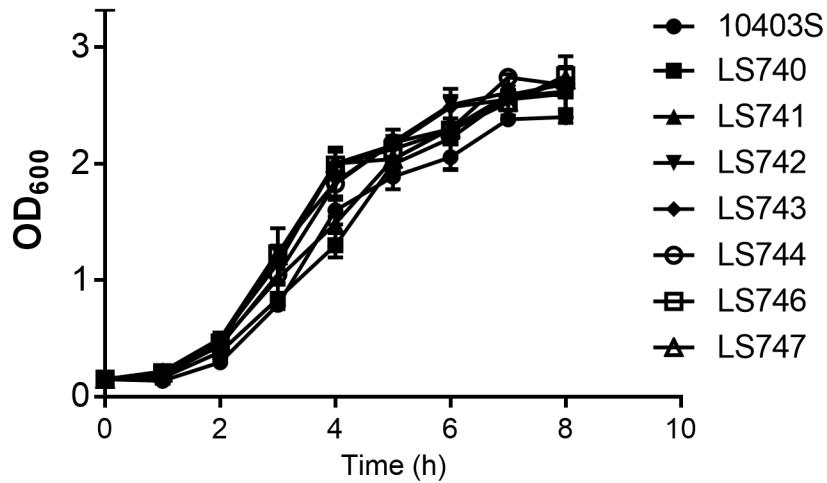


Supplementary Information to

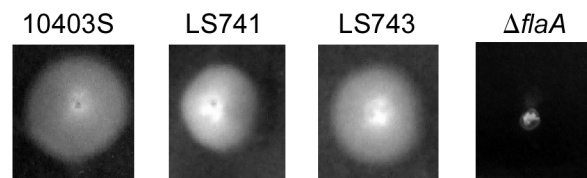
Characterization of the pathogenesis and immune response to *Listeria monocytogenes* strains isolated from a sustained national outbreak

Pallab Ghosh, Yan Zhou, Quentin Richardson and Darren E. Higgins

Supplementary Figures



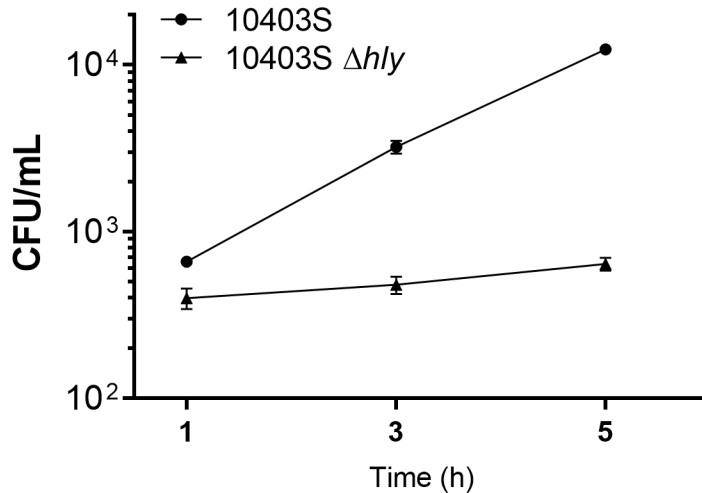
Supplementary Figure S1. *In vitro* growth of *L. monocytogenes* strains in BHI broth. Bacterial cultures were grown shaking at 37°C for 8 hours and the OD₆₀₀ was measured at 60-minute intervals. Data represents the mean \pm SD of three experiments.



Supplementary Figure S2. Motility of *L. monocytogenes* strains. Flagellar motility of *L. monocytogenes* 10403S, LS741, LS743, and the flagellar motility-deficient EGDe Δ *flaA* (Δ *flaA*) strains are shown on 0.3% agar medium grown at 30°C for 16 hours. Images shown are representative of two separate experiments with similar results.

