





Whole-Genome Sequence of *Limosilactobacillus fermentum* Strain DM075, Isolated from the Human Oral Cavity

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ABSTRACT Here, we report the complete genome sequence of nitric oxide (NO)-producing *Limosilactobacillus fermentum* strain DM075, which was isolated from human tongue coating samples from healthy donors in South Korea. The complete genome sequence of DM075 comprises a single circular 2,204,022-bp genome, with a GC content of 51.0%, and lacks antimicrobial resistance genes.

The relationship between oral microbiota and their production of nitric oxide (NO) has recently gained much attention (1–6). To isolate NO-producing *Lactobacillus* strains, we obtained tongue coating samples from healthy donors from South Korea, measured nitrate-reducing activity using a double agar overlay method based on the Griess reaction (4), and identified DM075 by complete 16S rRNA gene sequencing in February 2022.

For long-read sequencing, DM075 was anaerobically and statically cultivated in MRS broth for 24 h at 37°C. Genomic DNA (gDNA) was extracted using the Maxwell RSC system (Promega, USA) and sheared to 7 to 12 kb using the Megaruptor 3 system (Diagenode, USA), and small fragments (<3 kb) were removed using AMPure PB beads (Pacific Biosciences [PacBio], USA). The sequencing library was constructed using 3 μg of gDNA with the SMRTbell Express template preparation kit v2.0 (PacBio). The library was sequenced using the Sequel system (PacBio), yielding a total of 78,320 subreads (N_{50} , 10,359 bp) with an average length of 8,323 bp. The data were assembled according to the Microbial Assembly protocol in SMRT Link v10.1.0.119588 (PacBio), including read quality control, error correction, adapter filtering, circularity checking, and overlap trimming (7), which produced a single circular *oriC*-rotated genome. For short-read sequencing, 100 ng of gDNA was sheared using Adaptive Focused Acoustics technology (Covaris, USA), and an ~350-bp sequencing library was prepared using the TruSeq Nano DNA high-throughput library preparation kit (Illumina, USA). The HiSeq X Ten platform (Illumina) was used for the sequencing, which resulted in a total of 17,586,382 quality-filtered 2 × 151-bp paired-end reads (with ≥90% of bases having Phred quality scores of >30). The Illumina reads were then mapped to the PacBio-assembled genome using BWA-MEM v0.7.17 (8) after adapter and quality trimming with Trimmomatic v0.38 (9). The final error correction was conducted three times with Pilon v1.21 (10) with a default minDepth value of 0.01, resulting in a single circular 2,204,022-bp chromosome (Table 1).

The average nucleotide identity (ANI) was analyzed using OrthoANI (11), which yielded 99.28% sequence similarity with *Limosilactobacillus fermentum* CBA7106 (GenBank accession number [CP021964.1](https://doi.org/10.1093/nar/gkab111)). The genome annotation by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v6.1 (12) predicted 2,129 protein-coding genes, 15 rRNA genes, and 58 tRNA genes. Additional annotations were conducted using Prokka v1.14.6 (13), InterProScan v5.30-69.0 (14), and eggNOG DB v4.5 (15), which suggested the highest incidence of genes for DNA metabolism (16.5%), followed by amino acid metabolism (8.6%) and translation (6.5%). The absence of antibiotic resistance genes, one of the key prerequisites for strain safety, was verified using ResFinder v4.1 (16) and the Comprehensive

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TABLE 1 Summary of assembly and annotation statistics for *L. fermentum* DM075

Parameter	Finding
Genetic element	Chromosome
Length (bp)	2,204,022
GC content (%)	51.0
No. of coding sequences	2,129
No. of rRNAs	15
No. of tRNAs	58
Sequencing depth (×)	259.2
GenBank accession no.	CP100352

Antibiotic Resistance Database (CARD) v3.2.3 (17). All tools were run with default parameters unless otherwise specified.

