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| **Article Type** | Genome Announcement |
| **Article Title (within 20 words without abbreviations)** | Complete genome sequence of Enterococcus faecium strain AJ_C_05 with potential characteristics to break down carbohydrates, applicable to livestock industry |
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# Complete genome sequence of *Enterococcus faecium* strain AJ_C_05 with potential characteristics to break down carbohydrates, applicable to livestock industry

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## Abstract (up to 150 words)

*Enterococcus faecium* (*E. faecium*) strain AJ_C_05 was isolated from a Deonjang, a traditional Korean fermented food made from soybeans, and its complete genome was sequenced using the Oxford MinION platform. The complete genome of *E. faecium* strain AJ_C_05 has one circular chromosome and two plasmids. The chromosome of *E. faecium* strain AJ_C_05 consists of 2,623,546 base pairs (bp) with a guanine + cytosine (G+C) content of 38.3%, 2,680 coding sequences, 69 tRNAs, and 18 rRNAs. One plasmid is 164,853 bp long and has a G+C content of 35.5%, and the other plasmid is 11,235 bp long with G+C content of 33.1%. *E. faecium* strain AJ_C_05 possesses genes for enzyme determinants, such as alpha-galactosidase (EC 3.2.1.22), beta-glucosidase (EC 3.2.1.21), cellulases (EC 3.2.1.4), chitinase (EC 3.2.1.122), proteases (EC 3.4.21.112), and xylanase (EC 3.2.1.8). These characteristics of *E. faecium* strain AJ_C_05 appears to indicate its potential to be used as probiotic feed additives. This study offers valuable insight into the functionality of *E. faecium* isolated from soybean paste, which has the potential to promote digestion when used as a feed additive.

## Keywords (3 to 6)

Whole genome sequencing, *Enterococcus faecium*, Feed additive

## The main text

*Enterococcus faecium (E. faecium)* is recognized for its biological properties, which include the ability to inhibit harmful bacteria, good adhesion to the intestinal mucosa, and resistance to bile salts, stomach acid, and heat [1]. Because of these properties, it is widely used as probiotics for humans and animals. Previous studies have shown that adding *E. faecium* as a probiotics to weaning piglet feed improved growth and feed conversion [2]. *Enterococcus* is dominant microbiota of Doenjang, a traditional Korean fermented food made from soybeans [3].

In this study, we isolated *Enterococcus faecium* strain AJ_C_05 from a homemade soybean paste, Doenjang, collected from a local market in Cheonan-si, Chungcheongnam-do, Republic of Korea. *E. faecium* strain AJ_C_05 was aerobically cultured in Enterococcosel broth (MBcell, Seoul, South Korea) at 37℃ for 24 hours. The genomic DNA of the AJ_C_05 strain was extracted using the TaKaRa MiniBEST Bacterial Genomic DNA Extraction Kit Ver. 3.0 (TaKaRa, Shiga, Japan) following the manufacturer's instructions. The complete genome of *E. faecium* AJ_C_05 was sequenced using the Oxford Nanopore Technologies MinION platform. Briefly, a library was prepared using an Oxford Nanopore Ligation Sequencing Kit (SQK-LSK109) (Oxford Nanopore, Oxford, UK), and the sequencing process was performed on a MinION sequencing device equipped with a MinION flow cell (R9.4.1) (Oxford Nanopore). A total of 322,486,606 base pairs with 91,650 reads were obtained, and genome assembly was performed de novo using the Canu assembler (v. 1.8) and Flye assembler (v. 2.9.2) with ‘nano-corr’ options. The Homopolish polisher (v. 0.4.1) was utilized to correct errors in the assembled draft genome. Quality Assessment Tool for Genome Assemblies (QUAST) (v. 5.0.2) and Benchmarking Universal Single-Copy Orthologs (BUSCO) (v. 5.4.6) were used to quantitatively evaluate the completeness of the genome assembly. For genome functional annotation, the Rapid Annotation using Subsystem Technology (RAST) (v. 2.0) and EggNOG-mapper (v. 2.0) were utilized [4, 5] . In addition, ResFinder (v.4.4.0) and BLASTn method with the Virulence Factor Database (VFDB) were used to identify antibiotic resistance genes and virulence factors, respectively.