10403S PrfA	1	MNAQAEEFKKYLETNGIKPKQFHKKELIFNQWDPQEYCIFLYDGITKLTISENGTIMNL	60
LS741 PrfA	1	MNAQAEEFKKYLETNGIKPKQFHKKELIFNQWDPQEYCIFLYDGITKLTISENGTIMNL	60
LS743 PrfA	1	MNAQAEEFKKYLETNGIKPKQFHKKELIFNQWDPQEYCIFLYDGITKLTISENGTIMNL	60
Seq. identities		MNAQAEEFKKYLETNGIKPKQFHKKELIFNQWDPQEYCIFLYDGITKLTISENGTIMNL	
10403S PrfA	61	QYYKGAFVIMSGFIDTETSVMGYNLEVISEQATAYVIKINELKELLSKNLTHFFYVFQTL	120
LS741 PrfA	61	QYYKGAFVIMSGFIDTETSVMGYNLEVISEQATAYVIKINELKELLSKNLTHFFYVFQTL	120
LS743 PrfA	61	QYYKGAFVIMSGFIDTETSVMGYNLEVISEQATAYVIKINELKELLSKNLTHFFYVFQTL	120
Seq. identities		QYYKGAFVIMSGFIDTETSVMGYNLEVISEQATAYVIKINELKELLSKNLTHFFYVFQTL	
10403S PrfA	121	QKQVSYSLAKFNDFSINGKLGSI CGQLLILTYVYGKETPDGIKITLDNLTMQELGYSSGI	180
LS741 PrfA	121	QKQVSYSLAKFNDFSINGKLGSI CGQLLILTYVYGKETPDGIKITLDNLTMQELGYSSGI	180
LS743 PrfA	121	QKQVSYSLAKFNDFSINGKLGSI CGQLLILTYVYGKETPDGIKITLDNLTMQELGYSSGI	180
Seq. identities		QKQVSYSLAKFNDFSINGKLGSI CGQLLILTYVYGKETPDGIKITLDNLTMQELGYSSGI	
10403S PrfA	181	AHSSAVSRIISKLKQEKVIVYKNSCFYVQNL DYLKRYAPKLEW FYLACPATWGKLN	237
LS741 PrfA	181	AHSSAVSRIISKLKQEKVIVYKNSCFYVQNL DYLKRYAPKLEW FYLACPATWGKLN	237
LS743 PrfA	181	AHSSAVSRIISKLKQEKVIVYKNSCFYVQNL DYLKRYAPKLEW FYLACPATWGKLN	237
Seq. identities		AHSSAVSRIISKLKQEKVIVYKNSCFYVQNL DYLKRYAPKLEW FYLACPATWGKLN	

Supplementary Figure S3. Protein sequence alignment of PrfA in *L. monocytogenes* strains 10403S, LS741 and LS743. The known PrfA* mutations^{1,2} do not appear in 10403S or the LS741 and LS743 outbreak strains.



Supplementary Figure S4. Intracellular growth of *L. monocytogenes* in HeLa cells. HeLa cells were infected with the indicated strains for 1 hour. Gentamicin was then added and at 2-hour intervals post-infection, HeLa cells were lysed and intracellular bacteria were enumerated by plating dilutions of lysates. Data presented is the mean \pm SD of three independent experiments performed in duplicate.

Supplementary Table S1. Characteristics of the *L. monocytogenes* strains used in this study.

Strain or isolate	Alternative name	PFGE pattern [#]	ST ^{**}	Serotype	Outbreak associated	Source	Reference(s)	Biosample or accession no.
LS740	CFSAN012827	II	NA	1/2b	Yes, cantaloupe outbreak in 2011	Human	3	SAMN02769788
LS741	CFSAN012828	II	5	1/2b	Yes, cantaloupe outbreak in 2011	Human	3	SAMN02769789, AAALMK000000000.1
LS742	CFSAN012829	I	NA	1/2a	Yes, cantaloupe outbreak in 2011	Human	3	SAMN02769790
LS743	CFSAN012830	I	29	1/2a	Yes, cantaloupe outbreak in 2011	Human	3,4	SAMN02769791, JRZB000000000.1
LS744	CFSAN012831	IV	NA	1/2a	Yes, cantaloupe outbreak in 2011	Human	3	SAMN02769792
LS746	CFSAN012832	III	NA	1/2a	Yes, cantaloupe outbreak in 2011	Human	3	SAMN02769793
LS747	CFSAN012833	IV	NA	1/2a	Yes, cantaloupe outbreak in 2011	Human	3	SAMN02769794
10403S	N/A	N/A	85	1/2a	No	Human, lab reference strain	5,6	SAMN02641382, NC_017544
10403S <i>Δhly</i>	DP-L2161	N/A	85	1/2a	N/A		7	N/A
EGDe <i>ΔflaA</i>	DH-L1042	N/A	35	1/2a	N/A	Lab reference strain	8	N/A
LS741 <i>Δhly</i>	DH-L2148	II	5	1/2b	N/A		This study	N/A