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Data availability. The accession numbers for the 16S rRNA partial sequence and the genome sequence and raw sequencing reads for DM075 are as follows: GenBank, [OP579185.1](https://doi.org/10.1093/nar/gkw569) and [CP100352](https://doi.org/10.1093/nar/gkw569); BioProject, [PRJNA853106](https://doi.org/10.1093/nar/gkw569); BioSample, [SAMN29360653](https://doi.org/10.1093/nar/gkw569); SRA, [SRX15901026](https://doi.org/10.1093/nar/gkw569) and [SRX15901027](https://doi.org/10.1093/nar/gkw569).

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REFERENCES

- Rosier BT, Buetas E, Moya-Gonzalez EM, Artacho A, Mira A. 2020. Nitrate as a potential prebiotic for the oral microbiome. *Sci Rep* 10:12895. <https://doi.org/10.1038/s41598-020-69931-x>.
- Vanhatalo A, L'Heureux JE, Kelly J, Blackwell JR, Wylie LJ, Fulford J, Winyard PG, Williams DW, van der Giezen M, Jones AM. 2021. Network analysis of nitrate-sensitive oral microbiome reveals interactions with cognitive function and cardiovascular health across dietary interventions. *Redox Biol* 41:101933. <https://doi.org/10.1016/j.redox.2021.101933>.
- Xu J, Verstraete W. 2001. Evaluation of nitric oxide production by lactobacilli. *Appl Microbiol Biotechnol* 56:504–507. <https://doi.org/10.1007/s002530100616>.
- Doel JJ, Benjamin N, Hector MP, Rogers M, Allaker RP. 2005. Evaluation of bacterial nitrate reduction in the human oral cavity. *Eur J Oral Sci* 113: 14–19. <https://doi.org/10.1111/j.1600-0722.2004.00184.x>.
- Bhusal A, Muriana PM. 2021. Isolation and characterization of nitrate reducing bacteria for conversion of vegetable-derived nitrate to 'natural nitrite.' *Appl Microbiol* 1:11–23. <https://doi.org/10.3390/applmicrobiol1010002>.
- Sato-Suzuki Y, Washio J, Wicaksono DP, Sato T, Fukumoto S, Takahashi N. 2020. Nitrite-producing oral microbiome in adults and children. *Sci Rep* 10:16652. <https://doi.org/10.1038/s41598-020-73479-1>.
- Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <https://doi.org/10.1038/nmeth.2474>.
- Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv* 1303.3997. <https://doi.org/10.48550/arXiv.1303.3997>.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963. <https://doi.org/10.1371/journal.pone.0112963>.
- Yoon SH, Ha SM, Lim J, Kwon S, Chun J. 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie Van Leeuwenhoek* 110:1281–1286. <https://doi.org/10.1007/s10482-017-0844-4>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvermin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
- Jones P, Binns D, Chang HY, Fraser M, Li W, McAnulla C, McWilliam H, Maslen J, Mitchell A, Nuka G, Pesseat S, Quinn AF, Sangrador-Vegas A, Scheremetjew M, Yong SY, Lopez R, Hunter S. 2014. InterProScan 5: genome-scale protein function classification. *Bioinformatics* 30:1236–1240. <https://doi.org/10.1093/bioinformatics/btu031>.
- Huerta-Cepas J, Szklarczyk D, Forslund K, Cook H, Heller D, Walter MC, Rattei T, Mende DR, Sunagawa S, Kuhn M, Jensen LJ, von Mering C, Bork P. 2016. eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. *Nucleic Acids Res* 44:D286–D293. <https://doi.org/10.1093/nar/gkv1248>.
- Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 67:2640–2644. <https://doi.org/10.1093/jac/dks261>.
- Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, Huynh W, Nguyen AV, Cheng AA, Liu S, Min SY, Miroshnichenko A, Tran HK, Werfalli RE, Nasir JA, Oloni M, Speicher DJ, Florescu A, Singh B, Faltyn M, Hernandez-Koutoucheva A, Sharma AN, Bordeleau E, Pawlowski AC, Zubyk HL, Dooley D, Griffiths E, Maguire F, Winsor GL, Beiko RG, Brinkman FSL, Hsiao WWL, Domselaar GV, McArthur AG. 2020. CARD 2020: antibiotic resistome surveillance with the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Res* 48:D517–D525. <https://doi.org/10.1093/nar/gkz935>.