J Anim Sci Technol 2023;65(1):271-274 https://doi.org/10.5187/jast.2022.e91



Journal of Animal Science and Technology

pISSN 2672-0191 eISSN 2055-0391

Complete genome sequence of Limosilactobacillus fermentum JNU532 as a probiotic candidate for the functional food and feed supplements

Bogun Kim^{1#}, Ziayo Meng^{2#}, Xiaoyue Xu¹, Seungwoo Baek¹, Duleepa Pathiraja¹, In-Geol Choi^{1*} and Sejong Oh^{2*}



²Division of Animal Science, Chonnam National University, Gwangju 61186, Korea



Received: Oct 2, 2022 Revised: Oct 23, 2022 Accepted: Oct 24, 2022

#These authors contributed equally to this work.

*Corresponding author

In-Geol Choi
Department of Biotechnology, College
of Life Sciences and Biotechnology,
Korea University, Seoul 02841, Korea.
Tel: +82-2-3290-3152
E-mail: igchoi@korea.ac.kr

Sejong Oh Division of Animal Science, Chonnam National University, Gwangju 61186, Korea.

Tel: +82-62-530-2116 E-mail: soh@jnu.ac.kr

Copyright © 2023 Korean Society of Animal Sciences and Technology. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID

Bogun Kim https://orcid.org/0000-0002-5493-1674 Ziayo Meng https://orcid.org/0000-0002-6344-4747

Abstract

Lactic acid bacteria (LAB) have been reported to possess various beneficial properties and are commonly used as probiotics. LAB play a crucial role in milk fermentation, industrial lactic acid fermentation, and health and medicine. *Limosilactobacillus fermentum* isolated from fermented dairy and food products is considered as 'Generally Recognized as Safe' by FDA. *Limosilactobacillus fermentum* plays an important role in modulation of the intestinal microbiota, enhancing the host immune system and improving feed digestibility. We isolated a probiotic candidate that was identified and named *Limosilactobacillus fermentum* JNU532. In a previous report, cell-free culture of *L. fermentum* JNU532 exhibited anti-melanogenic and antioxidant activities. In this study, we present the complete genome assembly of the bacterial strain JNU532. The final genome consists of one circular chromosome (2,077,416 base pairs) with a guanine + cytosine (GC) ratio of 51.5%.

Keywords: Limosilactobacillus fermentum, Probiotics, Food, Feed, Supplements

Recently, the genus *Lactobacillus* was divided into several genera [1], with the species *Lactobacillus* fermentum being assigned to the genus *Limosilactobacillus*. Limosilactobacillus fermentum is one of heterofermentatives and is used in the fermentation of milk, plants, and silage. Limosilactobacillus fermentum strains not only enhance the nutritional value and flavor of food but also its functional properties. This species has strong pH tolerance and good bile tolerance, and it can also reduce cholesterol content in the human body [2]. In addition, *L. fermentum* can inhibit harmful intestinal microbiota, lessen the activity of food allergens, reduce mutagenic and carcinogenic activities, display immunomodulatory activity, and lower cholesterol [3,4]. *Limosilactobacillus fermentum* JNU532 was isolated from local fermented kimchi in this study. In our previous study, an *L. fermentum* JNU532—derived fermentation broth demonstrated antioxidant properties and the ability to reduce melanin production by inhibiting the activity of tyrosinase in B16F10 melanoma cells. Therefore, *L. fermentum* JNU532 may be

https://www.ejast.org 271

Xiaoyue Xu https://orcid.org/0000-0001-9654-8676 Seungwoo Baek https://orcid.org/0000-0002-2461-4221 Duleepa Pathiraja https://orcid.org/0000-0001-6239-5958 In-Geol Choi

https://orcid.org/0000-0001-7403-6274 Sejong Oh

https://orcid.org/0000-0002-5870-3038

Competing interests

No potential conflict of interest relevant to this article was reported.

Funding sources

This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, and Forestry (IPET) through High Value-added Food Technology Development Program, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (grant 321034051HD020) and was supported by the National Research Foundation (NRF) grant funded by the Korea government (2021R1A4A1031220).

Acknowledgements

Not applicable.

Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions

Conceptualization: Choi IG, Oh S.
Data curation: Kim B, Choi IG, Oh S.
Formal analysis: Kim B, Meng Z.
Methodology: Kim B, Meng Z.
Software: Kim B, Xu X, Baek S.
Validation: Pathiraja D.
Investigation: Kim B, Meng Z, Choi IG.
Writing - original draft: Choi IG, Oh S.
Writing - review & editing: Kim B, Meng Z, Xu X, Baek S, Pathiraja D, Choi IG, Oh S.

Ethics approval and consent to participate

This manuscript does not require RB/IACUC approval because there are no human and animal participants.

considered a natural depigmentation agent. [5].

L. fermentum JNU532 was cultivated in Man-Rogosa-Sharpe (MRS) medium (Becton, Dickinson, Franklin, USA) for 24 h at 37°C. Genomic DNA was extracted with a HiGeneTM Genomic DNA Prep kit (BIOFACT, Daejeon, South Korea), according to the manufacturer's instructions.

