*lmuB*), trimethoprim (*dfrG*), and chloramphenicol (*catA8*). Additionally, 5 transposases (*ISSsu9*, *ISBce13*, *IS1216E*, *IS1297*, *IS1476*) and 1 transposon (Tn552) were located in the plasmid. Besides, antimicrobial resistance genes, *liaR* and *dfrE*, against daptomycin and trimethoprim were identified in a chromosome. The genome obtained in this study would be useful to monitor antibiotic resistance among the genus *Enterococcus*.

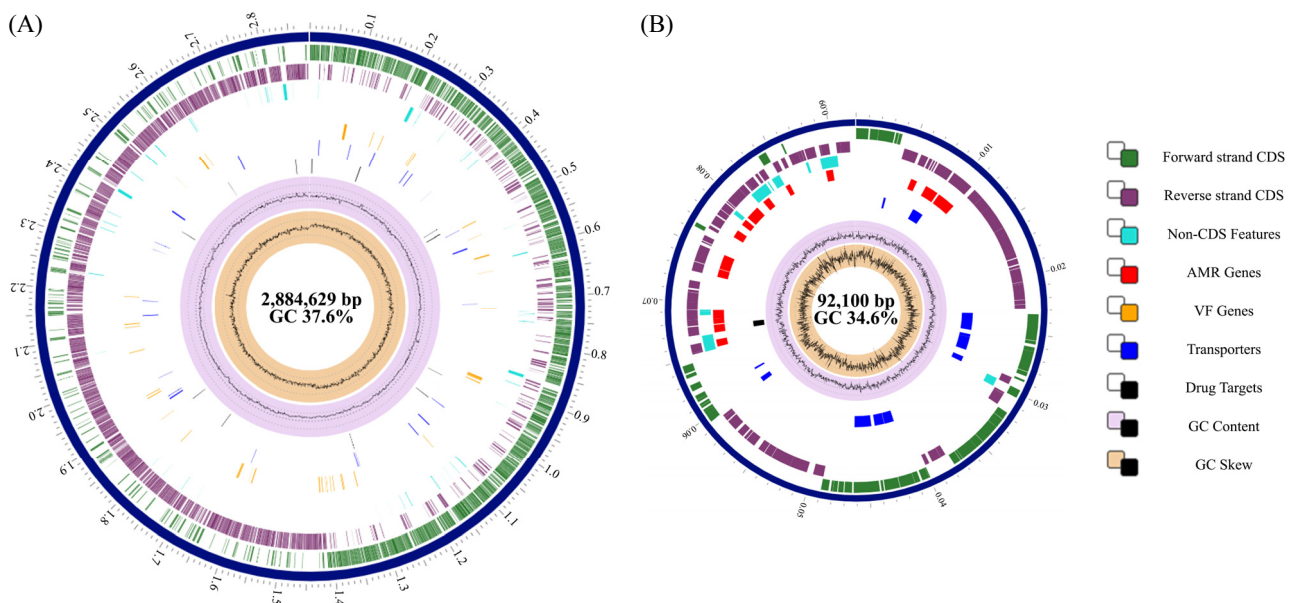


Fig. 1. Circular genome map of *Enterococcus faecalis* CF3A-2134. Chromosome (A) and plasmid (B). Genome features are presented as follows: forward strand CDS (green), reverse strand CDS (violet), non-CDS (fluorescent blue), AMR (antimicrobial resistance) genes (red), VF (virulence factor) genes (ocher), transporters (blue), drug targets, GC content and GC skew (black).

Nucleotide sequence and strain accession numbers

The strain is available at the Korean Collection for Type Cultures (KCTC) under deposition number BP1917276. The genome of strain CF3A-2134 has been deposited in GenBank/EMBL/DDBJ under the accession numbers, CP136403 and CP136404.

적 요

유전형 620에 속하는 다제내성 *Enterococcus faecalis* CF3A-2134 균주는 돼지 분변으로부터 분리되었으며 ciprofloxacin, gentamicin, streptomycin, tetracycline, quinupristin-dalfopristin, chloramphenicol에 내성을 보였다. CF3A-2134 균주의 전장 유전체는 2,884,629 bp 크기의 원형 염색체 1개와 92,100 bp 크기의 원형 플라스미드 1개로 구성되어 있으며 G+C 함량은 37.5%였다. 이 유전체에는 2,936개의 단백질 암호화 염기서열, 12개의 rRNA 유전자, 65개의 tRNA 유전자가 포함되어 있었다.

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Conflict of Interest

Jong-Chan Chae is Editor of KJM. He was not involved in the review process of this article. Also, authors have no conflicts of interest to report.

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