Population genetics and virulence potential of *Listeria monocytogenes* serotype 1/2b strains

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SUMMARY

Listeria monocytogenes **is a facultative intracellular pathogen that causes life-threatening infections, including meningitis and abortion in humans and ruminants. Serotypes 1/2a, 1/2b and 4b are highly associated with human infection, of which the serotypes 1/2b and 4b exhibit similar phylogenitic lineages, the genomic diversity within serotype 1/2b strains however remains unknown. Here, we used a multi-locus sequence typing (MLST) approach to examine the population genetics of 1/2b (n=31) and 4b isolates (n=31) in Japan, revealing that 54.8% of the serotype 1/2b isolates were sequence type (ST)-3, ST-5 or ST-87, while 94.7% of the serotype 4b isolates were ST-1, ST-2 or ST-6. Infection of the insect model** ***Galleria mellonella*** **showed greater variation of lethalityof the serotype 1/2b strains compared to 4b strains. The serotype 1/2b strains exhibited variation of listeriolysin O (LLO) secretion, and** ***hlyA*** **mutation in EGDe strain exhibited decreased lethality to the insect model, suggesting the link between the LLO secretion and the insect's lethality of the serotype 1/2b isolates. To gain insight into the genomic diversity of serotype 1/2b, three representative 1/2b isolates were genome sequenced. Comparative genome analysis revealed sequence variations in a prophage region and type II CRISPR loci among serotype 1/2b isolates, suggesting the idea of phage-mediated genomic diversification within this serotype. This is the first report of MLST-based population genetics of** ***L. monocytogenes*** **isolates in Japan. Our data revealed diverse virulence potentials among serotype 1/2b isolates, reflected in the population structure.**

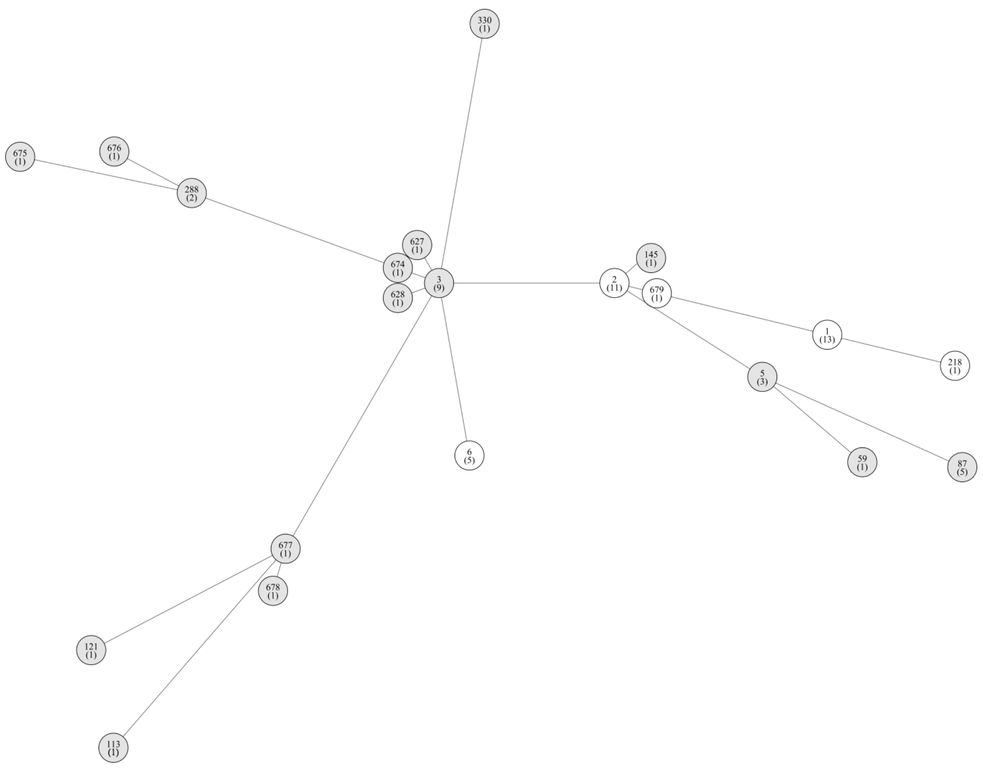

Fig. 1. Phylogenic analysis of *L. monocytogenes* serotypes 1/2b and 4b isolates from Japan based on an MLST approach. Minimum spanning tree of 21 STs from 62 *L. monocytogenes* isolates based on MLST allelic profiles. The ST numbers are shown in the circles. STs from Serotypes 1/2b and 4b are indicated in grey or open circles, respectively. The number of isolates belonging to each ST are shown in the parenthesis.

