Table 1. Attenuated *Listeria monocytogenes* strains platform strains

Strain	Genotype	Phenotype	Pathogenicity in C57BL/6 mice LD_{50},* cfu	Pathogenicity in Balb/c mice LD_{50},† cfu	Log attenuation‡	OVA-specific $CD8^+$ T cells,§ %	Log protection¶	Ref.
DP-L4056	Wt; 10403S, phage free	Wild-type	1×10^5	5×10^4	—	2.96 ± 1.5	4.86 ± 0.01	1
DP-L4056 (Heat killed)	Wt; 10403S, phage free	Wild-type	1×10^{10}	ND	5.0	0.03 ± 0.0	–0.76 ± 0.46	2
Single mutants								
DP-L4027	*hly* (Δ*LLO*)	Defective phagolysosome release	2×10^9	1×10^9	4.3	2.85 ± 0.9	1.74 ± 0.26	3
DP-L4029	*ΔactA*	No host actin nucleation; defective cell-to-cell spread	1×10^8	1×10^8	3.0	8.57 ± 1.4	4.29 ± 0.31	4
DP-L4017	LLO L461T	Cytotoxic; constitutive LLO activity at physiologic pH; defective cell-to-cell spread	1×10^7	ND	2.0	7.28 ± 1.3	4.73 ± 0.16	5
DP-L4042	LLO Δ26 (Δ*PEST*)	Cytotoxic; defective cell-to-cell spread	6×10^8	ND	3.8	2.87 ± 2.1	0.80 ± 0.11	6
DP-L4097	LLO S44A	Cytotoxic; defective cell-to-cell spread	7.5×10^7	ND	2.9	3.60 ± 1.6	2.22 ± 0.41	7
DP-L4364	Δ*lplA* (lipoate protein ligase)	Abortive infection; limited ability to proliferate intracellularly	1×10^8	5×10^6	3.0	3.01 ± 1.2	3.28 ± 0.17	8
DP-L4405	Δ*inlA*	Impaired InlA-mediated infection	1×10^5	ND	0	3.56 ± 1.1	ND	9
DP-L4406	Δ*inlB*	Impaired InlB-mediated infection	1×10^5	ND	0	3.11 ± 0.3	4.18 ± 0.37	9
Double mutants								

Strain	Genotype	Phenotype	Pathogenicity in C57BL/6 mice LD_{50},* cfu	Pathogenicity in Balb/c mice LD_{50},† cfu	Log attenuation‡	OVA-specific $CD8^+$ T cells,§ %	Log protection¶	Ref.
CS-L0001	Δ*actA*/Δ*inlB*	No host actin nucleation; defective cell-to-cell spread	1×10^8–5×10^8	1×10^8	3.0	8.36 ± 1.2	4.86 ± 0.00	This study
CS-L0002	Δ*actA*/Δ*lplA*	No host actin nucleation; defective cell-to-cell spread	1×10^9	ND	4.0	3.00 ± 1.3	4.86 ± 0.00	This study
CS-L0003	L461T/Δ*lplA*	Cytotoxic; Abortive infection; limited ability to proliferate intracellularly	1×10^9	ND	4.0	ND	4.86 ± 0.00	This study
DP-L4038	Δ*actA*/L461T	Cytotoxic; defective cell-to-cell spread	3×10^9	ND	4.5	3.31 ± 0.6	3.56 ± 0.33	7
DP-L4384	LLO S44A/L461T	Cytotoxic; defective cell-to-cell spread	1×10^8	ND	3.0	2.49 ± 1.5	0.66 ± 0.33	7

LLO, listeriolysin O; ND, not determined; —, not applicable.

*Pathogenicity in female C57BL/6 mice was determined of the indicated parental strain. Three to five mice were infected i.v. with 5-fold dilutions starting at 1×10^9 colony-forming units (cfu) of the indicated strain. Survival was monitored over 10 days and the LD_{50} was calculated. The recombinant ovalbumin (OVA)-expressing strains were shown to have an LD_{50} that was within a factor of five of the unmodified parent strains (data not shown).

†Pathogenicity in female Babl/c mice was determined with serial 5-fold dilutions of the indicated parental strain.

‡Log attenuation is calculated based on the LD_{50} for the indicated strain compared with wild-type *Listeria* in C57BL/6 mice.

§The percent OVA-specific $CD8^+$ T cells was determined 7 days after immunization in C57BL/6 mice vaccinated i.v. with a dose equal to 0.1 LD_{50} of the indicated *Listeria* OVA strain. OVA-specific IFN-γ secreting $CD8^+$ T cells were determined by intracellular cytokine staining. The average of at least three independent experiments is shown.

¶Log protection is calculated based on the difference between cfu per spleen of naïve and *Listeria*-vaccinated C57BL/6 mice. Female C57BL/6 mice were vaccinated with 0.1 LD_{50} of the indicated attenuated *Listeria* strains. Wild-type *Listeria* served as positive control and heat-killed *Listeria* (wt) as negative control. Vaccinated mice are

challenged with a 2× LD_{50} 28 days post the primary vaccination. Spleens were harvested 72 h post challenge and homogenized in PBS/0.5% Triton-X100 to determine CFU per organ. Limit of detection (LOD) is 50 CFU per organ. The presented data are combined from two independent experiments.

1. Bishop, D. K. & Hinrichs, D. J. (1987) *J. Immunol.* **139,** 2005–2009.

2. Lauvau, G., Vijh, S., Kong, P., Horng, T., Kerksiek, K., Serbina, N., Tuma, R. A. & Pamer, E. G. (2001) *Science* **294,** 1735–1759.

3. Jones, S. & Portnoy, D. A. (1994) *Infect. Immun.* **62,** 5608–5613.

4. Camilli, A., Tilney, L. G. & Portnoy, D. A. (1993) *Mol. Microbiol.* **8,** 143–157.

5. Glomski, I. J., Gedde, M. M., Tsang, A. W., Swanson, J. A. & Portnoy, D. A. (2002) *J. Cell Biol.* **156,** 1029–1038.

6. Decatur, A. L. & Portnoy, D. A. (2000) *Science* **290,** 992–995.

7. Glomski, I. J., Decatur, A. L. & Portnoy, D. A. (2003) *Infect. Immun.* **71,** 6754–6765.

8. O'Riordan, M., Moors, M. A. & Portnoy, D. A. (2003) *Science* **302,** 462–464.

9. Bakardjiev, A. I., Stacy, B. A., Fisher, S. J. & Portnoy, D. A. (2004) *Infect. Immun.* **72,** 489–497.