

Supplementary Information

Novel PCR Assays Complement Laser Biosensor-Based Method and Facilitate *Listeria* Species Detection from Food. *Sensors* 2015, 15, 22672-22691

Kwang-Pyo Kim ^{1,2,†}, **Atul K. Singh** ^{1,†}, **Xingjian Bai** ¹, **Lena Leprun** ^{1,‡} and **Arun K. Bhunia** ^{1,3,*}

¹ Molecular Food Microbiology Laboratory, Department of Food Science, Purdue University, West Lafayette, IN 47907, USA; E-Mails: kpkim@jbnu.ac.kr (K.-P.K.); aksingh@purdue.edu (A.K.S.); bai16@purdue.edu (X.B.); l.leprun@laposte.net (L.L.)

² Department of Food Science and Technology, College of Agriculture and Life Sciences, Chonbuk National University, Jeonbuk 561756, Korea

³ Department of Comparative Pathobiology, Purdue University, West Lafayette, IN 47907, USA

‡ Present address: CROUS de Dijon, Dijon Cedex 21012, France.

† These authors contributed equally to this study.

* Author to whom correspondence should be addressed; E-Mail: bhunia@purdue.edu; Tel.: +1-765-494-5443; Fax: +1-765-494-7953.

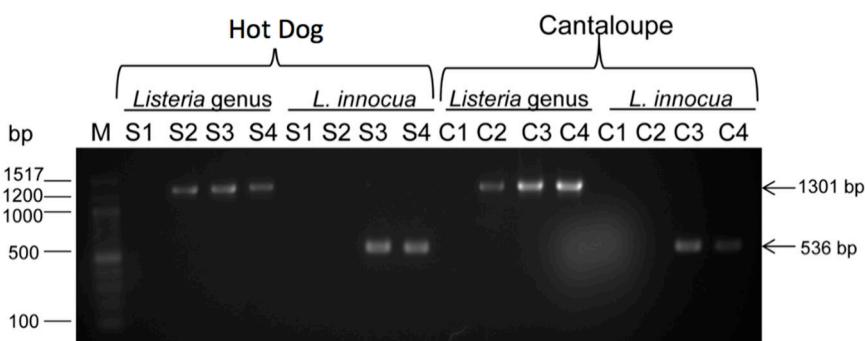


Figure S1. Application of *lap* gene-based *Listeria sensu stricto* specific primers (ELAP-F/LIS-R1) and 460 *Listeria innocua* (InnF1/InnR1)-specific primer sets for detection of *L. monocytogenes* and *L. innocua* in food system (hotdog sample, S and Cantaloupe, C). Uninoculated sample, S1/C1; sample inoculated with *L. monocytogenes* F4244, S2/C2; sample inoculated with *L. innocua* F4248, S3/C3; and sample inoculated with *L. monocytogenes* F4244 and *L. innocua* F4248, S4/C4. To detect *Listeria* genus and *L. innocua*, primer set ELAP-F1/LIS-R1 and Inn-F1/Inn-R1 were used in the PCR amplification for DNA extracted from enriched sample. M, 100 bp DNA ladder (NEB, Ipswich, MA).

Table S1. List of bacterial culture and specificity of *lap* gene-specific primer sets used in this study.

Isolates (serotype) ^a	Ribotype ^b	PCR Amplification with Primer Combinations			
		ELAP-F1/LIS-R1	Inn-F1/Inn-R1	IvaSee-F1/IvaSee-R1	Wel-F1/Wel-R1
<i>Listeria</i> strains					
<i>L. monocytogenes</i> ATCC15313 (1/2a)	DUP-1042	+	—	—	—
<i>L. monocytogenes</i> Scott A (4b)	DUP-1042	+	—	—	—
<i>L. monocytogenes</i> F4244(4b)	DUP-1044	+	—	—	—
<i>L. monocytogenes</i> ATCC35152 (1/2a)	DUP-1030	+	—	—	—
<i>L. monocytogenes</i> ATCC43257 (4b)	NT	+	—	—	—
<i>L. monocytogenes</i> ATCC19118 (4e)	DUP-1038	+	—	—	—
<i>L. monocytogenes</i> ATCC7644 (1/2c)	NT	+	—	—	—
<i>L. monocytogenes</i> ATCC19114 (4a)	DUP-1059	+	—	—	—
<i>L. monocytogenes</i> ATCC2540 (3b)	DUP-1052	+	—	—	—
<i>L. monocytogenes</i> ATCC19116 (4c)	DUP-1061	+	—	—	—
<i>L. monocytogenes</i> ATCC19117 (4d)	DUP-1042	+	—	—	—
<i>L. monocytogenes</i> ATCC19112 (1/2c)	DUP-1039	+	—	—	—
<i>L. monocytogenes</i> ATCC19115 (4b)	DUP-1042	+	—	—	—
<i>L. ivanovii</i> SE98	NT	+	—	+	—
<i>L. ivanovii</i> ATCC19119	DUP-1021	+	—	+	—
<i>L. ivanovii</i> V12 (5)	NT	+	—	+	—
<i>L. ivanovii</i> V35 (5)	NT	+	—	+	—
<i>L. ivanovii</i> V119	NT	+	—	+	—
<i>L. ivanovii</i> LM6	NT	+	—	+	—
<i>L. ivanovii</i> SLCC4769	NT	+	—	+	—
<i>L. ivanovii</i> V195 (5)	NT	+	—	+	—
<i>L. ivanovii</i> LA29	NT	+	—	+	—
<i>L. ivanovii</i> SE136	DUP-1021	+	—	+	—
<i>L. ivanovii</i> 2732 NVSL	NT	+	—	+	—
<i>L. ivanovii</i> 2875 NVSL	NT	+	—	+	—
<i>L. innocua</i> F4248	DUP-1006	+	+	—	—
<i>L. innocua</i> V57 (6a)	DUP-1005	+	+	—	—
<i>L. innocua</i> V58 (6b)	DUP-1009	+	+	—	—
<i>L. innocua</i> V11 (6a)	DUP-1009	+	+	—	—
<i>L. innocua</i> F4247	DUP-1018	+	+	—	—
<i>L. innocua</i> V22 (6a)	DUP-1005	+	+	—	—
<i>L. innocua</i> V24 (6b)	DUP-1005	+	+	—	—
<i>L. innocua</i> ATCC33090 (6a)	DUP-1009	+	+	—	—
<i>L. innocua</i> C91-2(L)	DUP-1005	+	+	—	—
<i>L. innocua</i> LA-1	DUP-1009	+	+	—	—
<i>L. seeligeri</i> SE31	NT	+	—	+	—
<i>L. seeligeri</i> SLCC3954	NT	+	—	+	—
<i>L. seeligeri</i> V45 (1/2b)	NT	+	—	+	—
<i>L. seeligeri</i> V34 (1/2b)	NT	+	—	+	—
<i>L. seeligeri</i> V13 (1/2b)	NT	+	—	+	—