The short-read sequencing library was prepared with an Illumina® DNA Prep kit (Illumina, San Diego, CA, USA). Sequencing was performed on the Illumina MiSeq platform (Illumina) using the Illumina MiSeq reagent kit V3 (300 bp, paired end). The long-read sequencing library was prepared using an Oxford Nanopore Ligation Sequencing Kit (Oxford Nanopore, Oxford, UK). Long-read sequencing was carried out on a MinION sequencing device (Oxford Nanopore) equipped with a MinION flow cell (R9.4.1, Oxford Nanopore). Illumina sequencing produced 1,244,607 paired end reads (433,289,083 bp), while 122,763 reads with an average length of 1,839 bases were obtained from Oxford Nanopore sequencing.

De novo genome sequence assembly was assembled performed using a Flye assembler (v. 2.9) [6] with default options. Adapter sequences from short reads were removed using TrimGalore (v. 0.6.7) [7] with the 'paired' parameter. Errors in the draft genome assembly were corrected with Pilon (v. 1.24) [8] with default parameters. Gene prediction of the chromosomal sequence was performed with Prokka (v. 1.14.5) [9]. The completeness of the genome assembly was assessed using BUSCO (v. 5.2.2) [10] with the OrthoDB v10 bacterial (bacteria_odb10) database.

The complete genome sequence of *L. fermentum* JNU532 consisted of one circular chromosome with a guanine + cytosine (GC) ratio of 51.5% (Table 1). A total of 2,113 genes, including 15 rRNA and 57 tRNA, were predicted in the genome (Fig. 1). The genomic information of *L.*

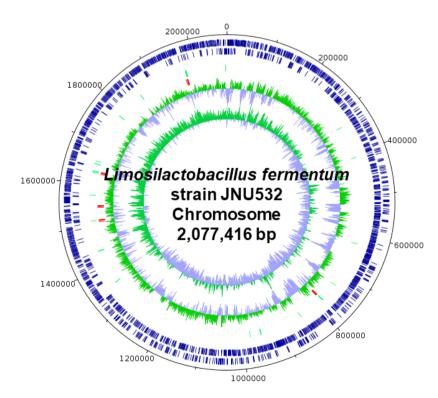


Fig. 1. Circular chromosome map of *Limosilactobacillus fermentum* JNU532. From the periphery to the center, marked features are as follows: protein—coding sequences on the forward strand, protein—coding sequences on the reverse strand, tRNA, rRNA, GC ratio, and GC skew. bp, base pair; G, guanine; C, cytosine; tRNA, transfer RNA; rRNA, ribosomal RNA.

Table 1. Genome features of Limosilactobacillus fermentum JNU532

	L. fermentum JNU532
Total genome length (bp)	2,077,416
GC content (%)	51.5
Depth	96.0
Genome completeness (%)	99.2
Protein-coding genes	2,041
tRNA	57
rRNA	15

bp, base pair; G, guanine; C, cytosine; tRNA, transfer RNA; rRNA, ribosomal RNA.

fermentum JNU532 could provide insight to future research on the characteristics of this strain for functional food and feed supplements (Table 1).

DATA AVAILBILITY

The complete genome sequence has been deposited in the National Center for Biotechnology Information (NCBI) GenBank under the accession number GCA_024800585.1. The BioProject accession number is PRJNA872884 and the BioSample accession number is SAMN30472492.

REFERENCES

- Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, et al. 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. 2020;70:2782–858. https://doi.org/10.1099/ijsem.0.004107
- Pan DD, Zeng XQ, Yan YT. Characterisation of Lactobacillus fermentum SM-7 isolated from koumiss, a potential probiotic bacterium with cholesterol-lowering effects. J Sci Food Agric. 2011;91:512-8. https://doi.org/10.1002/jsfa.4214
- Naghmouchi K, Belguesmia Y, Bendali F, Spano G, Seal BS, Drider D. Lactobacillus fermentum: a bacterial species with potential for food preservation and biomedical applications. Crit Rev Food Sci Nutr. 2020;60:3387-99. https://doi.org/10.1080/10408398.2019.1688250
- 4. Swain MR, Anandharaj M, Ray RC, Parveen Rani R. Fermented fruits and vegetables of Asia: a potential source of probiotics. Biotechnol Res Int. 2014;2014:250424. https://doi.org/10.1155/2014/250424
- Meng Z, Oh S. Antioxidant and antimelanogenic activities of kimchi-derived Limosilactobacillus fermentum JNU532 in B16F10 melanoma cells. J Microbiol Biotechnol. 2021;31:990-8. https://doi.org/10.4014/jmb.2104.04008
- 6. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. Assembly of long, error-prone reads using repeat graphs. Nat Biotechnol. 2019;37:540-6. https://doi.org/10.1038/s41587-019-0072-8
- Krueger F, James F, Ewels P, Afyounian E, Schuster-Boeckler B. FelixKrueger/TrimGalore: v0.6.7 - DOI via Zenodo [Internet]. Zenodo. 2021 [cited 2022 Feb 23]. https://zenodo.org/record/5127899
- 8. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLOS ONE. 2014;9:e112963. https://doi.org/10.1371/journal.pone.0112963

- 9. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30:2068-9. https://doi.org/10.1093/bioinformatics/btu153
- 10. Manni M, Berkeley MR, Seppey M, Simão FA, Zdobnov EM. BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. Mol Biol Evol. 2021;38:4647-54. https://doi.org/10.1093/molbev/msab199