Draft Genome Sequences of Six *Lactobacillus gasseri* and Three *Lactobacillus paragasseri* Strains Isolated from the Female Bladder

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ABSTRACT

*Lactobacilli* are dominant members of the healthy female bladder microbiota. Here, we report the complete genome sequences of six *Lactobacillus gasseri* and three *Lactobacillus paragasseri* strains isolated from catheterized urine samples. These *L. paragasseri* genomes are the first publicly available sequences of the species from the bladder.

*Lactobacillus* is a commensal bacterium in the human body and is a key component of the healthy urinary and vaginal microbiota (1). The family *Lactobacillaceae* has one of the highest rates of incidence compared to those of other bacterial families in the urinary tract (2). *Lactobacillus gasseri* is a predominant species in the human microbiota and is able to prevent other bacteria from growing in the same environment, protecting the host from pathogens (3). *Lactobacillus paragasseri* was classified as a novel species in 2018 (4) and, until now, has not been characterized in the urinary tract.

Catheterized urine samples were collected from women as part of prior institutional review board (IRB)-approved studies (5–9). Bacteria were isolated from these samples using the enhanced quantitative urine culture (EQUC) method (9) and stored at −80°C. We selected nine strains in our collection for whole-genome sequencing; these strains were identified as *L. gasseri* by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) mass spectrometry. Freezer stocks for each of the nine strains were first streaked on Columbia colistin-nalidixic acid agar with 5% sheep blood plates (catalog number 221353; BD) and incubated at 35°C in 5% CO2 for 48 hours. A single colony was then selected and grown in MRS liquid medium at 35°C in 5% CO2 for 48 hours. DNA was extracted with the Qiagen DNeasy UltraClean microbial kit, and the DNA was quantified by a Qubit fluorometer. DNA libraries were constructed (Nextera XT library prep kit) and sequenced using the MiSeq reagent kit v2, producing 250-bp paired-end reads (minimum, 266,494 pairs; maximum, 1,342,972 pairs; average, 524,976 pairs). The raw reads were trimmed using Sickle v1.33 (https://github.com/najoshi/sickle) and then assembled with SPAdes v3.13.0 (10) (parameters, “only-assembler” option for k = 55, 77, 99, and 127). The assembled contigs were evaluated for genome completeness and contamination by Checkm v1.0.12 (11), and genome coverage was calculated using BBMap v38.47 (https://sourceforge.net/projects/bbmap/). Genome annotations were performed using PATRIC v3.5.43 (12) and the NCBI
Prokaryotic Genome Annotation Pipeline (PGAP) v4.8 (13). The PGAP annotations are published with the deposited genome assemblies. A phylogenetic tree was derived with RAxML v8.2.11 in PATRIC using the PATRIC annotations and the codon tree method (12). Unless otherwise noted, default parameters were used for all software tools.

The nine bladder lactobacilli genomes vary in size from 1,041,937 bp (strain UMB1399) to 2,108,391 bp (strain UMB6975) in length, with an average GC content of 35.2%. Assembly statistics are listed in Fig. 1A. Genome assemblies for lactobacilli are particularly challenging given the presence of numerous short repeats throughout the genome (14). As part of NCBI's quality control process, average nucleotide identity is calculated (15), and three of the genomes (UMB0596, UMB1065, and UMB6975) were reclassified as strains of the species L. paragasseri (98 to 99% identical for over 94% of the genome to the type genome of L. paragasseri (strain JCM 5343 [GenBank accession number AP018549]). The nine bladder lactobacillus genomes were also compared with those of publicly available L. gasseri and L. paragasseri strains in PATRIC (as of July 2019). Figure 1B shows a phylogenetic analysis of these genome comparisons. There is a clear distinction between the six bladder L. gasseri and three bladder L. paragasseri strains. From our phylogenetic analysis, we have identified not only three new strains of L. paragasseri but also other strains presently classified as L. gasseri that are likely members of the L. paragasseri species (Fig. 1B; branches shown in orange).

