5-1-2022

Draft Genome Assemblies of 4 Lactobacillus jensenii and 3 Lactobacillus mulieris Strains from the Urinary Tract

Adriana Ene
Loyola University Chicago

Catherine Putonti
Loyola University Chicago

Recommended Citation

Ene, Adriana and Putonti, Catherine. Draft Genome Assemblies of 4 Lactobacillus jensenii and 3 Lactobacillus mulieris Strains from the Urinary Tract. Microbiology Resource Announcements, 11, 5: 1-3, 2022. Retrieved from Loyola eCommons, Biology: Faculty Publications and Other Works, http://dx.doi.org/10.1128/mra.00032-22

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology® Resource Announcements

GENOME SEQUENCES

Draft Genome Assemblies of 4 *Lactobacillus jensenii* and 3 *Lactobacillus mulieris* Strains from the Urinary Tract

Adriana Ene,[a] **Catherine Putonti**[a,b,c]

[a]Bioinformatics Program, Loyola University Chicago, Chicago, Illinois, USA
[b]Department of Biology, Loyola University Chicago, Chicago, Illinois, USA
[c]Department of Microbiology and Immunology, Loyola University Chicago, Stritch School of Medicine, Maywood, Illinois, USA

ABSTRACT Lactobacilli are dominant members of the healthy female bladder microbiota. Here, we report the draft genome sequences of 4 *Lactobacillus jensenii* and 3 *Lactobacillus mulieris* strains isolated from catheterized urine samples.

Lactobacillus bacteria are key members of the healthy urinary and vaginal microbiota (1, 2). One species, *Lactobacillus jensenii*, is generally considered beneficial to these communities and is capable of inhibiting or killing uropathogenic *Escherichia coli* (3). However, *L. jensenii* is isolated frequently from the bladders of females with urge urinary incontinence (UUI) (4). Recently, a new *Lactobacillus* species of the female urogenital tract was characterized—*Lactobacillus mulieris* (5). *L. jensenii* and *L. mulieris* are closely related taxa, and their genomes can best be distinguished by average nucleotide identity (ANI) (5). Using ANI, publicly available genomes of *L. jensenii* were reassigned as *L. mulieris* (6), and additional *L. mulieris* strains were sequenced (7, 8). In an effort to better characterize the genetic content of *L. jensenii* and *L. mulieris*, we have sequenced 4 urinary isolates of *L. jensenii* and 3 urinary isolates of *L. mulieris*.

Catheterized urine samples, collected from females as part of prior institutional review board (IRB)-approved studies (IRB approvals LUC206469, LUC207102, and LUC204195 from Loyola University Chicago and 17077AW from University of California San Diego) (9–13),

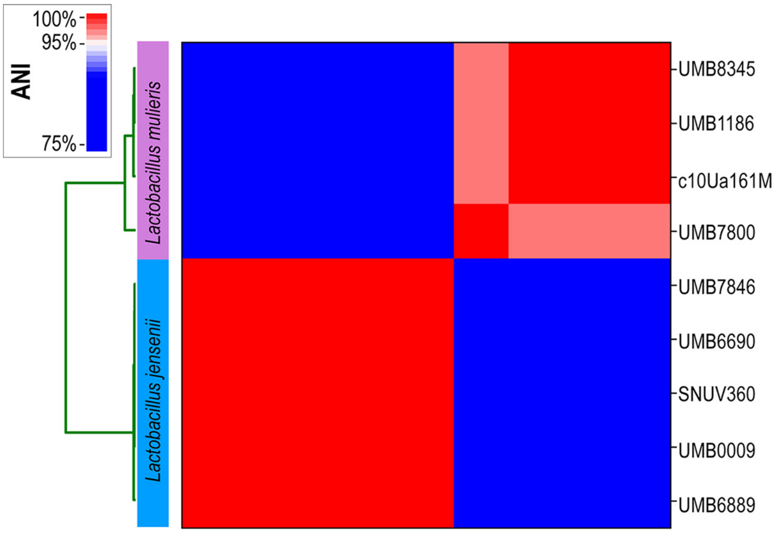

FIG 1 ANI analysis of genomes from 7 urinary isolates, *L. jensenii* SNUV360 (NZ_CP0188091), and *L. mulieris* c10Ua161M (GCA_007095465.1).