Table 1. Statistics of *L. monocytogenes* MLST data from serotypes 1/2b and 4b strains used in this study.

Serotype	No. isolate	No. haplotypes	Haplotype diversity, Hd	No. polymorphic sites	Nucleotide diversity, Pi	F_{ST}	G_{ST}
1/2b	31	16	0.89247	191	0.01463	0.02922	0.01914
4b	31	5	0.69247	14	0.00166	0.09417	0.0222
total	62	21	0.89794	2,335	0.00940	-	

3,288 bases-concatenated sequences were used to calculate the divergence within the serotypes 1/2b & 4b populations by DnaSP program.

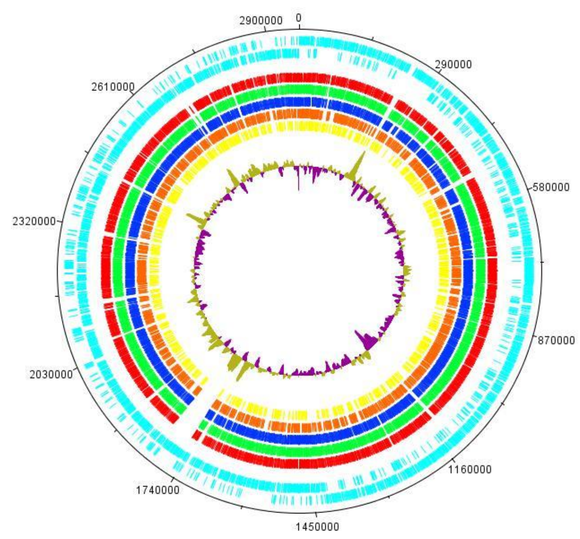

Fig. 2. Circular genome mapping of *L. monocytogenes* serotype 1/2b strains. The CDSs of the reference genome of strain SLCC2755 (accession No. AF532277) are shown in the two outer rings (light blue, clockwise and counterclockwise). The subsequent rings depict RAST-annotated CDSs in the genomes of Lm_0003 (red), Lm_0008 (green), Lm_0010 (blue), and EGDe (accession No. AL592022). The innermost ring depicts the GC content variation and GC skew from the mean (60%) of the reference genome. The arrow indicates the prophage-associated genes with highly variable sequences among the strains.

A

B

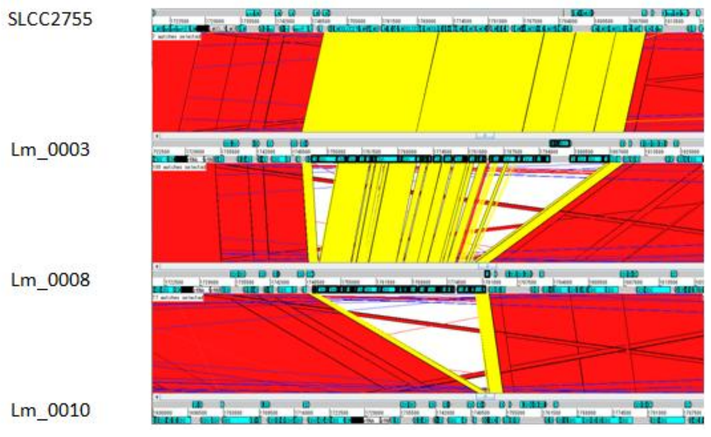

Fig. 3. Sequence variation of the type II CRISPR array (A) and prophage loci (B) in *L. monocytogenes* serotype 1/2b, imaged using the Artemis Comparison Tool (ACT). (A) Focused genetic alignment for the CRISPR array among the SLCC2755, Lm_0003, Lm_0008, and Lm_0010 strains (B) Focused genetic alignment for the prophage locus among the SLCC2755, Lm_0003, Lm_0008, and Lm_0010 strains.

