



Complete Genome Sequences of Three *Lactobacillus gasseri* Urine Isolates Obtained from Postmenopausal Women

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ABSTRACT *Lactobacillus gasseri* frequently colonizes the lower urinary tract of healthy women. However, the role of *L. gasseri* in urinary tract health and the genes required for urinary tract colonization are poorly understood. Herein, we announce the complete genome sequences of three *Lactobacillus gasseri* isolates collected from the urine of postmenopausal women.

Lactobacillus gasseri is a commensal bacterium that is resistant to low pH and bile salts and can colonize the human gut, vagina, and urinary tract (1). While its role in the urinary microbiome (urobiome) is comparatively poorly understood, *L. gasseri* is considered protective in the vaginal and gut microbiomes, where it controls the chemical niche through production of antimicrobial compounds, including bacteriocins (i.e., gassericin), lactic acid, and hydrogen peroxide (1). However, while *L. gasseri* is largely seen as a commensal organism, it has recently been found to occur more often in the urobiome of women with urgency urinary incontinence, indicating that much is still unknown about its role in the urobiome (2).

Before this work, only nine complete *L. gasseri* genomes had been deposited in the NCBI database, of which none were urine isolates. The dissemination of complete genome assemblies for urinary isolates of *L. gasseri* will improve our understanding of genetic adaptations to the urinary niche, including extrachromosomal elements like conjugative plasmids. As such, we are excited to report the complete genomic assemblies for three *L. gasseri* isolates collected from the urine of postmenopausal women as part of institutional review board-approved studies (STU 032016-006 and MR 17-120).

“Clean-catch” midstream urine was collected from three women, two without clinical urinary tract infection (UTI) history and one with UTI history but no UTI at the time of collection. Urine was plated onto De Man, Rogosa, and Sharpe (MRS) agar. The plates were incubated microaerophilically at 35°C using the GasPak EZ Campy pouch system (BD) for 3 days. Species were identified using Sanger sequencing of the PCR-amplified 16S rRNA gene (8F and 1492R primers) as described previously (3) and MegaBLAST (BLAST v2.10.0) (4). After identification, isolated colonies were cultured in MRS broth under the same conditions. Then, the DNeasy blood and tissue kit (Qiagen) was used to extract genomic DNA, which was then analyzed using the 260/280-nm absorbance ratio as well as with agarose gel electrophoresis.

For the long reads, Oxford Nanopore libraries were created using the ligation sequencing kit (SQK-LSK109) and barcode expansion kit 1-12 (EXP-NBD104) before being sequenced using a MinION device with R9 FLO-MIN106 flow cells. The long reads were analyzed using ONT MinKNOW for live base calling, barcode trimming, and demultiplexing and were then quality trimmed using NanoFilt v2.6.0 (5). The trimmed reads were checked for quality using NanoStat v1.2.0, with reads of <200 bp and Phred scores of <7 being discarded. Illumina library prep was performed using the Nextera DNA Flex library prep kit, after which 2 × 150-bp paired-end reads were generated using a

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TABLE 1 Accession numbers, assembly parameters, and isolate characteristics of three urinary *Lactobacillus gasseri* strains obtained from postmenopausal women with different UTI histories

Strain	Host health state	BioSample accession no.	SRA accession no ^a	No. of raw reads	No. of trimmed reads	ONT read N ₅₀ (bp)	Read depth (x)	GenBank accession no.	Type of contig (circular)	Total length (bp)	GC content (%)	No. of CDs ^b
Lg637_C84	Never UTI	SAMN23313746	SRX13999865 (I), SRX13998806 (O)	8,108,286 (I), 258,821 (O)	8,019,100 (I), 258,365 (O)	6,123	590 (I), 443 (O)	CP087763 CP087764	Chromosome Plasmid	1,860,447 81,131	35.0 32.4	1,717 87
Lg1199_C173	Never UTI	SAMN23313747	SRX13999866 (I), SRX13998807 (O)	7,865,502 (I), 595,961 (O)	7,777,799 (I), 594,839 (O)	3,426	532 (I), 613 (O)	CP087959 CP087960 CP087961 CP087962	Chromosome Plasmid Plasmid Plasmid	1,928,547 74,843 54,525 23,715	34.8 32.7 34.9 33.4	1,781 73 66 34
Lg1266_C155	History of rUTI, no current UTI	SAMN23313748	SRX13999867 (I), SRX13998808 (O)	7,354,438 (I), 588,684 (O)	7,272,480 (I), 587,615 (O)	3,758	569 (I), 714 (O)	CP087761 CP087762	Chromosome Plasmid	1,776,837 43,072	35.1 35.9	1,628 51

^a O, ONT; I, Illumina.^b CDs, coding sequences.

