Complete genome sequence of a potential probiotic *Lacticaseibacillus* rhamnosus strain LDTM 7511, isolated from Korean infant feces

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한국인 유아 분변에서 분리한 프로바이오틱스 후보 균주 Lacticaseibacillus rhamnosus LDTM 7511의 유전체 염기서열

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Lacticaseibacillus rhamnosus strain LDTM 7511 isolated from Korean infant feces have been found to have beneficial effects in alleviating intestinal inflammation and modulating gut bacterial dysbiosis in a dextran sulfate sodium-induced colitis murine model. Here, we report the complete genome sequence of Lacticaseibacillus rhamnosus strain LDTM 7511. The genome consisted of 3,007,472 bp with a GC content of 46.7% that was predicted to contain 2,818 genes, including 2,670 protein-coding genes. Functional annotation revealed the potential probiotic traits within the genome.

Keywords: Lacticaseibacillus rhamnosus, complete genome, probiotics

Nomadic lactobacilli, isolated from a broad range of habitats, possess metabolic flexibility for adaptation due to their relatively larger genome size than host-specified species (Duar et al., 2017). Lacticaseibacillus rhamnosus, a representative nomadic species, belongs to a phylogenetic cluster within the L. casei group in the family Lactobacillaceae (Zheng et al., 2020). of the most well-researched probiotic strains, and its beneficial effects on the host have been reported (Gorbach et al., 2017). In a previous study, L. rhamnosus strain LDTM 7511 was isolated from Korean infant feces, and we demonstrated its antiinflammatory and gut microbiota modulatory effects in a mouse colitis model, suggesting its potential as a microbial therapeutic for patients with inflamed gut (Yeo et al., 2020).

Lacticaseibacillus rhamnosus strain GG (ATCC 53103) is one

With the approval of the Ethics Committee of Seoul National University (IRB no. 1702/011-004), infant fecal samples were collected, diluted, and plated onto de Man Rogosa and Sharp agar (Difco), and incubated for 48 h at 37°C in an anaerobic chamber (Coy Laboratory Products) with an atmosphere consisting of 5% CO₂, 10% H₂, and 85% N₂. From the physiological traits of lactic acid bacteria, the L. rhamnosus LDTM 7511 was isolated (Yeo et al., 2020). Genomic DNA was extracted with QIAamp DNA Mini Kit (Qiagen) and purified using the QIAquick PCR Purification Kit (Qiagen). The SMRTbell library was constructed using the PacBio DNA Template Prep Kit 1.0 and sequenced in the PacBio RS II (Pacific Biosciences) sequencing platform

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(Macrogen Corporation). Subsequent steps are based on the PacBio Sample Net-Shared Protocol, which is available at http://pacificbiosciences.com/.

Quality-filtered 251,576 reads (mean subread, 5,941; total number of bases, 1,494,635,082; N₀, 7,514; genome coverage, 496.9×) were de novo assembled with HGAP3 v. 3.0 and annotated in NCBI Prokaryotic Genomic Annotation Pipeline (PGAP v. 5.1). The genome pairwise comparison on DSMZ Type Strain Genome Server (Meier-Kolthoff et al., 2022) identified 79.6% dDDH between strain LDTM 7511 and L. rhamnosus JCM 1136^T (GenBank accession no. GCF 001435405.1), which was 97.25% average nucleotide identity (http://www. ezbiocloud.net). The complete genome sequence of the strain LDTM 7511 was 3,007,472 bp, with a GC content of 46.7% (Table 1). 2,353 genes were predicted to belong to the Cluster of Orthologous Group (COG) families in the EggNOG database v. 5.0 (Huerta-Cepas et al., 2019), of which 101 genes were attributed to multiple functional descriptions. The strain LDTM 7511 genome contained genes related to probiotic properties, such as stress resistance (D-alanylation of lipoteichoic acid protein, HF522 13795 and HF522 13810; Clp ATPase, HF522 05075 and HF522 07365), cell surface adherence (sortase family,

Table 1. Genomic features of L. rhamnosus LDTM 7511

Genomic feature	Value
Contig	1
Genome size (bp)	3,007,472
GC content (%)	46.7
Genes	2,818
Protein-coding genes (CDSs)	2,670
tRNA genes	59
rRNA genes	15
Non-coding RNA genes	3

HF522_06845; fibronectin-binding protein, HF522_10320), stress tolerance and microbial interactions (S-ribosylhomocysteine lyase, HF522_13950), as well as immunomodulation (HF522_13795) (Lebeer *et al.*, 2008). Secondary metabolite biosynthesis gene cluster analysis revealed the presence of genes encoding carnocin CP52 and enterocin X-chainβ in the BAGEL4 database (van Heel *et al.*, 2018) and gene clusters responsible for lactococcin G transporter and type III polyketide synthase (T3PKS) in antiSMASH v. 6.0 (Blin *et al.*, 2021) (Fig. 1). No intact prophage regions were detected using the PHASTER tool (Arndt *et al.*, 2016). Putative genes associated with antibiotic

