Draft Genome Sequences of Five Enterococcus faecium Isolates from Traditional Montenegrin Brine Cheese

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ABSTRACT Enterococcus faecium is a multifaceted bacterial species. It is part of the natural human microbiota, it grows in a variety of traditional foods, and emerging multiresistant clones are a leading cause of nosocomial infections. Here, we present draft genomes of five E. faecium isolates originating from traditional Montenegrin brine cheeses.

Enterococci, belonging to the group of lactic acid bacteria, are often present in traditional food products (1). Due to the emergence of multidrug-resistant enterococci as a leading cause of nosocomial infections (2), only specific strains of Enterococcus faecium can be used as probiotics or feed additives (3). The genus Enterococcus has not obtained the status generally recognized as safe (GRAS) (4). E. faecium strains exhibiting probiotic and other beneficial potentials must be differentiated from pathogenic strains, as determined by the European Food Safety Authority (EFSA) (5, 6). Whole-genome sequencing (WGS) analysis of E. faecium strains has the potential to distinguish between safe and potentially harmful strains, thereby minimizing possible risks for consumers (7, 8).

Bacteria were isolated from white brine-ripened traditional cheese from Montenegro, as described previously (9, 10). Colonies morphologically suspected to be enterococci were subcultured for species identification by WGS.

The GeneMatrix bacterial and yeast genomic DNA purification kit (EURx, Gdańsk, Poland) was used for the isolation of genomic DNA from overnight cultures grown in M17 or MRS broth (HiMedia, India). The Nextera XT kit (Illumina, Inc., San Diego, CA, USA) was used for WGS library preparation, and paired-end sequencing (2 × 300 bp) was performed on a MiSeq system (Illumina) as described previously (11). Default parameters were used for all software unless otherwise specified. Raw reads were quality controlled using FastQC v0.11.9. Trimmomatic v0.36 (12) was used to remove adapter sequences and to trim the last 10 bp of each sequence, as well as sequences with a quality score of < 20. Reads were assembled using SPAdes v3.11.1 (13). Contigs were filtered for a minimum coverage of 5× and a minimum length of 200 bp using SeqSphere+ software v6.0.0 ( Ridom GmbH, Würzburg, Germany).

WGS of five E. faecium isolates generated 1,053,512 to 1,933,204 reads, with a mean coverage of 39× to 48×. The NCBI Prokaryotic Genome Annotation Pipeline identified 2,721 to 2,840 genes, 2,639 to 2,690 coding sequences, 168 to 192 pseudogenes, 14 to 19 rRNA genes, and 61 to 68 tRNA genes (Table 1). Species identification was done with a BLAST search against the NCBI 16S rRNA database (14), Mash distance analysis (15), and ribosomal multilocus sequence typing (rMLST) (16). All methods identified all isolates as E. faecium. Strains were characterized by MLST (17) and core genome MLST (cgMLST) using SeqSphere+ with default settings, as described (18). All isolates had >97% good cgMLST targets and were sequence type 1453 (ST1453) and the...
new cgMLST complex type 2909 (CT founder strain INF40) (19) and antiSMASH 5.0 (20) revealed that all isolates carried genes of the bacteriocin biosynthetic gene clusters (i.e., type III polyketide synthase, the RiPP cluster, and enterolysin A). Analysis of genomes with ResFinder (21), PlasmidFinder (22), and VirulenceFinder (23) from the Center of Genomic Epidemiology revealed that all isolates carried the intrinsic resistance genes msrC and aac(6’)-Ii and had mutations in pgbS conferring resistance to ampicillin C, carried the plasmids rep1 and repUS15, and carried virulence factors acm and efaAfm.

**Data availability.** The Enterococcus faecium whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under accession no. JAAHCA000000000 (INF40), JAAHCB000000000 (INF29), JAAHCC000000000 (INF9), JAAHCD000000000 (INF39), and JAAHCE000000000 (INF12). The versions described in this paper are the first versions (accession no. JAAHCA010000000 to JAAHCE010000000). The raw sequence reads have been deposited in the Sequence Read Archive (SRA) under accession no. SRR11111643 (INF12), SRR11111644 (INF39), SRR11111645 (INF9), SRR11111646 (INF29), and SRR11111647 (INF40).

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**REFERENCES**


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**TABLE 1 Characteristics and accession numbers of E. faecium isolates from Montenegrin brine cheese**

<table>
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<tr>
<th>Strain</th>
<th>Genome size (bp)</th>
<th>No. of reads</th>
<th>Total no. of genes</th>
<th>No. of RNAs</th>
<th>Avg coverage (%)</th>
<th>No. of contigs</th>
<th>Contig N₅₀ (bp)</th>
<th>G+C content (%)</th>
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<th>SRA accession no.</th>
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