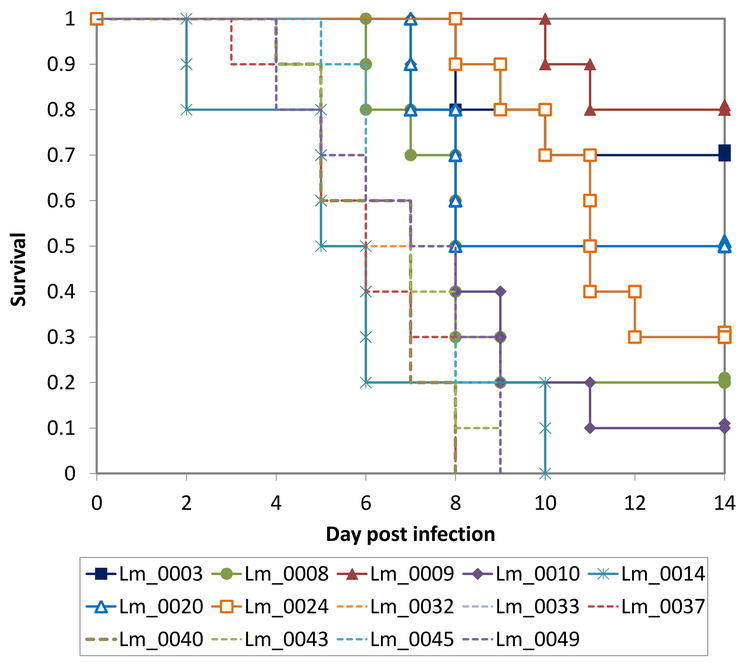

Fig. 4. Survival of *Galleria mellonella* upon *L. monocytogenes* infection. The insects (n=10 per group) were dorsolaterally infected with 10^6 cells of representative isolates of *L. monocytogenes*; serotype 1/2b (with symbols): Lm_0003 (ST-3), Lm_0008 (ST-3), Lm_0009 (ST-3), Lm_0010 (ST-5), Lm_0014 (ST-87), Lm_0020 (ST-288), and Lm_0024 (ST-675); serotype 4b (dotted lines without symbols): Lm_0032 (ST-1), Lm_0033 (ST-1), Lm_0037 (ST-1), Lm_40 (ST-2), Lm_0043 (ST-2), Lm_0045 (ST-2), and Lm_0049 (ST-6). The survival of the insects was scored daily for up to 14 days post infection.

Table 2. Survival of *G. mellonella* upon infection with *L. monocytogenes*

Inoculum			MST[*1]	SM	SD	Mean square	*p*-value [*2]
serotype	strain	ST					(1/2b *vs* 4b)
1/2b	Lm_0003	3	12.5	10.44	2.24	4.32	0.0037
	Lm_0008	3	13.5				
	Lm_0009	3	8.8				
	Lm_0010	5	8.9				
	Lm_0014	87	7.2				
	Lm_0024	675	11.4				
	Lm_0020	288	10.8				
4b	Lm_0037	1	6.0	6.60	0.39	0.13	
	Lm_0043	2	6.7				
	Lm_0049	6	6.9				
	Lm_0045	2	7.2				
	Lm_0032	1	6.5				
	Lm_0033	1	6.6				
	Lm_0040	2	6.3				

[*1] MST, median survival time

[*2] Student *t*-test was used to calculate the statistical significance betweeen serotypes 1/2b and 4b.

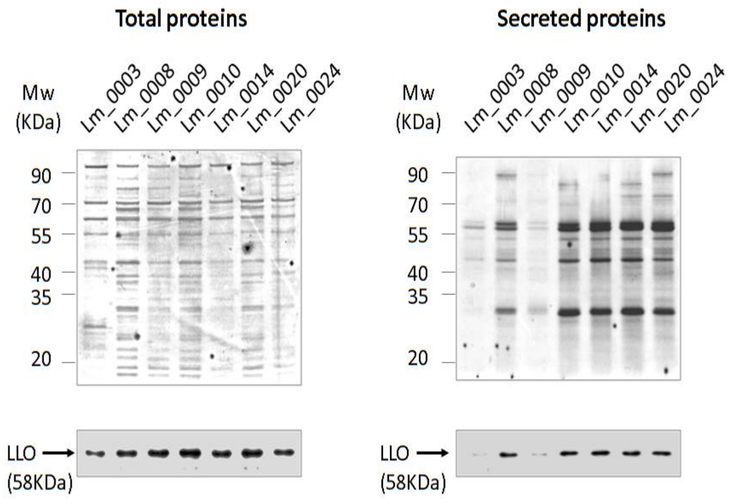

Fig. 5. Variation of LLO secretion in *L. monocytogenes* 1/2b. Seven representative 1/2b strains used for lethality assay (Fig. 2) were cultivated in modified welshimer broth to an OD_{600} of 1.35-1.40. Bacterial cells and supernatants were obtained, from which proteins were extracted, respectively (total proteins, right panels; secreted proteins, left panels). Both total and secreted protein samples originated from equal numbers of bacterial cultures were loaded in SDS-PAGE, which were subjected to CBB stain (upper panels) or western blot to detect LLO (arrowed, bottom panels).