

Complete genome sequence of *Enterococcus faecalis* strain DM01, a potential starter culture candidate for soybean fermentation

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콩발효 종균후보 *Enterococcus faecalis* strain DM01의 유전체 염기서열

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Enterococcus faecalis strain DM01 was isolated from meju, a traditional Korean fermented soybean product. The strain did not exhibit resistance to eight antibiotics (ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, penicillin G, tetracycline, and vancomycin), biofilm formation, and hemolytic activity. The genome of strain DM01 consists a single circular 2,785,968-bp chromosome with a G + C content of 37.68%. The complete genomic sequence of strain DM01 provides genetic information to support the absence of antibiotic resistance, biofilm formation, and hemolytic activity.

Keywords: *Enterococcus faecalis*, meju, soybean fermentation, starter

Enterococcus faecalis belongs to lactic acid bacteria has been used as a starter culture for cheese production (Foulquie Moreno *et al.*, 2006). Some strains have been used as probiotics to improve human health and to enhance farm animals growth (Franz *et al.*, 2011; Yamaguchi *et al.*, 2013). The pharmaceutical company SymbioPharm (Germany) sells a medicine Symbioflor 1 containing *E. faecalis* culture for the illness of upper respiratory system. Recently, *E. faecalis* has been reported as a predominantly isolated lactic acid bacterium of

fermented soybean foods (Jeong *et al.*, 2014; Park *et al.*, 2017).

We isolated *E. faecalis* DM01 from a commercial meju, a traditionally manufactured Korean fermented soybean product used for the production of traditional soybean sauce and paste. The strain sustained growth on tryptic soy agar (TSA; BD Diagnostic Systems) containing 7% (w/v) NaCl of final concentration. The strain exhibited proteolytic activity on TSA containing 6% NaCl and 2% skim milk (Sigma-Aldrich), and lipolytic activity on TSA containing 4% NaCl and 1% tributyrin (Sigma-Aldrich) (Jeong *et al.*, 2014). Both activities are the prerequisite of starter culture candidate for soybean fermentation. When the European Food Safety Authority guidelines for the safe use of microorganisms as food/feed materials were applied (EFSA, 2004), strain DM01 was susceptible to eight antibiotics (ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, penicillin G, tetracycline, and vancomycin) and exhibited neither hemolysis nor biofilm formation. To shed light on the genetic background behind safety of *E. faecalis* DM01 as a fermentation starter candidate, its complete genome was sequenced.

Whole-genome sequencing was performed using the PacBio Single-Molecule Real-Time (SMRT) sequencing system by ChunLab, Inc. One contig was generated from reads from the PacBio system (90,291 reads and 968.28× coverage) for *E.*

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faecalis DM01 with the HGAP4 algorithm in SMRT Link version 7.0.1 (Pacific Bioscience) and CodonCode Aligner (CodonCode Co.). Genome annotation was carried out using NCBI Prokaryotic Genome Annotation Pipeline version 4.6 (Tatusova *et al.*, 2016). Open reading frames were predicted using Glimmer 3 (Tatusov *et al.*, 1997), followed by annotation through a search against the Clusters of Orthologous Groups database (Tatusov *et al.*, 1997).

The complete genome of strain DM01 consists of a single circular 2,785,968-bp chromosome with a G + C content of 37.68% (Table 1). The genome is predicted to contain 2,611 open reading frames, 59 tRNA genes, and 12 rRNA genes. In total, 2,432 genes were functionally assigned to categories based on the Clusters of Orthologous Groups. The majority of the genes are related to carbohydrate transport and metabolism [248 genes (10.2%)], followed by transcription [199 genes (8.2%)] and amino acid transport and metabolism [177 genes (7.3%)]. The high proportion of genes in carbohydrate and amino acid utilization indicates that strain DM01 has the ability to use of soybean carbohydrates and proteins. The genome of strain DM01 does not encode the virulence factors such as α -hemolysin, β -hemolysin, biofilm formation, and enterotoxin. Although strain DM01 showed sensitive to eight antibiotics (ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, penicillin G, tetracycline, and vancomycin), two potential antibiotic resistance genes annotated to encode chloramphenicol resistance protein (FLL49_RS11675) and erythromycin resistance ATP-binding protein (FLL49_RS06530) were identified. It is well known that annotated antibiotic resistance genes do not always link with phenotypic resistance (Jeong *et al.*, 2017). The phenotypic susceptibility of strain DM01 to chloramphenicol and erythromycin insinuates that

both genes are involved in the general metabolic process of its possessor. The complete genome sequence of *E. faecalis* DM01 provides genetic information to support the absence of antibiotic resistance, biofilm formation, and hemolytic activity.

Nucleotide sequence accession number(s)

E. faecalis DM01 has been deposited in the Korean Collection for Type Cultures under accession number KCTC 43096, and its complete genome sequence has been deposited in DDBJ/ENA/GenBank under accession number CP041344.

적 요

메주로부터 분리한 *Enterococcus faecalis* DM01 균주는 8종의 항생제(ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, penicillin G, tetracycline, vancomycin) 저항성, 바이오필름 및 용혈현상을 나타내지 않았다. GC 함량 37.68%, 2,785,968 bp 크기의 단일 chromosome을 보유하고 있는 DM01 균주의 유전체 정보는 콩발효식품 제조용 종균 적용에 요구되는 항생물질 저항성, 바이오필름 및 용혈현상 관련 안전성을 뒷받침하는 것으로 확인되었다.

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Table 1. Genome features of *Enterococcus faecalis* strain DM01

Features	Value
Genome size (bp)	2,785,968
G + C content (%)	37.68
Open reading frames	2,611
CDS assigned by COG	2,432
rRNA genes	12
tRNA genes	59

Abbreviations: CDS, coding DNA sequence; COG, Cluster of Orthologous Groups of proteins.

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