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High-Quality Whole Genome Sequence of a Linezolid-Resistant and Vancomycin-Susceptible *Enterococcus faecalis* Isolate ES-2-1 from a Pig Stool in South Korea

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We report the whole genome sequence of a linezolid-resistant and vancomycin-susceptible *Enterococcus faecalis* strain, ES-2-1, which was isolated from a pig stool in South Korea. The assembled genome of ES-2-1 consists of a 2,648,168-bp circular chromosome containing the *optrA* gene (encoding the ABC-F type ribosomal protection protein), an 84,891-bp plasmid containing numerous antimicrobial resistance genes, and an 82,106-bp cryptic plasmid. The ES-2-1 strain belongs to sequence type 1024 (ST1024) and carries multidrug resistant genes including the *optrA* (*o*xazolidinone *p*henicol *t*ransferable *r*esistance *A*) gene, which confers linezolid resistance.

Keywords: **Whole genome sequence, linezolid resistance, *Enterococcus faecalis*, pig stool, South Korea**

Linezolid has been approved by the Korea Food and Drug Agency (FDA) in December 2000. Its antibiotic activity was demonstrated against many bacterial species, including aerobic and anaerobic Gram-positive bacilli, anaerobic Gram-positive cocci, some Gram-negative anaerobes, *Nocardia* species, and mycobacterial species [1]. As one of the last-resort antimicrobials, it has been applied in the clinics for treating severe infections by methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* species (VRE) [1, 2]. In general, however, Gram-negative bacteria have a proton motive force efflux pump, AcrAB, which provides intrinsic resistance to linezolid as well as other oxazolidinone-class antimicrobials [1, 3]. Here, a linezolid-resistant and vancomycin-susceptible *Enterococcus faecalis* isolate, ES-2-1, was isolated from a pig stool in South Korea, 2021, and further analyzed by whole-genome sequencing (WGS). To this end, a single colony of ES-2-1 was inoculated into Trypticase soy broth (Becton Dickinson and Co., USA) and incubated at 37℃ with aeration and shaking (220 rpm) for 18 h. The bacterial genomic DNA was extracted using HiYield genomic DNA mini kit (Real Biotech Corporation, Taiwan) and subjected to WGS at a commercially available company (Sanigen Co., Ltd., Republic of Korea) [4].

Briefly, two separate genomic DNA libraries were constructed by the requirements of the Illumina and Oxford Nanopore systems. A combination of long-read Nanopore MinION and short-read Illumina Miseq platforms was used for obtaining the complete genome sequence of ES-2-1. For Illumina sequencing, the extracted genomic DNA was fragmented by sonication using a Covaris M220 (Covaris, USA). The sheared DNA were then used to prepare a WGS library with an average insert size of 550 bp using a TruSeq Nano DNA Sample Prep kit (Illumina, USA). The library was sequenced on an Illumina Miseq platform (Illumina) using the 300 bp paired-end sequencing mode. For Nanopore sequencing,

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a MinION sequencing library was prepared using the Nanopore Ligation Sequencing Kit (SQK-LSK110; Oxford Nanopore, UK). The library was sequenced with an R9.4.1 MinION flow cell (Flongle) for a 24 h run using MinKNOW with the default settings (MinKNOW core 5.0.0, Guppy 6.0.6). Illumina and Nanopore data were assembled with different processes. For Illumina sequencing, the data were processed to remove low quality bases and adapter sequences with the optimized settings using Trimmomatic v0.39 (LEADING: 10, TRAILING: 10, SLIDING WINDOW: 4:20, MINLEN: 200). Subsequently, additional phiX control were removed from pre-assembled data. Trimmed sequences were aligned against phiX genome with bowtie2 v2.3.5.1 with the default options and filtered out by samtools v1.9. For Nanopore sequencing, the data were base-called with guppy base-caller v3.1.5. NanoFilt v2.8.0 was used to filter obtained reads with average Phred quality score lower than 7 and length lower than 1,000. Unicycler v0.4.8 was used to construct genome combined with Filtered MiSeq and MinION data. After, genome was annotated using Prokka v1.14.6 and their CDS were identified.

As a result, a total 2,374,962 reads were generated (Total read bases, 709,328,945 bases; G+C content, 37.65%; Q30, 78.19%) for ES-2-1. The species were identified by calculating the average nucleotide identity value of its genome assembly (GCF_033815475.1) and one of the publicly available *Enterococcus faecalis* genome assembly (GCA_033815475.1) in National Center for Biotechnology Information (NCBI) GenBank database. Multilocus sequence typing (MLST) was done at the web site (https://cge.cbs.dtu.dk/services/MLST/) available from the Center for Genomic Epidemiology (CGE). The chromosomal point mutations and/or the acquired genes related to antimicrobial resistance (AMR) were identified by using the ResFinder software (https://cge.cbs.dtu.dk/services/ResFinder/) from CGE. To assess the accuracy of ResFinder, we compared its gene prediction output with that of AMRFinderPlus (https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder/), which is another publicly available gene prediction software from NCBI. To determine the antimicrobial resistant phenotype of the ES-2-1 strain, we carried out the minimal inhibitory concentration (MIC) analysis using KRVP2F Sensititre (TREK Diagnostic Systems, USA) [5]. High-quality complete genome sequence of ES-2-1 was submitted to the NCBI GeneBank database (NCBI accession numbers CP138655.1, CP138656.1, and CP138657.1).

