





Complete Genome Sequence of a Serotype 7 Listeria monocytogenes Strain, FSL R9-0915

📵 Tracey Lee Peters, a 📵 Lauren K. Hudson, a Daniel W. Bryan, a 📵 Yaxiong Song, a Henk C. den Bakker, b Zuzana Kucerova, c Thomas G. Denesa

^aDepartment of Food Science, University of Tennessee, Knoxville, Tennessee, USA

ABSTRACT Listeria monocytogenes serotype 7 lacks glycosidic constituents in wall teichoic acids. Here, we present the complete genome sequence of L. monocytogenes serotype 7 strain FSL R9-0915 and an analysis of genes known to affect L. monocytogenes antigenicity. This strain is used as a control strain in Listeria phage host range analyses.

isteria monocytogenes is a Gram-positive foodborne bacterial pathogen that causes the potentially fatal illness listeriosis (1). L. monocytogenes strains are serotyped based on somatic and flagellar antigens (2). Of the 13 established serotypes, serotype 7 is one of the least commonly studied. Here, we present the complete genome sequence of L. monocytogenes strain FSL R9-0915 (alternative identifiers [IDs] are KC983, KC1716, NTCC 1627, and H3293). FSL R9-0915 was originally isolated from an unknown source on 17 December 1969 by Jeanette Donker-Voet from the Netherlands. This strain was maintained as part of a serotyping collection as a representative serotype 7 strain (3) and was provided to the Centers for Disease Control and Prevention (CDC) on 6 March 1997 upon request by Robert Weaver. This strain is resistant to all wild-type Listeria phages surveyed; thus, it is a useful control strain for Listeria phage host range studies (4, 5).

FSL R9-0915 was grown overnight in brain heart infusion broth (at 37°C, with shaking); genomic DNA was extracted using the Qiagen DNeasy minikit (Hilden, Germany), per the manufacturer's instructions. For Illumina sequencing, the library was prepared using a Nextera XT kit and sequenced with a NextSeq 550 instrument (150-bp pairedend reads; 3,474,670 total reads were generated; average length of 137.31 bp). For Nanopore sequencing, the library was prepared using an Oxford Nanopore rapid barcoding kit (SQK-RBK0004) and sequenced with a MinION instrument (130,765 total reads were generated; average read length of 5,446.79 bp). FastQC v0.11.7 (6) was used to determine read quality, and Trimmomatic v0.35 (7) was used to trim Illumina reads (parameters were ILLUMINACLIP:NexteraPE-PE.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36). A hybrid assembly was constructed with Unicycler v0.4.8-beta (8) (with pilon polishing), which automatically removes overlaps and circularizes and reorients the assembly to begin at the dnaA gene. QUAST v4.6.3 (9) was used to determine assembly statistics (e.g., length and G+C content), and BBMap v38.08 (10) and SAMtools v1.8 (11) were used to map reads to the assembly and determine average read coverage. The assembly was annotated with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP; v4.13) (12). Default parameters were used for all software unless otherwise specified.

The complete genome assembly of FSL R9-0915 consists of two contigs as follows: one chromosome (2,946,104 bp; G+C content of 38.01%; 153.1× Illumina read

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tdenes@utk.edu. Received 2 October 2020

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bCenter for Food Safety, University of Georgia, Griffin, Georgia, USA

cCenters for Disease Control and Prevention, National Listeria Reference Laboratory, Atlanta, Georgia, USA



TABLE 1 Amino acid differences between genes involved in wall teichoic acid biosynthesis for L. monocytogenes strains FSL R9-0915 and 10403S

Gene homolog ide	entifier for strain:	- Feature	No. of amino acid changes	Residue	Amino acid change by strain		
10403S	EGD-e				10403S	FSL R9-0915	Predicted effect ^a
LMRG_00541	lmo1079	YHFO family protein	5	422	lle	Thr	RNS
				684	Gly	Asp	RNS
				697	Val	lle	CNS
				909	Leu	Arg	RNS
				948	Lys	Arg	CNS
LMRG_00542	lmo1080	rmlT	8	40	Ser	Ala	RNS
				49	Pro	Ser	RNS
				393	Ser	Ala	RNS
				427	Asp	Asn	RNS
				456	Asn	Asp	RNS
				461	Asp	Glu	CNS
				498	lle	Val	CNS
				544	Val	lle	CNS
LMRG_00543	lmo1081	rmlA	0	3-1-1	vai	iic	CIVS
LMRG_00544	lmo1082	rmlC	1	38	Ala	Val	CNS
LMRG_00545	lmo1083	rmlB	2	24	His	Tyr	RNS
LIVING_00545	111101065	ППБ	2	54 54		•	CNS
				5 4 55	Val	lle	
LMRG_00546	1 1001	10	2		71		Truncated proteir
	lmo1084	rmID	2	28	Thr	Asn	CNS
		ci i c		73	Asn	Asp	RNS
LMRG_01697	lmo2550	Glycosyltransferase	1	300	Pro	Leu	CNS
LMRG_01698	lmo2549	Putative flippase	0				
LMRG_01692	lmo2555	Glycosyltransferase	1	195	Asp	Asn	RNS
LMRG_01693	lmo2554	Glycosyltransferase	10	129	Glu	Ala	RNS
				150	Thr	Gly	RNS
				157	Glu	Ala	RNS
				160	Gly	Glu	RNS
				222	Asn	Ser	CNS
				225	Asn	Lys	RNS
				237	Thr	Ser	CNS
				264	Ser	Cys	CNS
				314	Ala	Ser	RNS
				332	Gly	Asp	RNS
LMRG_01694	lmo2553	Flippase-like domain-	5	48	Leu	Ser	RNS
		containing protein		190	lle	Val	CNS
		3.		338	Gln	Lys	RNS
				350	Thr	Ala	RNS
				352	Ala	Val	CNS
LMRG_02769	lmo1695	mprF	5	164	Asp	Asn	RNS
		трп	,	555	Glu	Asn	RNS
				668	Asp	Glu	CNS
				714	lle	Val	CNS
				714	Asp	Glu	CNS

^a RNS, radical nonsynonymous mutation; CNS, conservative nonsynonymous mutation; nonsynonymous substitutions were classified based on amino acid charge and polarity (16).

coverage) and one plasmid (50,101 bp; G+C content of 35.48%; 253.0× Illumina read coverage). The PGAP annotation identified 2,977 genes, including 2,888 coding DNA sequences (CDSs) and 89 RNA genes (18 rRNAs, 67 tRNAs, and 4 noncoding RNAs [ncRNAs]). A single-nucleotide frameshift deletion (Table 1) was identified in the FSL R9-0915 rmlB homolog (a dTDP-D-glucose-4,6-dehydratase; nucleotide position 1147464) compared with serotype 1/2a strain 10403S (GenBank accession number NC_017544.1); this would result in a truncated protein. These findings support studies that suggest that serotype 7 strains evolved from a serotype 1/2 genetic background (13) by accumulating mutations in genes (e.g., rmlB) that could disrupt glycosylation of wall teichoic acids (WTAs), such as loss of rhamnose in WTAs (14, 15). Additionally, previous wheat germ agglutination assays confirm that FSL R9-0915 lacks N-acetylglucosamine in its WTAs (4).



Although no identified mutations are clearly responsible for the lack of N-acetylglucosamine in WTAs, several amino acid changes were found in relevant genes (Table 1).

Data availability. The sequencing data and assembly for FSL R9-0915 are located under BioProject number PRJNA664209 (BioSample SAMN16231355; raw reads SRR12695183 and SRR12695179; annotated assembly CP062124 and CP062125).

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