Editor Irene L. G. Newton, Indiana University, Bloomington

Address correspondence to Catherine Putonti, cputonti@luc.edu.

The authors declare no conflict of interest.

Received 17 January 2022
Accepted 24 March 2022
Published 7 April 2022

TABLE 1 *L. jensenii* and *L. mulieris* draft genome statistics

Strain name	SRA accession no.	No. of pairs of raw reads	Genome accession no.	Coverage (×)	No. of contigs	Length (bp)	N_{50} (bp)	GC content (%)	Symptom status[a]
L. jensenii UMB0009	SRR17382894	1,490,462	JAKEYG000000000	288.37	56	1,487,531	49,572	34.26	OAB−/UTI−
L. jensenii UMB6889	SRR17382891	1,500,599	JAKEYJ000000000	262.94	63	1,662,558	49,065	34.17	OAB−/UTI−
L. jensenii UMB6690	SRR17382892	1,388,309	JAKEYI000000000	233.47	55	1,741,648	56,078	34.44	rUTI
L. jensenii UMB7846	SRR17382889	1,537,023	JAKEYL000000000	250.11	45	1,803,482	73,859	34.29	OAB+/UTI−
L. mulieris UMB1186	SRR17382893	101,961.5	JAKEYH000000000	257.99	78	1,696,445	43,865	34.16	UTI+
L. mulieris UMB7800	SRR17382890	1,400,104	JAKEYK000000000	237.14	54	1,694,839	56,825	34.13	rUTI
L. mulieris UMB8354	SRR17382888	595,635	JAKEYM000000000	108.19	63	1,604,117	71,306	33.94	OAB+/UTI−

[a] Participant symptom status abbreviations are as follows: OAB, overactive bladder symptoms; UTI, urinary tract infection; and rUTI, recurrent UTI.

were cultured using the enhanced quantitative urine culture (EQUC) method (13) and stored at −80°C. Seven strains identified as *L. jensenii* by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (as previously described [9]) were selected for whole-genome sequencing. Freezer stocks were first streaked onto Columbia colistin and nalidixic acid (CNA) agar with 5% sheep blood plates (BD 221353) and incubated at 35°C in 5% CO_2 for 48 h. A single colony was then selected and grown in MRS + 1% Tween liquid medium at 35°C in 5% CO_2 for 48 h. The Qiagen blood and tissue kit was used for DNA extraction, following the manufacturer's protocol with the additional lysis treatment prior to extraction. This lysis treatment includes a suspension of the cell pellet in lysis buffer (see details in reference 7) and incubation at 35°C for 30 min. The DNA was quantified using a Qubit fluorometer and sent to the Microbial Genomic Sequencing Center (Pittsburgh, PA) for library preparation (using the Illumina DNA prep kit and Integrated DNA Technologies [IDT] 10-bp unique dual index [UDIs]) and sequencing on the Illumina NextSeq 2000 platform (paired-end, 150-bp reads); demultiplexing, quality control, and adapter trimming were performed using bcl-convert (v. 3.9.3; https://support-docs.illumina.com/SW/BCL_Convert/Content/SW/FrontPages/BCL_Convert.htm). Raw reads were first trimmed for quality using bbduk (v. 38.92) (https://sourceforge.net/projects/bbmap/) with the following parameters: ftl = 15, ftr = 135, minlength = 30, qtrim=rl, maq = 20, maxns = 0, statscolumns = 5, and trimq = 20. Filtered reads were assembled via SPAdes v. 3.15.2 using the "only-assembler" option for k = 55, 77, 99, and 127 (14). Genome annotations were performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v. 5.3 (15).

The seven genomes were compared to representative strains of the two species, namely, *L. jensenii* SNUV360 (NZ_CP018809.1) and *L. mulieris* c10Ua161M (GCA_007095465.1), using pyANI (v. 0.2) (16). Based upon this ANI analysis (Fig. 1), we can assign their taxonomy, as follows: 4 strains of *L. jensenii* and 3 strains of *L. mulieris*. The draft genome assemblies of these seven bladder lactobacilli genomes vary in size from 1,487,531 bp (*L. jensenii* UMB0009) to 1,803,482 bp (*L. jensenii* UMB7846), with an average GC content of 34.20%. Full assembly statistics are listed in Table 1. Subsequent sequencing of these two species will provide insight into their role in the urinary microbiota.