Table S1. Cont.

Isolates (serotype) ^a	Ribotype ^b	PCR Amplification with Primer Combinations			
		ELAP-F1/LIS-R1	Inn-F1/Inn-R1	IvaSee-F1/IvaSee-R1	Wel-F1/Wel-R1
<i>L. welshimeri</i> ATCC35897	NT	+	–	–	+
<i>L. welshimeri</i> LM156	NT	+	–	–	+
<i>L. welshimeri</i> 105 ₂ (2L)	NT	+	–	–	+
<i>L. grayi</i> LM37	NT	–	–	–	–
<i>L. grayi</i> ATCC19120	NT	–	–	–	–
<i>L. marthii</i> BAA-1595	NT	+	–	NT	NT
<i>L. rocourtiae</i> CIP109804	NT	–	–	NT	NT
<i>Non-Listeria</i> cultures					
<i>Enterobacter aerogenes</i>	NT	–	–	–	–
<i>Serratia marcescens</i>	NT	–	–	–	–
<i>Hafnia alvei</i>	NT	–	–	–	–
<i>Lactobacillus casei</i>	NT	–	–	–	–
<i>Lactobacillus acidophilus</i> NRRL B131910	NT	–	–	–	–
<i>Bacillus cereus</i> ATCC3432	DUP-6078	–	–	–	–
<i>Escherichia coli</i> EDL933 (O157:H7)	DUP-3064	–	–	–	–
<i>Salmonella enterica</i> serovar Typhimurium ATCC13096	NT	–	–	–	–

^a Total strain 55 bacterial strains were used in this study, comprising of 47 strains of *Listeria* species (*L. monocytogenes*, n = 13; *L. ivanovii*, n = 12; *L. innocua*, n = 10; *L. seeligeri*, n = 5; *L. welshimeri*, n = 3; *L. grayi*, n = 2; *L. marthii*, n = 1; *L. rocourtiae*, n = 1); Non-*Listeria* strains, n = 8; NT, not tested;

^b Ribotyping was performed in an automated RiboPrinter (Qualicon, Inc.) with the *EcoRI* restriction enzyme (Gray *et al.*, 2005). Ribopatterns were compared with the RiboPrinter database for culture identification.

Table S2. Cross validation matrix obtained after image analysis of the scatter of eight *Listeria* species colonies grown on brain-heart infusion (BHI) agar medium, related to Figure 4.

<i>Listeria</i> species ^a	<i>L. monocytogenes</i>	<i>L. innocua</i>	<i>L. grayi</i>	<i>L. seeligeri</i>	<i>L. marthii</i>	<i>L. welshimeri</i>	<i>L. rocourtiae</i>	<i>L. ivanovii</i>
<i>L. monocytogenes</i>	97.7	0	0	0	0	0	2.3	0
<i>L. innocua</i>	0	100	0	0	0	0	0	0
<i>L. grayi</i>	0	0	66.4	1.2	0	5.4	0.3	26.7
<i>L. seeligeri</i>	0	0	1.8	94.9	0	3.3	0	0
<i>L. marthii</i>	1.7	0	1.2	0	95.2	0	0	2
<i>L. welshimeri</i>	0	0	6.6	2.9	0	88.9	0	1.6
<i>L. rocourtiae</i>	0	0	0	0	0	0	100	0
<i>L. ivanovii</i>	0	0	45.3	1.3	0	3.2	0	50.1

^a Cross-validation matrix was generated with image classifier after analysis of around 80 scatter pattern per species.