Complete Genome Sequences of Six *Lactobacillus iners* Strains Isolated from the Human Vagina

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ABSTRACT  *Lactobacillus iners* is a common member of the human vaginal microbiota, with a genome size smaller than that of other lactobacilli. Here, we report the complete genome sequences of six *L. iners* strains isolated from different vaginal swab specimens. Three strains were found to harbor ~100-kbp plasmids, which were not known previously.

*Lactobacillus iners* is a Gram-positive, facultative anaerobic bacterium that is a member of the human vaginal microbiota (1, 2). Surveys have demonstrated that a substantial portion of reproductive-age women have a vaginal microbiota dominated by *L. iners* (3, 4). The species' relationship to vaginal health is somewhat complicated (5). While it is capable of lowering vaginal pH via the production of lactic acid, a hallmark of vaginal health (6), it is also frequently found coresident with species that have been associated with bacterial vaginosis, a common vaginal condition associated with adverse health outcomes (7–9). The species has also been shown to provide suboptimal protection against sexually transmitted infections, such as those caused by chlamydia (10, 11). Here, we report the complete genome sequences of six *L. iners* strains.

The six *L. iners* strains were isolated from archived and deidentified midvaginal swab specimens collected from either African American (n = 3) or Caucasian women (n = 3). The swab specimens were originally collected after obtaining informed consent from all participants, who also provided consent for storage of the samples and their use in future research studies related to women’s health. The original studies were approved by the University of Maryland School of Medicine Institutional review board. The swab specimens were resuspended in 1 ml of brucella broth supplemented with hemin and vitamin K, and then 25 μl of the suspension was plated onto human blood bilayer agar with Tween 80. After 48 to 72 hours of aerobic or anaerobic (5%:10%:85% H₂/CO₂/N₂ gas mixture) incubation at 37°C, the strains were isolated. Large genomic DNA fragments were extracted using the MasterPure complete DNA purification kit (Lucigen, Middleton, WI, USA). Sequencing libraries were prepared using the SMRTbell express template prep kit 2.0 (Pacific Biosciences of California, Menlo Park, CA, USA), size selected on a BluePippin instrument (Sage Science, Beverly, MA, USA), and sequenced on a PacBio Sequel II instrument (Pacific Biosciences) with a single-molecule real-time (SMRT) cell 8M.

An average of 310,000 reads were obtained for each strain (median read length, 10.1 kbp), providing an average genome coverage of 2,258× (range, 1,203× to 3,682×). Long read assembly was performed using the Canu assembler v1.8 on the raw PacBio long reads.

Citation France MT, Rutt L, Narina S, Arbaugh S, McComb E, Humphrys MS, Ma B, Hayward MR, Costello EK, Relman DA, Kwon DS, Ravel J. 2020. Complete genome sequences of six *Lactobacillus iners* strains isolated from the human vagina. Microbiol Resour Announc 9:e00234-20. https://doi.org/10.1128/MRA.00234-20.

Editor Catherine Putonti, Loyola University Chicago

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Received 3 March 2020

Accepted 17 April 2020

Published 14 May 2020
reads with a target genome size of 1.3 Mbp and a minimum read length of 1 kbp (12). For three of the strains, a single large contig was produced, circularized using Simple-Circularise (https://github.com/Kzra/Simple-Circularise; v1 default settings), and then oriented using Circlator v1.5.5 with default settings (13), such that the origin of replication was at the start. The remaining three strains had an additional contig encoding a 100-kbp plasmid, which was also circularized.

The average genome size of the six L. iners was 1.36 Mbp and ranged from 1.32 Mbp to 1.40 Mbp, with an average GC content of 33.3% (Fig. 1). Despite being isolated from six different subjects, the L. iners genome sequences were fairly similar, with an average nucleotide identity (ANI) of 98.7% and overlap of 91.6%, as estimated by pyani v0.2.10 with the -ANIm setting (14). The three plasmid sequences were also largely similar, with an ANI of 98.1% and overlap of 85.1%. Gene prediction and annotation were performed using Prokka v1.12 with default settings (15). The average number of coding sequences per genome was 1,254 and ranged from 1,203 to 1,331. Five of the six L. iners genomes were found to encode 6 rRNA operons, while the genome of strain C0254C1 encoded one fewer. All of the genomes encoded 71 tRNA genes, except the genome of strain C0059G1, which encoded 73. The plasmid was found to encode between 97 and 100 genes, including several related to conjugal transfer.

**Data availability.** The six genome sequences and three plasmid sequences have been deposited in the NCBI GenBank with accession numbers CP049223 to CP049231. Raw sequencing reads were submitted to the NCBI Sequence Read Archive under the BioProject PRJNA608123.

**ACKNOWLEDGMENTS**

We acknowledge the Institute for Genome Science’s Genomic Resources Center, including Luke Tallon, Jack Boylan, and Holly Roussey, for technical assistance.
This work was supported, in part, by the National Institute of Allergy and Infectious Diseases and the National Institute for Nursing Research of the National Institutes of Health under award numbers U19AI084044, UH2AI083264, and R01NR015495 and by the Bill and Melinda Gates Foundation (OPP1189217). D.A.R. is supported by the Chan Zuckerberg Biohub Microbiome Initiative and the Thomas C. and Joan M. Merigan Endowment at Stanford University.

J.R. is a cofounder of LUCA Biologics, a biotechnology company focusing on translating microbiome research into live biotherapeutic drugs for women’s health. All other authors declare no competing interests.

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