[#]Pulsed-field gel electrophoresis (PFGE) pattern combinations based on the restriction enzymes *AscI* and *ApaI* are reported as described³.

^{**}ST, sequence type based on a 7-gene multilocus sequence typing (MLST) comparison as observed in the Institut Pasteur *L. monocytogenes* MLST database (<https://bigsd.bpasteur.fr/listeria/listeria.html>).

NA: not analyzed

N/A: not applicable

Supplementary Table S2. *Escherichia coli* strains and plasmids used in this study.

Strain or plasmid	Strain genotype or plasmid description	Reference(s)
pKSV7	Allelic exchange vector for <i>L. monocytogenes</i>	9
pKSV7 Δhly	Allelic exchange vector for the in-frame deletion of <i>hly</i> in LS741	7
DP-E2154	<i>E. coli</i> harboring pKSV7 Δhly	7

Supplementary References

- 1 Eiting, M., Hagelucken, G., Schubert, W. D. & Heinz, D. W. The mutation G145S in PrfA, a key virulence regulator of *Listeria monocytogenes*, increases DNA-binding affinity by stabilizing the HTH motif. *Mol. Microbiol.* **56**, 433-446 (2005).
- 2 Becavin, C. *et al.* Comparison of widely used *Listeria monocytogenes* strains EGD, 10403S, and EGD-e highlights genomic variations underlying differences in pathogenicity. *mBio* **5**, 10.1128/mBio.00969-00914 (2014).
- 3 Laksanalamai, P. *et al.* Genomic characterization of *Listeria monocytogenes* strains involved in a multistate listeriosis outbreak associated with cantaloupe in US. *PLoS One* **7**, e42448; 42410.41371/journal.pone.0042448 (2012).
- 4 Burall, L. S., Grim, C., Gopinath, G., Laksanalamai, P. & Datta, A. R. Whole-genome sequencing identifies an atypical *Listeria monocytogenes* strain isolated from pet foods. *Genome Announc.* **2**, e01243-01214; 01210.01128/genomeA.01243-01214 (2014).
- 5 Edman, D. C., Pollock, M. B. & Hall, E. R. *Listeria monocytogenes* L forms. I. Induction maintenance, and biological characteristics. *J. Bacteriol.* **96**, 352-357 (1968).
- 6 Bishop, D. K. & Hinrichs, D. J. Adoptive transfer of immunity to *Listeria monocytogenes*. The influence of *in vitro* stimulation on lymphocyte subset requirements. *J. Immunol.* **139**, 2005-2009 (1987).
- 7 Jones, S. & Portnoy, D. A. Characterization of *Listeria monocytogenes* pathogenesis in a strain expressing perfringolysin O in place of listeriolysin O. *Infect. Immun.* **62**, 5608-5613 (1994).
- 8 Gründling, A., Burrack, L. S., Bower, H. G. & Higgins, D. E. *Listeria monocytogenes* regulates flagellar motility gene expression through MogR, a transcriptional repressor required for virulence. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 12318-12323 (2004).
- 9 Smith, K. & Youngman, P. Use of a new integrational vector to investigate compartment-specific expression of the *Bacillus subtilis* spoIIM gene. *Biochimie* **74**, 705-711 (1992).