The *E. faecium* strain AJ_C_05 consists of the one circular chromosome and two plasmids. The chromosome genome comprises 2,623,546 base pairs (bp) with a guanine + cytosine (G+C) content of 38.3%, 2,680 coding sequences, 69 tRNAs, and 18 rRNAs. One plasmid is 164,853 bp long with a G+C content of 35.5% and the other is 11,235 bp long with a G+C content of 33.1% (Table 1, Fig. 1A). We compared the whole genome of *E. faecium* strain AJ_C_05 with that of *Enterococcus faecium* T110 (NCBI accession number: PRJNA207757), a strain known for its probiotic properties isolated from human microbiome in Japan [6]. *E. faecium* T110 has a chromosome size of 2,693,877 bp, a G+C content of 38.4%, and 2,639 protein-coding genes. The two strains have similar chromosome

size, G+C content, and protein-coding genes, and an ANI value of 97.80%, indicating a high degree of similarity. Excluding an ‘unknown function’, the most COGs categories of *E. faecium* AJ_C_05 were ‘carbohydrate transport and metabolism [G]’, ‘Transcription [K]’, ‘Replication, recombination and repair [L]’, ‘Translation, ribosomal structure and biogenesis [J]’, ‘Amino acid transport and metabolism [E]’ and ‘Cell wall/membrane/envelope biogenesis [M]’ (Fig. 1B).

*E. faecium* strain AJ_C_05 possesses genes for enzyme determinants, such as cellulases (EC 3.2.1.4), proteases (EC 3.4.21.112), and xylanase (EC 3.2.1.8). Xylanase is an enzyme that specifically targets xylans, which are key carbohydrate components in the cell walls of grains [7]. Cellulase can hydrolyze non-starch polysaccharides (NSP) and destroy cell walls [8]. Protease contributes to reducing nitrogen emissions into the environment by optimizing protein utilization [9]. In addition, *E.faecium* strain AJ_C_05 contains the genes for enzymes such as beta-glucosidase (EC 3.2.1.21), chitinase (EC 3.2.1.122), and alpha-galactosidase (EC 3.2.1.22) that can break down carbohydrates.

The complete genome for *E. faecium* strain AJ_C_05 reveals that the antibiotic resistance gene aac (6')-Ii is located on the chromosome. However, its transfer to other bacteria is hindered because of its presence on the chromosome. The pilF, encoding PilA-type pilus structure, was detected on the plasmid I. Despite being identified as a virulence factor, it may also contribute to bacterial colonization on cell surface structures, potentially benefiting probiotic gut colonization [10]. The characteristics of *E. faecium* strain AJ_C_05 suggests its potential for use as probiotic feed additives. This study provides valuable insights into the functionality of the newly observed *E. faecium* strain AJ_C_05 isolated from Doenjang, which has the potential to aid digestion when used as a bacterial feed additive for livestock.

# References (Vancouver or NLM style)


1. Kopit LM, Kim EB, Siezen RJ, Harris LJ, Marco ML. Safety of the surrogate microorganism Enterococcus faecium NRRL B-2354 for use in thermal process validation. Applied and Environmental Microbiology. 2014;80(6):1899-909. https://doi.org/10.1128/AEM.03859-13

2. Mallo J, Riopérez J, Honrubia P. The addition of Enterococcus faecium to diet improves piglet's intestinal microbiota and performance. Livest Sci. 2010;133(1-3):176-8. https://doi.org/10.1016/j.livsci.2010.06.057

3. Jeong D-W, Kim H-R, Jung G, Han S, Kim C-T, Lee J-H. Bacterial community migration in the ripening of doenjang, a traditional Korean fermented soybean food. Journal of Microbiology and Biotechnology. 2014;24(5):648-60. https://doi.org/10.4014/jmb.1401.01009

4. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC genomics. 2008;9:1-15. https://doi.org/10.1186/1471-2164-9-75

5. Cantalapiedra CP, Hernández-Plaza A, Letunic I, Bork P, Huerta-Cepas J. eggNOG-mapper v2: functional annotation, orthology assignments, and domain prediction at the metagenomic scale. Molecular biology and evolution. 2021;38(12):5825-9. https://doi.org/10.1093/molbev/msab293

6. Natarajan P, Parani M. First complete genome sequence of a probiotic Enterococcus faecium strain T-110 and its comparative genome analysis with pathogenic and non-pathogenic Enterococcus faecium genomes. Journal of genetics and genomics= Yi chuan xue bao. 2014;42(1):43-6. https://doi.org/10.1016/j.jgg.2014.07.002

7. Li N, Yang P, Wang Y, Luo H, Meng K, Wu N, et al. Cloning, expression, and characterization of protease-resistant xylanase from Streptomyces fradiae var. k11. Journal of Microbiology and Biotechnology. 2008;18(3):410-6.

8. Adeola O, Cowieson A. Board-invited review: opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. Journal of animal science. 2011;89(10):3189-218. https://doi.org/10.2527/jas.2010-3715

9. Pan L, Zhao P, Yang Z, Long S, Wang H, Tian Q, et al. Effects of coated compound proteases on apparent total tract digestibility of nutrients and apparent ileal digestibility of amino acids for pigs. Asian-Australasian journal of animal sciences. 2016;29(12):1761. https://doi.org/10.5713/ajas.16.0041

10. Li B, Zhan M, Evivie SE, Jin D, Zhao L, Chowdhury S, et al. Evaluating the safety of potential probiotic Enterococcus durans KLDS6. 0930 using whole genome sequencing and oral toxicity study. Frontiers in Microbiology. 2018;9:1943. https://doi.org/10.3389/fmicb.2018.01943

