



Complete genome sequence of *Bacillus licheniformis* strain 0DA23-1, a potential starter culture candidate for soybean fermentation

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콩발효 종균후보 *Bacillus licheniformis* 0DA23-1의 유전체 염기서열

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Bacillus licheniformis strain 0DA23-1, a potential fermentation starter candidate, was isolated from doenjang, a Korean high-salt-fermented soybean food. Strain 0DA23-1 contains a single circular 4,405,373-bp chromosome with a G + C content of 45.96%. The complete genome of strain 0DA23-1 does not include any of the virulence factors found in the well-known pathogens *Bacillus cereus* and *Staphylococcus aureus*. Additionally, no genes associated with resistance to eight antibiotics (chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, streptomycin, tetracycline, and vancomycin), hemolysis, or biofilm formation were identified.

Keywords: *Bacillus licheniformis*, doenjang, soybean fermentation, starter

The genus *Bacillus* is the predominant bacterial group found in the fermented soybean foods of East Asia, including Korea (Kim *et al.*, 2009; Lee *et al.*, 2010). *Bacillus licheniformis*, a predominantly identified *Bacillus* species, exhibits growth on tryptic soy agar (TSA; Difco) supplemented with 14% (w/v) NaCl and enzyme, including amylase, protease, and lipase, activities (Jeong *et al.*, 2014, 2017b). Because of these properties,

B. licheniformis has the potential to be applied as a fermentation starter culture in soybean foods, including the Korean doenjang, a high-salt-fermented soybean paste ripened at NaCl concentrations of > 12% (w/w). Recently, we isolated *B. licheniformis* '0DA23-1' from a commercial doenjang. This strain sustained growth on TSA containing 14% NaCl, and exhibited proteolytic and lipolytic activities on TSA containing 3% and 4% NaCl, respectively. When the safety of strain 0DA23-1 was evaluated using the European Food Safety Authority guidelines for the safe use of microorganisms as food/feed materials (EFSA, 2004; Jeong *et al.*, 2017a), it was found to be susceptible to eight antibiotics (chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, streptomycin, tetracycline, and vancomycin) and did not exhibit either hemolysis or biofilm formation (data not shown). To confirm the genetic background of *B. licheniformis* 0DA23-1 as a safe fermentation starter candidate, its complete genome was sequenced.

Whole-genome sequencing was performed using the PacBio Single-Molecule Real-Time sequencing system by ChunLab, Inc. One contig was generated from a hybrid assembly of reads from the PacBio system (173.26 × coverage) for *B. licheniformis* 0DA23-1. The reads were assembled using CLC Genomics Workbench ver. 7.5.1 (CLC Bio) and CodonCode Aligner (CodonCode Co.). Gene predictions were performed using

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Glimmer 3 (Tatusov *et al.*, 1997), followed by annotation through a search against the Clusters of Orthologous Groups (COG) database (Tatusov *et al.*, 1997).

The complete genome of strain 0DA23-1 consists of a single circular 4,405,373-bp chromosome with a G + C content of 45.96% (Table 1). The genome is predicted to contain 4,518 protein coding sequences, 81 tRNA genes, and 24 rRNA genes. In total, 4,104 genes were functionally assigned to categories based on COG. The majority of the genes are related to transcription [331 genes (8.1%)], followed by carbohydrate transport and metabolism [327 genes (8.0%)] and amino acid transport and metabolism [312 genes (7.6%)]. The high proportion of genes in nutrient utilization indicates that strain 0DA23-1 has the ability to degrade a wide range of soybean carbohydrates and proteins. The genome of strain 0DA23-1 does not encode any of the virulence factors found in the food pathogens *Bacillus cereus* and *Staphylococcus aureus*. Additionally, no genes involved in the resistance to eight antibiotics (chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, streptomycin, tetracycline, and vancomycin), hemolysis, or biofilm formation were identified from the annotated genes based on COG. The genomic traits of strain 0DA23-1 satisfied the requirements of a functional and safe fermentation starter candidate that can be applied to produce fermented soybean foods. The complete genome sequence of strain 0DA23-1 provides information that will aid in the selection of functional and safe *Bacillus* starter culture candidates for soybean food fermentation.

Nucleotide sequence accession numbers

B. licheniformis 0DA23-1 has been deposited in the Korean

Table 1. Genome features of *Bacillus licheniformis* strain 0DA23-1

Features	Value
Genome size (bp)	4,405,373
G + C content (%)	45.96
Open reading frames	4,518
CDS assigned by COG	4,104
rRNA genes	24
tRNA genes	81

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

Collection for Type Cultures under accession number KCTC 43013, and its complete genome sequence has been deposited in DDBJ/ENA/GenBank under accession number CP031126.

적 요

된장으로부터 콩발효 종균후보 *Bacillus licheniformis* 0DA23-1 가 분리되었다. 0DA23-1 균주는 GC 함량 45.96%, 약 4.4 Mb 크기의 단일 chromosome을 보유하고 있었고, 식중독균 *Bacillus cereus* 및 *Staphylococcus aureus*가 보유한 위해성 유전자는 유전체로부터 확인되지 않았다. 또한, 8종의 항생물질(chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, streptomycin, tetracycline, vancomycin) 저항성 및 혈청분해 활성, 바이오필름 생성 관련 유전자도 확인되지 않았다.

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