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GENOME SEQUENCES

Whole-Genome Sequences of Two *Listeria monocytogenes* Biofilm Formers

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ABSTRACT *Listeria monocytogenes* strains from different lineages show different biofilm-forming abilities. In this study, two strains of *L. monocytogenes* were whole genome sequenced using single-molecule real-time (SMRT) technology and characterized.

Listeria monocytogenes forms complex biofilms that persist for years in food processing environments (1). Strain and lineage characteristics have been shown to be important for biofilm formation and invasive capacity (2, 3). To further characterize the ability of *L. monocytogenes* to form biofilms, we closed and compared the genomes of two *L. monocytogenes* strains that are known biofilm formers (4).

Strain NRRL B-33043 (B-33043, lineage II, serotype 1/2a) and NRRL B-33260 (B-33260, lineage II, serotype 1/2c) (USDA Agricultural Research Service [ARS]) were obtained from a meat slaughter facility (5). Strains were kept in a CryoCare organism preservative system (Key Scientific, Stamford, TX) at −80°C until experiments. Strains were cultured in tryptic soy broth (Becton, Dickinson, Franklin Lane, NJ) overnight at 35°C. Genomic DNA from isolates B-33043 and B-33260 was extracted using the Maxwell RSC cultured cells DNA kit (Promega, Madison, WI) with the addition of RNase A after lysis. The genomes were closed using the Pacific Biosciences (PacBio) Sequel sequencing platform. In brief, the DNA from the isolates was part of a 4-plex to construct a multiplexed microbial SMRTbell library using the SMRTbell template preparation kit v1.0 (PacBio, Menlo Park, CA). The isolates were ligated with a barcode using the SMRTbell barcoded adapter complete preparation kit-96 (PacBio). The multiplexed SMRTbell library was sequenced on the PacBio Sequel sequencer using PacBio Sequel v2.0 chemistry on one Sequel single-molecule real-time (SMRT) Cell 1M v2 (PacBio), with a 10-h movie collection time.

Raw sequencing data were demultiplexed by running the demultiplex barcodes application with symmetric mode in SMRT Link v7.0.1 (PacBio). The genomes were *de novo* assembled using the PacBio hierarchical genome assembly process (HGAP) v4.0 with default parameters (6). The assemblies were then checked for even sequencing coverage in SMRT Link v7.0.1 and polished by resequencing in SMRT Link v7.0.1 to ensure >99.99% mean consensus concordance. The genomes were circularized by Circlator v1.5.5 (7), and each closed genome was rotated to start at the *dnaA* gene. Genomes were annotated using the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok). Genomes were aligned with mauveAligner v2.3.1 using the progressive algorithm with default settings (8). Genome comparisons were undertaken using the BLAST Ring Image Generator (BRIG) (9) and multivirulence locus sequence typing (MVLST) (https://sites.google.com/site/mvlstdatabase) was carried out according to available schemes, to assign sequence types (STs) and virulence types (VTs), respectively. The incompatibility types and *rep* family of the plasmids were determined using the PlasmidFinder v2.0 server (10) (https://cge.cbs.dtu.dk/services/PlasmidFinder-2.0). Plasmids' relaxase types and

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TABLE 1 Assembly and annotation results for *Listeria monocytogenes* strains

GenBank accession no.	Isolate	DNA type	Total no. of reads	Coverage (×)	N_{50} (bp)	No. of contigs	Length (bp)	No. of coding sequences	No. of tRNAs	No. of rRNAs	No. of noncoding RNAs	GC content (%)
CP068979	CFSAN100569	Chromosome	114,992	350	9,107	1	2,979,947	3,109	67	6	4	38.0
CP068980	pCFSAN100569	Plasmid 1				1	108,807					37.6
CP068981	pCFSAN100569	Plasmid 2				1	88,315					36.5
CP068977	CFSAN100570	Chromosome	72,291	185	9,136	1	2,976,500	2,941	67	6	4	38.0
CP068978	pCFSAN100570	Plasmid				1	25,550					36.5

