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Article Title (within 20 words without abbreviations)	Complete genome sequence of *Enterococcus faecium* strain AK_C_05 with potential characteristics applicable in livestock industry
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Authors' contributions Please specify the authors' role using this form.	Conceptualization: Doo H, Kim HB, Lee JH Data curation: Keum GB, Choi Y, Kang J Formal analysis: Kim ES, Kim S, Keum GB, Ryu S Methodology: Cho JH, Song M Validation: Kim S, Kwak J, Pandey S Writing - original draft: Doo H, Cho JH, Song M Writing - review & editing: Doo H, Cho JH, Song M, Kim ES, Kim S, Keum GB, Kwak J, Pandey S, Ryu S, Choi Y, Kang J, Kim HB, Lee JH
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Complete genome sequence of *Enterococcus faecium* strain AK_C_05 with potential characteristics applicable in livestock industry

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(Unstructured) Abstract (up to 350 words)

The *Enterococcus faecium* (*E. faecium*) strain AK_C_05 was isolated from cheonggukjang, the Korean traditional food, collected from a local market in South Korea. In this report, we presented the complete genome sequence of *E. faecium* strain AK_C_05. The genome of *E. faecium* strain AK_C_05 genome consisted of one circular chromosome (2,691,319 bp) with a guanine + cytosine (GC) content of 38.3% and one circular plasmid (177,732 bp) with a guanine + cytosine (GC) content of 35.48%. The Annotation results revealed 2,827 protein-coding sequences (CDSs), 18 rRNAs, and 68 tRNA genes. It possesses genes, which encodes enzymes such as alpha-galactosidase (EC 3.2.1.22), beta-glucosidase (EC 3.2.1.21) and alpha-L-arabinofuranosidase (EC 3.2.1.55) enabling efficient utilization of carbohydrates. Based on Clusters of Orthologous Groups analysis, *E. faecium* strain AK_C_05 showed specialization in carbohydrate transport and metabolism indicating the ability to generate energy using a variety of carbohydrates.

Keywords (3 to 6):

Enterococcus faecium, Livestock, Carbohydrates

The main text

The Enterococci bacteria belong to lactic acid bacteria (LAB) group, which can be found in fermented foods[1]. Especially, *Enterococcus faecium* is also utilized as probiotics, which could enhance the microbial balance in animals[2]. Despite of safety concerns regarding its use as probiotics, recent research has explored the use of *Enterococcus faecium* as a feed additive for livestock to enhance growth performance[1, 3].

In the present study, the *E. faecium* strain AK_C_05 was isolated from homemade cheonggukjang, the Korean traditional food, collected from a local market in Cheonan (36.802917° N, 127.149796° E), Chungcheongnam-do, South Korea. Then, the whole genome sequencing was performed to understand the genomic characteristics of *E. faecium* strain AK_C_05 as a potential probiotic in the livestock industry. The *E. faecium* strain AK_C_05 was cultivated in Enterococcosel broth (MBcell, Seoul, South Korea) at 37℃ for 24 hours. Genomic DNA was extracted from the cultured *E. faecium* pellet using CTAB DNA extraction method. The complete genome of the *E. faecium* AK_C_05 was sequenced using the Oxford Nanopore Technologies MinION platform at eGnome (Seoul, South Korea). Briefly, library preparation was performed using Native barcoding Sequencing Kit (SQK_NBD114.24, V14) following the manufacturer's instructions (Oxford Nanopore Technologies, Oxford, UK). The prepared library was loaded into the MinION MK1b sequencing device (Oxford Nanopore) equipped with a MinION flow cell (MIN114, R10.4.1, Oxford Nanopore). The Oxford Nanopore sequencing produced 79,247 of long reads, resulting in a total of 572,297,864 base pairs. De novo assemble was performed using a Flye assembler v2.9.2, followed by polishing using the Homopolish polisher v0.4.1. The quality of genome assembly was assessed using Quality Assessment Tool for Genome Assemblies (QUAST) v5.2.0[4]. The quantitative assessment of the genome completeness was conducted using the Benchmarking Universal Single-Copy Orthologs (BUSCO) v5.4.6[5]. To annotate and predict the protein coding genes, rRNA, and tRNA genes of *E. faecium strain* AK_C_05, the Rapid Annotation using Subsystem Technology (RAST) v2.0 tool was utilized[6]. The functional categorization of all predicted protein coding genes was performed using the Clusters of Orthologous Groups (COGs)-based EggNOG-mapper v2.0[7]. Furthermore, the presence of virulence factors and antibiotic resistance in *E. faecium* strain AK_C_05 was predicted using the BLASTn method, with reference to the Virulence Factor Database (VFDB) and the Comprehensive Antibiotic Resistance Database (CARD)[8, 9].