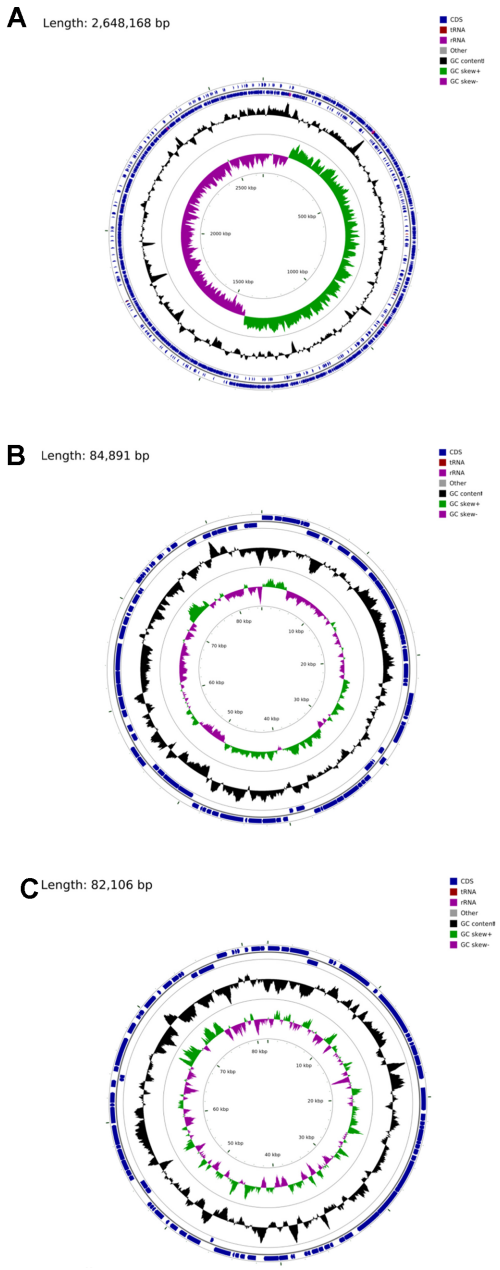

Fig. 1. The complete genome of *Enterococcus faecalis* ES-2-1. Circular maps of contig 1 (**A**) contig 2 (**B**) and contig 3 (**C**). Each circular map was drawn by applying the contig annotation information. Marked characteristics are shown from outside to the center; coding sequence (CDS) on forward stand, CDS on reverse stand, tRNA, rRNA, G+C content and GC skew.

Table 1. Genetic and phenotypic characteristics of *Enterococcus faecalis* ES-2-1.

Features	Value
No. of contigs	3
Genome size (bp)	2,468,168 (Chromosome), 84,891 (Plasmid 1), 82,106 (Plasmid 2)
G+C content (%)	37.82 (Chromosome), 34.86 (Plasmid 1), 34.69 (Plasmid 2)
No. of CDSs	2,647 (Chromosome) 80 (Plasmid 1) 85 (Plasmid 2)
No. of tRNA genes	58 (Chromosome)
No. of rRNA genes	12 (Chromosome)
MLST	ST1024
AMR profile	Linezolid, Ciprofloxacin, Chloramphenicol, Florfenicol, Gentamicin, Kanamycin, Streptomycin, Quinupristin/Dalfopristin, Tylosin, Tetracycline
AMR genes	*gyrA (S83I), parC (S80I), optrA, fexA, catA, tet(L), tet(M), abc-f, erm(A), erm(B), ant(6)-Ia, ant(9)-Ia, aac(6')-Ie/aph(2")-Ia, aph(3')-IIIa, lnu(B), spw, sat4, lsa(A), lsa(E), tcrB*
NCBI accession numbers	CP138655.1 (Chromosome), CP138656.1 (Plasmid 1), CP138657.1 (Plasmid 2)

Notes: CDSs, coding sequences; MLST, multilocus sequence typing; ST, sequence type; AMR, antimicrobial resistance.

The assembled genome of the ES-2-1 strain yielded three circular contigs (Fig. 1). Contig 1 comprised a single chromosome (2,648,168 bp, 37.82% G+C contents) which includes 2,647 coding sequences (CDSs), 58 tRNA, and 12 rRNA (Table 1). Contig 2 comprised a single plasmid (84,891 bp, 34.68% G+C contents) containing 80 CDSs. Contig 3 comprised a single plasmid (82,106 bp, 34.69% G+C contents) containing 85 CDSs. The ES-2-1 strain was identified as *E. faecalis* belonging to ST1024. The MIC analysis showed that the microorganism displayed multiple resistance to linezolid, ciprofloxacin, chloramphenicol, florfenicol, gentamicin, kanamycin, streptomycin, quinupristin/dalfopristin, tylosin, and tetracycline (data not shown). Corresponding to its AMR phenotype, the ES-2-1 genome carries the single point mutations in *gyrA* (S83I) and *parC* (S80I) and harbors several AMR genes such as the *optrA* (***o***xazolidinone ***p***henicol ***t***ransferable ***r***esistance ***A***) gene, which confers linezolid resistance (Table 1). Notably, however, the ES-2-1 genome neither harbors amino acid changes in the ribosomal proteins (L3, L4, and L22) nor acquires any other transferable linezolid resistance genes such as the *cfr* and *poxtA* genes [3, 6, 7].

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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