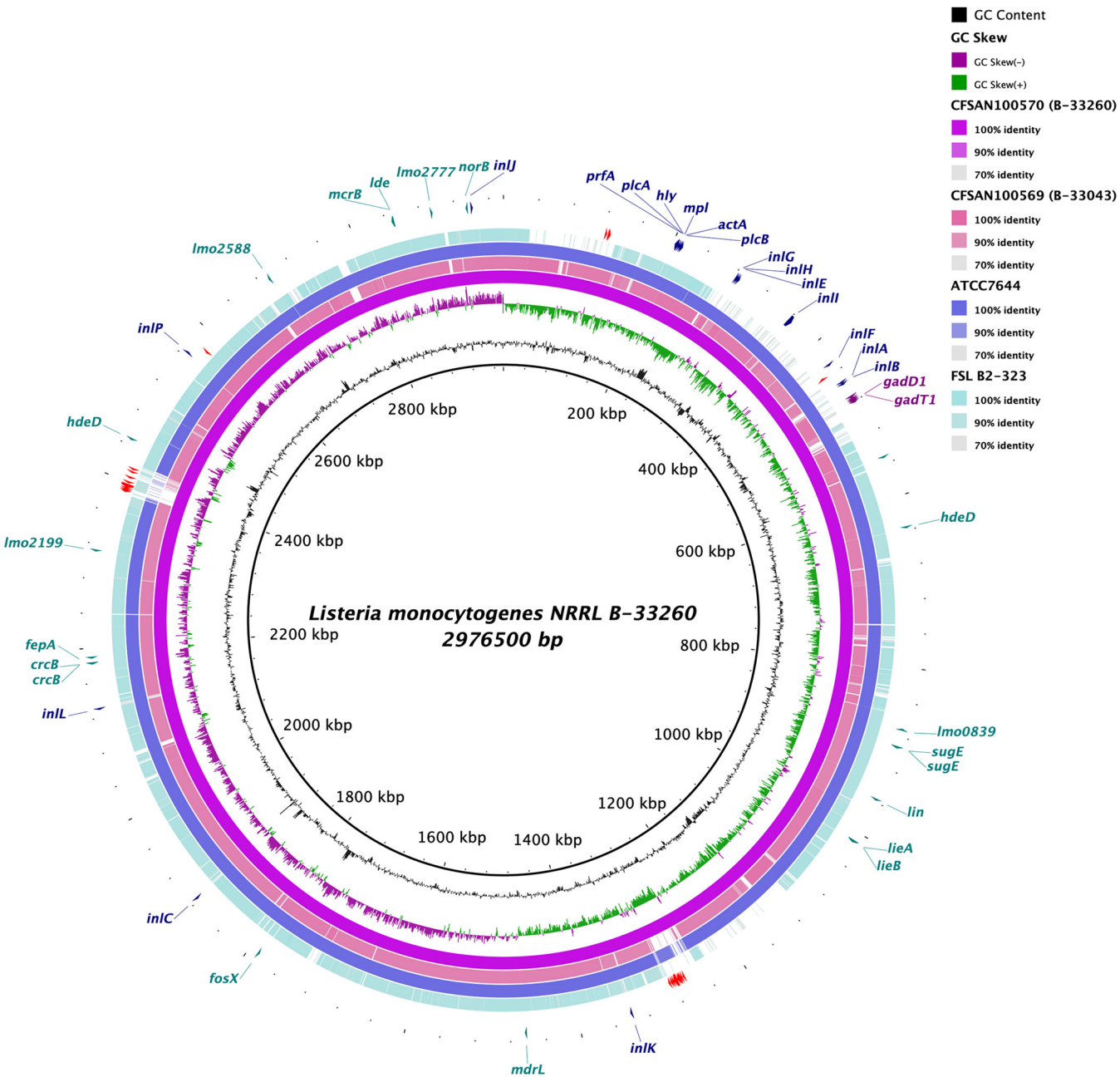

FIG 1 Genome comparison of *Listeria monocytogenes* isolates. The legend shows the color gradients for similarities. The prophage regions, virulence genes in LIPI1 and the internalin family, genes in SSIs, and resistance genes are marked in red, navy, purple, and teal, respectively.

conjugative transferability were predicted through MOB-typer in MOB-suite (11), available at the GalaxyTrakr server (https://galaxytrakr.org).

Assembly and annotation results are shown in Table 1. The sequenced isolates belong to the same lineage (II), but they do not share the same ST and serotype (ST2 and serotype 1/2a for strain B-33043 and ST0 and serotype 1/2c for strain B-33260). All four strains compared carried at least one intact prophage, and they all contained *Listeria* pathogenicity island 1 (LIPI1). A hypervariable genetic hot spot, *lmo0443* to *lmo0449* (annotated in EGD-e genome), containing the stress survival islets (SSIs) (SSI-1 and SSI-2) was present in all four genomes compared (Fig. 1, purple labels).

Data availability. The whole-genome sequences of strains B-33260 and B-33043 have been deposited in DDBJ/ENA/GenBank under the accession numbers CP068977, CP068978, CP068979, CP068980, and CP068981. The NCBI Sequence Read Archive (SRA)

accession numbers for the raw reads are SRX13755894 (BioSample accession number SAMN17311437) and SRX13755907 (BioSample accession number SAMN17311438).

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