Data availability. For all seven strains, the raw reads and draft assemblies have been deposited in GenBank. Table 1 lists the SRA accession numbers and genome assembly accession numbers.

ACKNOWLEDGMENTS

For prior participant recruitment, we acknowledge the Loyola Urinary Education and Research Collaborative (LUEREC) and the individuals who provided the samples for this study. This strains were obtained from Alan J. Wolfe.

REFERENCES

1. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SSK, McCulle SL, Karlebach S, Gorle R, Russell J, Tacket CO, Brotman RM, Davis CC, Ault K, Peralta L, Forney LJ. 2011. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A 108:4680–4687. https://doi.org/10.1073/pnas.1002611107.
2. Fok CS, Gao X, Lin H, Thomas-White KJ, Mueller ER, Wolfe AJ, Dong Q, Brubaker L. 2018. Urinary symptoms are associated with certain urinary microbes in urogynecologic surgical patients. Int Urogynecol J 29:1765–1771. https://doi.org/10.1007/s00192-018-3732-1.
3. Hütt P, Lapp E, Štšepetova J, Smidt I, Taelma H, Borovkova N, Oopkaup H, Ahelik A, Rööp T, Hoidmets D, Samuel K, Salumets A, Mändar R. 2016. Characterisation of probiotic properties in human vaginal lactobacilli strains. Microb Ecol Health Dis 27:30484. https://doi.org/10.3402/mehd.v27.30484.

4. Price TK, Hilt EE, Thomas-White K, Mueller ER, Wolfe AJ, Brubaker L. 2020. The urobiome of continent adult women: a cross-sectional study. BJOG 127:193–201. https://doi.org/10.1111/1471-0528.15920.
5. 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.
6. Putonti C, Shapiro JW, Ene A, Tsibere O, Wolfe AJ. 2020. Comparative genomic study of *Lactobacillus jensenii* and the newly defined *Lactobacillus mulieris* species identifies species-specific functionality. mSphere 5:e00560-20. https://doi.org/10.1128/mSphere.00560-20.
7. Ramotowski L, Miller-Ensminger T, Voukadinova A, Wolfe AJ, Putonti C. 2020. Draft genome sequence of Lactobacillus mulieris UMB9245, isolated from the female bladder. Microbiol Resour Announc 9:e00408-20. https://doi.org/10.1128/MRA.00408-20.
8. Tsibere O, Miller-Ensminger T, Voukadinova A, Wolfe AJ, Putonti C. 2020. Draft genome sequence of *Lactobacillus mulieris* UMB7784, isolated from the female urinary tract. Microbiol Resour Announc 9:e00403-20. https://doi.org/10.1128/MRA.00403-20.
9. 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.
10. Pearce MM, Zilliox MJ, Rosenfeld AB, Thomas-White KJ, Richter HE, Nager CW, Visco AG, Nygaard IE, Barber MD, Schaffer J, Moalli P, Sung VW, Smith AL, Rogers R, Nolen TL, Wallace D, Meikle SF, Gai X, Wolfe AJ, Brubaker L, Pelvic Floor Disorders Network. 2015. The female urinary microbiome in urgency urinary incontinence. Am J Obstet Gynecol 213:347.e1–347.11. https://doi.org/10.1016/j.ajog.2015.07.009.
11. 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.
12. Thomas-White KJ, Hilt EE, Fok C, Pearce MM, Mueller ER, Kliethermes S, Jacobs K, Zilliox MJ, Brincat C, Price TK, Kuffel G, Schreckenberger P, Gai X, Brubaker L, Wolfe AJ. 2016. Incontinence medication response relates to the female urinary microbiota. Int Urogynecol J 27:723–733. https://doi.org/10.1007/s00192-015-2847-x.
13. Price TK, Dune T, Hilt EE, Thomas-White KJ, Kliethermes S, Brincat C, Brubaker L, Wolfe AJ, Mueller ER, Schreckenberger PC. 2016. The clinical urine culture: enhanced techniques improve detection of clinically relevant microorganisms. J Clin Microbiol 54:1216–1222. https://doi.org/10.1128/JCM.00044-16.
14. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski 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.
15. 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.
16. Pritchard L, Glover RH, Humphris S, Elphinstone JG, Toth IK. 2016. Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens. Anal Methods 8:12–24. https://doi.org/10.1039/C5AY02550H.