The complete genome of the *E. faecium* strain AK_C_05 contain one circular chromosome (2,691,319 bp) with a guanine + cytosine (GC) content of 38.3% and one circular plasmid (177,732 bp) with a guanine + cytosine (GC) content of 35.48%. A total of 2,827 predicted protein-coding sequence, 18 rRNA genes, and 68 tRNA genes

were identified in *E. faecium* strain AK_C_05. The most abundant COGs category, excluding Function unknown [S], was Carbohydrate transport and metabolism [G], which accounted for 235 genes, representing 10.4% of the total genes identified. The genome feature and map of *E. faecium* strain AK_C_05 were presented in Table 1, Figure 1A and 1B.

Based on its specific focus on carbohydrate transport and metabolism, *E. faecium* strain AK_C_05 possesses genes and enzymes, such as alpha-galactosidase (EC 3.2.1.22), beta-glucosidase (EC 3.2.1.21) and alpha-L-arabinofuranosidase (EC 3.2.1.55), that enable efficient utilization of carbohydrates and the capacity to derive energy from diverse carbohydrate substrates. This characteristic makes *E. faecium* strain AK_C_05 a potential candidate for application in the livestock industry. The complete genome of *E. faecium* strain AK_C_05 has indicated the presence of the antibiotic resistance gene aac (6')-Ii in the chromosome and not in the plasmid, confirming that there is no potential for transmission of the resistance gene to other microorganisms. In the plasmid of *E. faecium* strain AK_C_05, the filA gene was detected, while no other virulence factors were identified. Interestingly, the filA gene's ability to facilitate adhesion to the cell wall is regarded as a beneficial trait for probiotics[10]. Overall, our results indicate that *E. faecium* AK_C_05 could be a promising functional probiotic for improving growth performance in the livestock industry.

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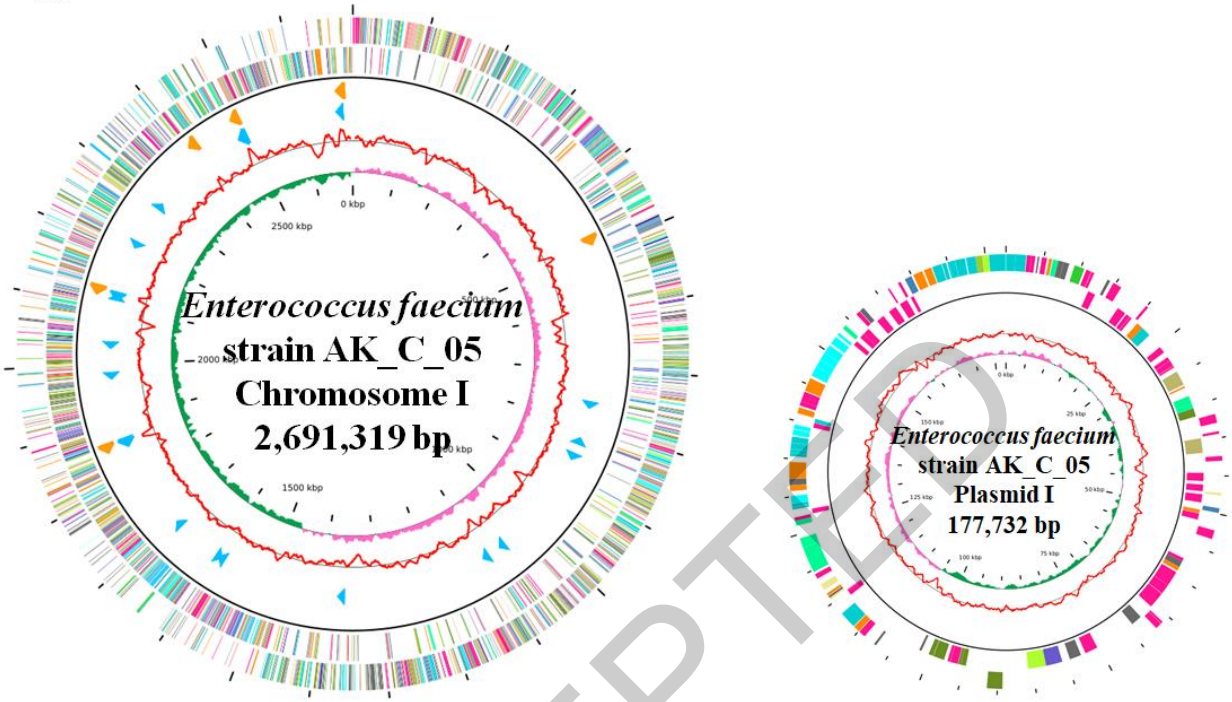
Tables and Figures