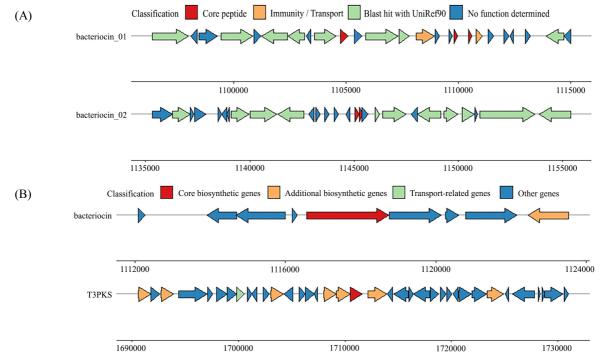


Fig. 1. The gene clusters responsible for secondary metabolite synthesis in the genome of *L. rhamnosus* strain LDTM 7511. (A) Predicted two potential bacteriocin-producing clusters in BAGEL4. (B) Predicted bacteriocin and type III polyketide synthase (T3PKS) regions in antiSMASH.

resistances or virulence genes, such as hemolysin-related genes and toxin-related genes, were not identified within the LDTM 7511 genome in the Comprehensive Antibiotic Resistance Database (CARD; McArthur *et al.*, 2013) and the Virulence Factor Database (VFDB; Chen *et al.*, 2005). This present genomic information will provide a scientific basis for the probiotic features of *L. rhamnosus* strain LDTM 7511.

Nucleotide sequence accession numbers

The complete genome sequence for strain LDTM 7511 has been deposited under GenBank accession no. CP051227.1 (BioProject accession no. PRJNA623728 and BioSample accession no. SAMN14558312), and the strain has been deposited at Korean Collection for Type Cultures (KCTC) under the accession no. KCTC18735P.

적 요

Lacticaseibacillus rhamnosus 균주 LDTM 7511은 마우스 대장염 모델에서 염증 완화 및 장내 균총의 불균형 조절 효과가 우수한 균주로, 한국인 유아 분변에서 분리되었다. 3,007,472 bp, GC 함량 46.7%인 균주 LDTM 7511의 유전체는 2,670개의 단백질 암호화 유전자를 포함하여 2,818 유전자를 보유하는 것으로 예측되었다. 또한 유전자의 기능 예측 분석을 통해 LDTM 7511 균주 유전체에서 프로바이오틱스 특성과 연관성이 있는 유전자들을 확인하였다.

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Conflict of Interest

The authors declare that they have no conflict of interests.

Ethical Statement

The study was approved by the Ethics Committee of Seoul National University (IRB no. 1702/011-004).

References

- Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, and Wishart DS. 2016. PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res.* 44, W16–W21.
- Blin K, Shaw S, Kloosterman AM, Charlop-Powers Z, van Wezel GP, Medema MH, and Weber T. 2021. antiSMASH 6.0: improving cluster detection and comparison capabilities. *Nucleic Acids Res.* 49, W29–W35.
- Chen L, Yang J, Yu J, Yao Z, Sun L, Shen Y, and Jin Q. 2005. VFDB: a reference database for bacterial virulence factors. *Nucleic Acids Res.* 33. D325–D328.
- Duar RM, Lin XB, Zheng J, Martino ME, Grenier T, Pérez-Muñoz ME, Leulier F, Gänzle M, and Walter J. 2017. Lifestyles in transition: evolution and natural history of the genus *Lactobacillus*. *FEMS Microbiol. Rev.* 41, S27–S48.
- Gorbach S, Doron S, and Magro F. 2017. *Lactobacillus rhamnosus* GG, pp. 79–88. *In* Floch MH, Ringel Y, and Walker WA. (eds.), The Microbiota in Gastrointestinal Pathophysiology: Implications for Human Health, Prebiotics, Probiotics, and Dysbiosis. Academic Press, Cambridge, Massachusetts, USA.
- Huerta-Cepas J, Szklarczyk D, Heller D, Hernández-Plaza A, Forslund SK, Cook H, Mende DR, Letunic I, Rattei T, Jensen LJ, *et al.* 2019. eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Res.* 47, D309–D314.
- **Lebeer S, Vanderleyden J, and De Keersmaecker SC.** 2008. Genes and molecules of lactobacilli supporting probiotic action. *Microbiol. Mol. Biol. Rev.* **72**, 728–764.
- McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, Bhullar K, Canova MJ, De Pascale G, Ejim L, *et al.* 2013. The comprehensive antibiotic resistance database. *Antimicrob. Agents Chemother.* 57, 3348–3357.
- Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, and Göker M. 2022. TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. *Nucleic Acids Res.* **50**, D801–D807.
- van Heel AJ, de Jong A, Song C, Viel JH, Kok J, and Kuipers OP. 2018. BAGEL4: a user-friendly web server to thoroughly mine RiPPs and bacteriocins. *Nucleic Acids Res.* 46, W278–W281.
- Yeo S, Park H, Seo E, Kim J, Kim BK, Choi IS, and Huh CS. 2020. Anti-inflammatory and gut microbiota modulatory effect of *Lactobacillus rhamnosus* strain LDTM 7511 in a dextran sulfate sodium-induced colitis murine model. *Microorganisms* 8, 845.
- Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, O'Toole PW, Pot B, Vandamme P, Walter J, et al. 2020. A taxonomic note on the genus Lactobacillus: description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. Int. J. Syst. Evol. Microbiol. 70, 2782–2858.