**Data availability.** This whole-genome shotgun project has been deposited in GenBank under the accession numbers VNFS0000000 (UMB4205), VNFT0000000 (UMB2965), VNFU0000000 (UMB1196), VNFY0000000 (UMB0607), VNGC0000000 (L. gasseri UMB2965, L. gasseri UMB3077, L. gasseri 505, L. gasseri 4M13, L. gasseri JCM 5344, L. paragasseri UMB6975, L. gasseri AL5, L. gasseri 494_LGAS, L. gasseri 497_LGAS, L. gasseri JV-V03, L. gasseri UMB0099, L. paragasseri UMB1065, L. gasseri JCM 1130, L. gasseri JCM 5343, L. gasseri AL3, L. gasseri UMB0056, L. paragasseri UMB0596, L. gasseri 130918, L. gasseri CECT 57114, L. rhamnosus DSM 14870, L. gasseri UMB0045a, L. gasseri UMB0045b, L. gasseri UMB0045c, L. gasseri UMB0045d, L. gasseri UMB0045e, L. gasseri UMB0045f, L. gasseri UMB0045g, L. gasseri UMB0045h, L. gasseri UMB0045i, L. gasseri UMB0045j, L. gasseri UMB0045k, L. gasseri UMB0045l, L. gasseri UMB0045m, L. gasseri UMB0045n, L. gasseri UMB0045o, L. gasseri UMB0045p, L. gasseri UMB0045q, L. gasseri UMB0045r, L. gasseri UMB0045s, L. gasseri UMB0045t, L. gasseri UMB0045u, L. gasseri UMB0045v, L. gasseri UMB0045w, L. gasseri UMB0045x, L. gasseri UMB0045y, L. gasseri UMB0045z, L. gasseri UMB0045aa, L. gasseri UMB0045ab, L. gasseri UMB0045ac, L. gasseri UMB0045ad, L. gasseri UMB0045ae, L. gasseri UMB0045af, L. gasseri UMB0045ag, L. gasseri UMB0045ah, L. gasseri UMB0045ai, L. gasseri UMB0045aj, L. gasseri UMB0045ak, L. gasseri UMB0045al, L. gasseri UMB0045am, L. gasseri UMB0045an, L. gasseri UMB0045ao, L. gasseri UMB0045ap, L. gasseri UMB0045aq, L. gasseri UMB0045ar, L. gasseri UMB0045as, L. gasseri UMB0045at, L. gasseri UMB0045au, L. gasseri UMB0045av, L. gasseri UMB0045aw, L. gasseri UMB0045ax, L. gasseri UMB0045ay, L. gasseri UMB0045az, L. gasseri UMB0045aa, L. gasseri UMB0045ab, L. gasseri UMB0045ac, L. gasseri UMB0045ad, L. gasseri UMB0045ae, L. gasseri UMB0045af, L. gasseri UMB0045ag, L. gasseri UMB0045ah, L. gasseri UMB0045ai, L. gasseri UMB0045aj, L. gasseri UMB0045ak, L. gasseri UMB0045al, L. gasseri UMB0045am, L. gasseri UMB0045an, L. gasseri UMB0045ao, L. gasseri UMB0045ap, L. gasseri UMB0045aq, L. gasseri UMB0045ar, L. gasseri UMB0045as, L. gasseri UMB0045at, L. gasseri UMB0045au, L. gasseri UMB0045av, L. gasseri UMB0045aw, L. gasseri UMB0045ax, L. gasseri UMB0045ay, L. gasseri UMB0045az, L. gasseri UMB0045aa, L. gasseri UMB0045ab, L. gasseri UMB0045ac, L. gasseri UMB0045ad, L. gasseri UMB0045ae, L. gasseri UMB0045af, L. gasseri UMB0045ag, L. gasseri UMB0045ah, L. gasseri UMB0045ai, L. gasseri UMB0045aj, L. gasseri UMB0045ak, L. gasseri UMB0045al, L. gasseri UMB0045am, L. gasseri UMB0045an, L. gasseri UMB0045ao, L. gasseri UMB0045ap, L. gasseri UMB0045aq, L. gasseri UMB0045ar, L. gasseri UMB0045as, L. gasseri UMB0045at, L. gasseri UMB0045au, L. gasseri UMB0045av, L. gasseri UMB0045aw, L. gasseri UMB0045ax, L. gasseri UMB0045ay, L. gasseri UMB0045az, L. gasseri UMB0045aa, L. gasseri UMB0045ab, L. gasseri UMB0045ac, L. gasseri UMB0045ad, L. gasseri UMB0045ae, L. gasseri UMB0045af, L. gasseri UMB0045ag, L. gasseri UMB0045ah, L. gasseri UMB0045ai, L. gasseri UMB0045aj, L. gasseri UMB0045ak, L. gasseri UMB0045al, L. gasseri UMB0045am, L. gasseri UMB0045an, L. gasseri UMB0045ao, L. gasseri UMB0045ap, L. gasseri UMB0045aq, L. gasseri UMB0045ar, L. gasseri UMB0045as, L. gasseri UMB0045at, L. gasseri UMB0045au, L. gasseri UMB0045av, L. gasseri UMB0045aw, L. gasseri UMB0045ax, L. gasseri UMB0045ay, L. gasseri UMB0045az, L. gasseri UMB0045aa, L. gasseri UMB0045ab, L. gasseri UMB0045ac, L. gasseri UMB0045ad, L. gasseri UMB0045ae, L. gasseri UMB0045af, L. gasseri UMB0045ag, L. gasseri UMB0045ah, L. gasseri UMB0045ai, L. gasseri UMB0045aj, L. gasseri UMB0045ak, L. gasseri UMB0045al, L. gasseri UMB0045am, L. gasseri UMB0045an, L. gasseri UMB0045ao, L. gasseri UMB0045ap, L. gasseri UMB0045aq, L. gasseri UMB0045ar, L. gasseri UMB0045as, L. gasseri UMB0045at, L. gasseri UMB0045au, L. gasseri UMB0045av, L. gasseri U
(UMB3077), and VNGD0000000 (UMB1399) for the six L. gasseri strains and VNFF00000000 (UMB0596), VNFF00000000 (UMB1065), and VNFF00000000 (UMB6975) for the three L. paragasseri strains. The versions described in this paper are the first versions. Raw sequence data are publicly available for the six L. gasseri strains (SRA accession numbers SRR9695707, SRR9695712, SRR9695713, SRR9695714, SRR9695719, and SRR9695724) and the three L. paragasseri strains (accession numbers SRR9695720, SRR9695721, and SRR9695723).

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