



Draft Genome Sequence of *Lactobacillus mulieris* UMB9245, Isolated from the Female Bladder

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ABSTRACT *Lactobacillus jensenii* is an anaerobic bacterium found in the urogenital tract that is known to prevent common vaginal infections. Recently, it was divided into two species, *L. jensenii* and *L. mulieris*. Here, we report the draft genome sequence of *L. mulieris* UMB9245, with a genome length of 1,723,383 bp assembled into 52 contigs.

Species of *Lactobacillus*, particularly *L. crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii*, are common members of the “healthy” female urogenital microbiota (1, 2). Evidence suggests that *L. jensenii* can prevent common infections, e.g., urinary tract infections (UTIs) and sexually transmitted infections, among others (3–5). *L. jensenii* acts as a barrier against pathogens and protects against various urogenital infections by out-competing foreign invaders (6, 7). Among the lactobacilli, *L. jensenii* is one of the species most commonly isolated from the urogenital tract (8, 9). Recently, the original species designation of *L. jensenii* was split into two species with the designation of a new species, *L. mulieris* (10). The *L. mulieris* type species was isolated from a urine sample; here, we present the draft genome sequence of another urinary *L. mulieris* strain. *L. mulieris* UMB9245 was isolated from a catheterized urine sample obtained from a female patient with a UTI.

L. mulieris UMB9245 was isolated from a prior institutional review board (IRB)-approved study (University of California, San Diego, IRB no. 170077AW) using the expanded quantitative urinary culture (EQUC) protocol (11). The genus and species were confirmed by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry, as described previously (11). The isolates were then stored at –80°C until sequencing. The isolate was streaked onto a Columbia naladixic acid (CNA) agar plate and incubated for 24 h at 35°C in 5% CO₂. A single colony was selected and grown in a liquid culture using MRS medium supplemented with 1 ml/liter Tween 80 under the same conditions as described above. DNA extraction was done using the Qiagen DNeasy blood and tissue kit following the manufacturer’s protocol for Gram-positive bacteria, with the following alterations: we used 230 μl of lysis buffer (180 μl of 20 mM Tris-Cl, 2 mM sodium EDTA, and 1.2% Triton X-100 and 50 μl of lysozyme) in step 2 and altered the incubation time in step 5 to 10 min. The DNA was quantified using a Qubit fluorometer and sent to the Microbial Genomic Sequencing Center at the University of Pittsburgh for library preparation (Illumina Nextera chemistry) and sequencing on the Illumina NextSeq 550 platform, which produced 2,972,316 pairs of 2 × 150-bp reads. Raw reads were trimmed using Sickle v1.33 (<https://github.com/najoshi/sickle>) and assembled using SPAdes v3.13.0 with the “only-assembler” option for k values of 55, 77, 99, and 127 (12). The genome coverage was calculated using BBMap v38.47 (<https://sourceforge.net/projects/bbmap>). We initially annotated our genome

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assembly using PATRIC v3.6.3 (13). The publicly available genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (14).

The *L. mulieris* UMB9245 draft genome is 1,723,383 bp long, assembled into 52 contigs with a coverage of 405×, GC content of 34.18%, and N_{50} value of 66,175 bp. Our annotations identified 6 rRNAs (3 5S rRNAs, 2 16S rRNAs, and 1 23S rRNA) and 53 tRNAs. Furthermore, three intact prophages were identified by PHASTER (15), two resembling *Lactobacillus jensenii*-infecting phage Lv-1 (GenBank accession no. [NC_011801](https://doi.org/10.1128/NC_011801)) and one resembling *Lactobacillus johnsonii*-infecting phage Lj928 (GenBank accession no. [NC_005354](https://doi.org/10.1128/NC_005354)). Each of the predicted intact phage sequences was then queried against the nonredundant/nucleotide database via BLASTn, confirming the PHASTER-predicted homology to Lv-1 (70% query coverage and 93.21% sequence identity for one phage and 3% query coverage and 74.97% sequence identity for another phage) and Lj928 (15% query coverage and 69.02% sequence identity). One CRISPR array, containing 42 spacer sequences, was detected by CRISPRFinder (16).