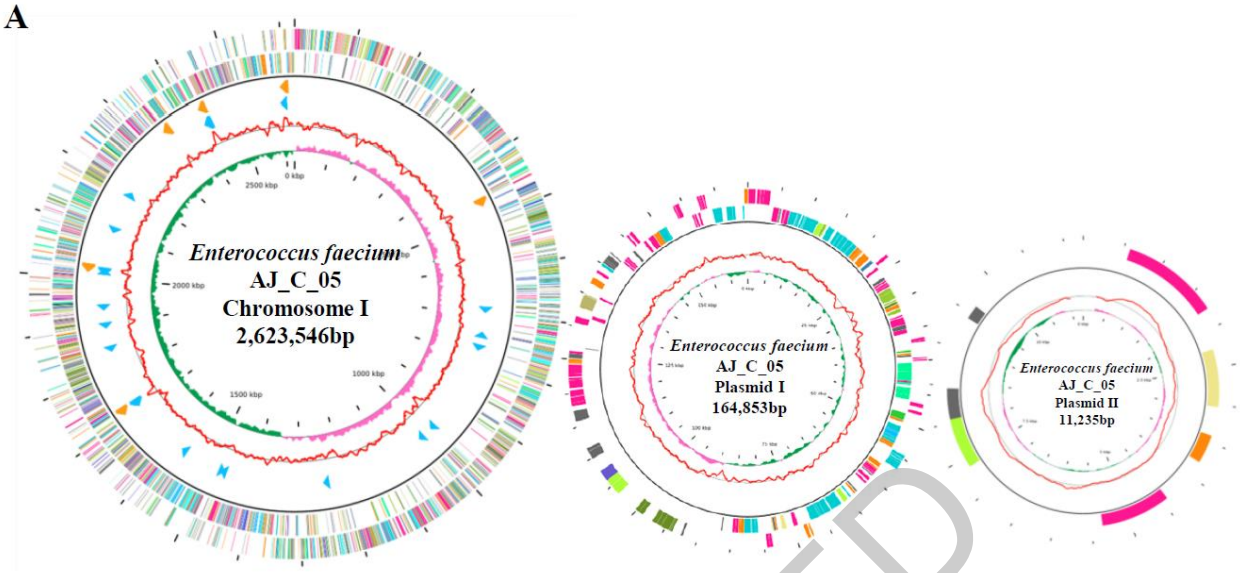
# Table

**Table 1. Genome features of *Enterococcus faecium* strain AJ_C_05**

| Property | Term | | |
|---|---|---|---|
| | **Chromosome** | **Plasmid1** | **Plasmid2** |
| Contig length(bp) | 2,623,546bp | 164,853bp | 11,235bp |
| Guanine + cytosine (%) | 38.3 | 35.5 | 33.1 |
| Protein-coding genes | 2680 | 278 | 24 |
| tRNA genes | 69 | - | - |
| rRNA genes | 18 | - | - |
| Genbank Accession No. | CP138459.1 | CP138458.1 | CP138460.1 |

# Figure

**A**




| Type | Length (bp) | GC-content (%) | CDSs | tRNA | rRNA |
|---|---|---|---|---|---|
| Chromosome | 2,623,546 | 38.3 | 2680 | 69 | 18 |
| Plasmid I | 164,853 | 35.5 | 278 | - | - |
| Plasmid II | 11,235 | 33.1 | 24 | - | - |

**B**

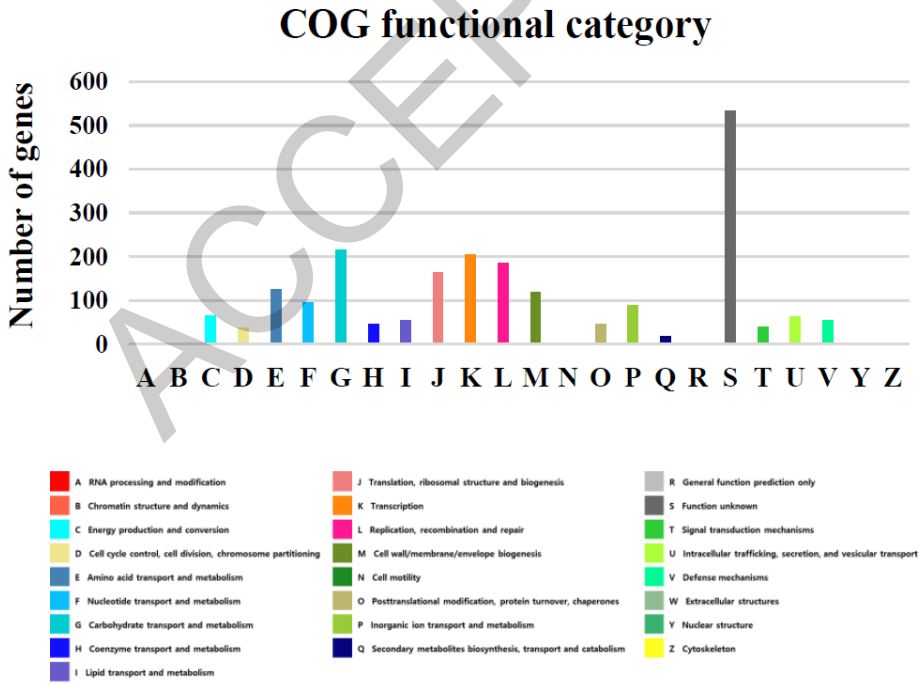





**Figure 1. Genome map of *Enterococcus faecium* strain AJ_C_05 and the functional categorization of predicted protein coding genes.**

The exterior circle delineates the locations of all identified gene coding sequences (ORFs), while the inner circle, highlighted in red, showcases the guanine + cytosine (GC) content. Variations in pink and green represent the GC skew. The representations of rRNA and tRNA operons are marked with orange and sky-blue arrows, correspondingly. The color-coded ORFs are aligned with their Clusters of Orthologous Groups (COG) designations as shown in Figure 1A. The COG functional classifications of the anticipated protein coding genes are illustrated in Figure 1B.