A



Type	Contig size (bp)	GC-content (%)	Protein-coding genes	tRNA	rRNA
Chromosome	2,691,319	38.3	2,629	68	18
Plasmid	177,732	35.48	198	-	-

B

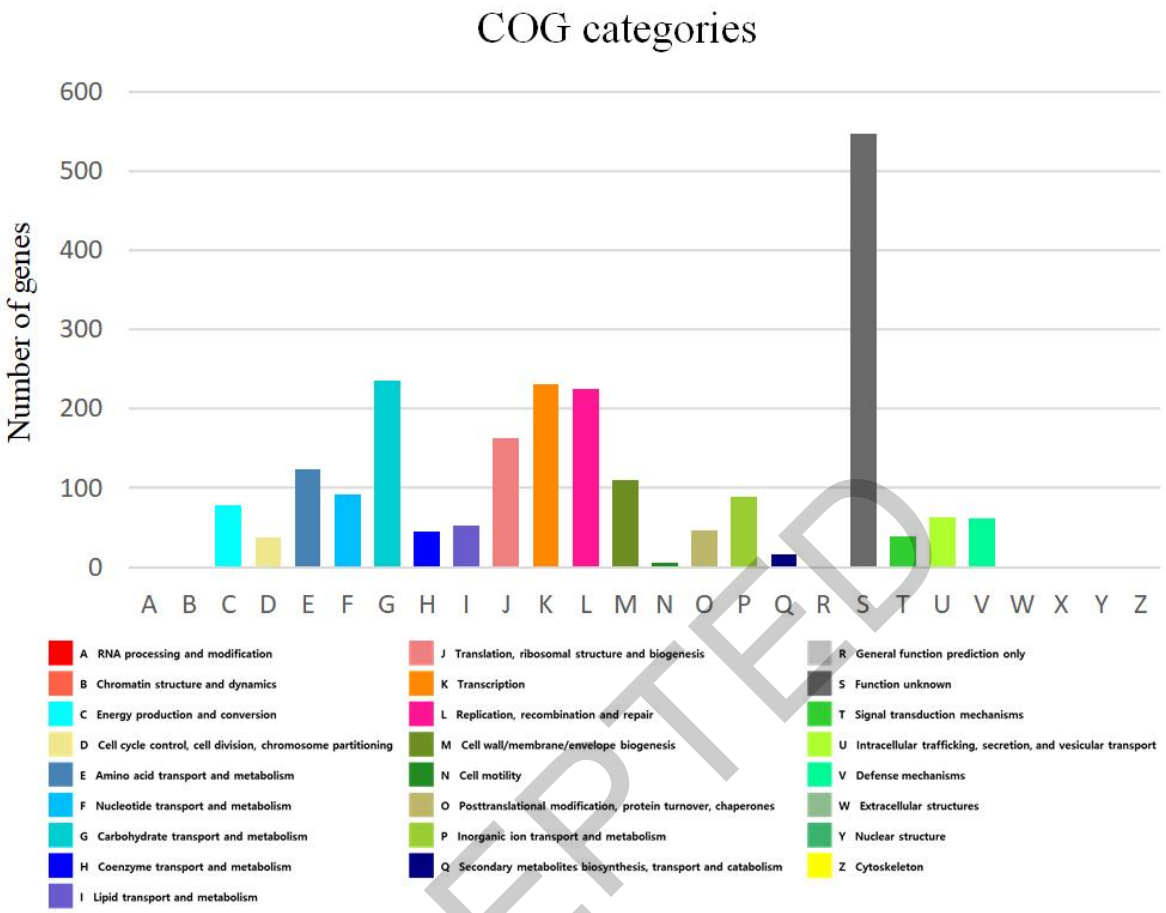


Figure 1. Genome map of *Enterococcus faecium* strain AK_C_05 and the functional categorization of predicted protein coding genes. The outer ring represents the positions of all annotated gene coding regions (ORFs), while the inner ring in red indicates the guanine + cytosine (GC) content. Peaks in pink and green indicate GC skew. The orange and sky-blue arrows represent rRNA and tRNA operons, respectively. The annotated ORFs are color-coded based on their Clusters of Orthologous Groups (COG) assignments in Figure 1A. The COG functional categories of the predicted protein coding genes are represented in Figure 1B.

136

137 **Table 1. Genome features of *Enterococcus faecium* strain AK_C_05**

Property	Term	
	Chromosome	**Plasmid**
Contig length (bp)	2,691,319 bp	177,732 bp
No. of contig	1 (chromosome)	1 (plasmid)
Guanine + cytosine (G + C)	38.3	35.48
Protein-coding genes	2,629	198
rRNA genes	18	-
tRNA genes	68	-
Genbank Accession No.	CP128995	CP128994

138