Future analyses of the newly defined species *L. mulieris* will further our understanding of this bacterium, particularly with respect to its potential role in the urinary microbiome.

Data availability. This whole-genome shotgun project has been deposited in GenBank under accession no. [JAAVSE00000000](https://doi.org/10.1128/JAAVSE00000000). The version described in this paper is the first version, JAAVSE01000000. The raw sequencing reads have been deposited in the SRA under accession no. [SRR11441021](https://doi.org/10.1128/SRR11441021).

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REFERENCES

- Lepargneur J-P. 2016. *Lactobacillus crispatus* as biomarker of the healthy vaginal tract. *Ann Biol Clin (Paris)* 74:421–427. <https://doi.org/10.1684/abc.2016.1169>.
- 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>.
- Spurbeck RR, Arvidson CG. 2010. *Lactobacillus jensenii* surface-associated proteins inhibit *Neisseria gonorrhoea* adherence to epithelial cells. *Infect Immun* 78:3103–3111. <https://doi.org/10.1128/IAI.01200-09>.
- Yamamoto HS, Xu Q, Fichorova RN. 2013. Homeostatic properties of *Lactobacillus jensenii* engineered as a live vaginal anti-HIV microbicide. *BMC Microbiol* 13:4. <https://doi.org/10.1186/1471-2180-13-4>.
- Antonio MA, Hawes SE, Hillier SL. 1999. The identification of vaginal *Lactobacillus* species and the demographic and microbiologic characteristics of women colonized by these species. *J Infect Dis* 180:1950–1956. <https://doi.org/10.1086/315109>.
- Boris S, Suárez JE, Vázquez F, Barbés C. 1998. Adherence of human vaginal lactobacilli to vaginal epithelial cells and interaction with uropathogens. *Infect Immun* 66:1985–1989. <https://doi.org/10.1128/IAI.66.5.1985-1989.1998>.
- Hudson PL, Hung KJ, Bergerat A, Mitchell C. 2020. Effect of vaginal *Lactobacillus* species on *Escherichia coli* growth. *Female Pelvic Med Reconstr Surg* 26:146–151. <https://doi.org/10.1097/SPV.0000000000000827>.
- Thomas-White K, Forster SC, Kumar N, Van Kuiken M, Putonti C, Stares MD, Hilt EE, Price TK, Wolfe AJ, Lawley TD. 2018. Culturing of female bladder bacteria reveals an interconnected urogenital microbiota. *Nat Commun* 9:1557. <https://doi.org/10.1038/s41467-018-03968-5>.
- Petrova MI, Lievens E, Malik S, Imholz N, Lebeer S. 2015. *Lactobacillus* species as biomarkers and agents that can promote various aspects of vaginal health. *Front Physiol* 6:81. <https://doi.org/10.3389/fphys.2015.00081>.
- Rocha J, Botelho J, Ksiezarek M, Perovic SU, Machado M, Carriço JA, Pimentel LL, Salsinha S, Rodríguez-Alcalá LM, Pintado M, Ribeiro TG, Peixe L. 2020. *Lactobacillus mulieris* sp. nov., a new species of *Lactobacillus delbrueckii* group. *Int J Syst Evol Microbiol* 70:1522–1527. <https://doi.org/10.1099/ijsem.0.003901>.
- Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ, Mueller ER, Brubaker L, Gai X, Wolfe AJ, Schreckenberger PC. 2014. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. *J Clin Microbiol* 52:871–876. <https://doi.org/10.1128/JCM.02876-13>.
- 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>.
- Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, III, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci Rep* 5:8365. <https://doi.org/10.1038/srep08365>.
- Tatusova T, DiCuccio M, Badretdin 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>.
- Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. 2016. PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res* 44:W16–W21. <https://doi.org/10.1093/nar/gkw387>.
- Couvin D, Bernheim A, Toffano-Nioche C, Touchon M, Michalik J, Néron B, Rocha EPC, Vergnaud G, Gautheret D, Pourcel C. 2018. CRISPRCasFinder, an update of CRISPRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins. *Nucleic Acids Res* 46:W246–W251. <https://doi.org/10.1093/nar/gky425>.