NextSeq 500 instrument. Read quality checking and trimming was performed using CLC Genomics Workbench v12.0.3, discarding all reads with a Phred score below 20 and a length of below 15 bp.

Closed hybrid assemblies were created using the normal mode of Unicycler v0.4.8 (6), SPAdes v3.13.0 (7), Racon v1.4.10 (8), and Pilon v1.2.3 (9, 10). The closed genomes were then rotated to the start of either the *dnaA* or *repA* gene, when present. The genomes were analyzed for quality using QUAST v5.0.2 (11) and checked for completeness using the bacteria ortholog set on the gVolante v1.2 (12) server with Bandage v0.8.1 (13) and BUSCO v1 (14). All genomes were complete. Finally, the number of coding sequences and GC content were determined using Geneious Prime v2020.0.5, and the genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline v4.11 (15, 16). For all analyses, default parameters were utilized.

Data availability. The genome sequences are available at GenBank (BioProject accession number [PRJNA761982](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA761982)). The BioSample and SRA accession numbers may be found in Table 1.

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REFERENCES

- Selle K, Klaenhammer TR. 2013. Genomic, and phenotypic evidence for probiotic influences of *Lactobacillus gasseri* on human health. *FEMS Microbiol Rev* 37:915–935. <https://doi.org/10.1111/1574-6976.12021>.
- Pearce MM, Hilt EE, Rosenfeld AB, Zilliox MJ, Thomas-White K, Fok C, Kliethermes S, Schreckenberger PC, Brubaker L, Gai X, Wolfe AJ. 2014. The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. *mBio* 5:e01283-14. <https://doi.org/10.1128/mBio.01283-14>.
- De Nisco NJ, Neugent M, Mull J, Chen L, Kuprasertkul A, de Souza Santos M, Palmer KL, Zimmern P, Orth K. 2019. Direct detection of tissue-resident bacteria and chronic inflammation in the bladder wall of postmenopausal women with recurrent urinary tract infection. *J Mol Biol* 431:4368–4379. <https://doi.org/10.1016/j.jmb.2019.04.008>.
- Zhang Z, Schwartz S, Wagner L, Miller W. 2000. A greedy algorithm for aligning DNA sequences. *J Comput Biol* 7:203–214. <https://doi.org/10.1089/10665270050081478>.
- De Coster W, D'Hert S, Schultz DT, Cruts M, Van Broeckhoven C. 2018. NanoPack: visualizing and processing long-read sequencing data. *Bioinformatics* 34:2666–2669. <https://doi.org/10.1093/bioinformatics/bty149>.
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Vaser R, Sović I, Nagarajan N, Šikić M. 2017. Fast and accurate de novo genome assembly from long uncorrected reads. *Genome Res* 27:737–746. <https://doi.org/10.1101/gr.214270.116>.
- 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>.
- Sharon BM, Hulyalkar NV, Nguyen VH, Zimmern PE, Palmer KL, De Nisco NJ. 2021. Hybrid de novo genome assembly for the generation of complete genomes of urinary bacteria using short- and long-read sequencing technologies. *J Vis Exp*. <https://doi.org/10.3791/62872>.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Nishimura O, Hara Y, Kuraku S. 2017. gVolante for standardizing completeness assessment of genome and transcriptome assemblies. *Bioinformatics* 33:3635–3637. <https://doi.org/10.1093/bioinformatics/btx445>.
- Wick RR, Schultz MB, Zobel J, Holt KE. 2015. Bandage: interactive visualization of de novo genome assemblies. *Bioinformatics* 31:3350–3352. <https://doi.org/10.1093/bioinformatics/btv383>.
- Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31:3210–3212. <https://doi.org/10.1093/bioinformatics/btv351>.
- Tatsova T, DiCuccio M, Badretidin A, Chetvernin 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>.
- Haft DH, DiCuccio M, Badretidin A, Brover V, Chetvernin V, O'Neill K, Li W, Chitsaz F, Derbyshire MK, Gonzales NR, Gwadz M, Lu F, Marchler GH, Song JS, Thanki N, Yamashita RA, Zheng C, Thibaud-Nissen F, Geer LY, Marchler-Bauer A, Pruitt KD. 2018. RefSeq: an update on prokaryotic genome annotation and curation. *Nucleic Acids Res* 46:D851–D860. <https://doi.org/10.1093/nar/gkx1068>.