NOTE *Public Health*

Multiplex PCR serogrouping of *Listeria monocytogenes* isolated in Japan

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ABSTRACT. PCR serogrouping methods were used to examine strains of *L. monocytogenes* isolated in Japan. Among 187 strains, 99.5% were classified into 4 PCR serogroups corresponding to conventional serotypes. Only one isolate had a new PCR profile, which may be a variant of serogroup IVb.

KEY WORDS: *Listeria monocytogenes*, PCR serogrouping, serotyping

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Listeria monocytogenes is a foodborne pathogen that causes invasive listeriosis in the immunocompromised, the elderly, pregnant women, embryos and newborns. Characterization of *L. monocytogenes* isolates is important for epidemiological investigation; serotyping [10], pulsed-field gel electrophoresis (PFGE) [3] and multilocus sequence typing (MLST) [9] are common methods utilized for characterization. Serotyping is firstly used for the bacterial characterization based on the variation of the somatic (O) and flagellar (H) antigens. Recently, molecular serotyping methods, such as PCR serogrouping, have been developed for rapid and accurate discrimination of *L. monocytogenes* [2, 5, 6]. In the present study, PCR serogrouping was performed on *L. monocytogenes* strains isolated in Japan, using those methods.

A total of 187 strains of *L. monocytogenes* isolated between 1989 and 2012 in Japan were investigated. The isolates were recovered from foods, food processing environments and human clinical specimens (159, 17 and 11 isolates, respectively). The food samples included both domestic and imported beef, pork, poultry, meat products, fish, fish products, natural cheese and other items from 13 countries (Table 1).

The strains were serotyped and compared using both PCR serogrouping and conventional immunological serotyping methods. PCR serogrouping was performed using a multiplex PCR assay that targets the six fragments, *lmo1118*, *lmo0737*, *orf2110*, *orf2819*, *prs* (*Listeria* genus specific) and *pfrA* (*L. monocytogenes* specific), and additional PCR targeting *flaA* as previously described by Doumith *et al*. [2], Kerouanton *et al*. [5] and Leclercq *et al*. [6]. Conventional immunological serotyping was performed with commercially available *Listeria* antisera (Denka Siken Co., Tokyo, Japan).

One hundred eighty-six of the isolates (99.5%) were classified into four PCR serogroups: IIa, IIb, IIc and IVb (Table 2). Fifty-two isolates of serotypes 1/2a and 3a, 27 isolates of serotypes 1/2b and 3b, and 36 isolates of serotype 1/2c were assigned to PCR serogroup IIa, IIb and IIc, respectively. One isolate (serotype 1/2a) that had a IIc PCR profile and was *flaA* gene positive was classified into PCR serogroup IIa; the *flaA* gene is present only in serotype 1/2a and 3a strains, and these have previously been classified into PCR serogroup IIa [1, 5]. On the other hand, one isolate (serotype 1/2c) that had a IIa PCR profile and was negative for the *flaA* gene was assigned to PCR serogroup IIc. Seventy-one isolates of serotypes 4ab, 4b/e and 4d were assigned to PCR serogroup IVb. Eight atypical isolates were assigned to PCR serogroup IVb-v1 [6]. These isolates had been recovered from imported food samples: 7 chickens from Brazil between 1998 and 2007, and 1 beef from Australia in 2012. Although the *lmo0737* fragment sequences of the IVb-v1 strains were 100% identical to those of the IVb-v1 strains reported previously [6], the present IVb-v1 strains had 4 different nucleotides compared with that of EGD-e (Gene bank accession no. NC_003210). PFGE analysis was performed using the restriction enzymes *Asc*I and *Apa*I according to the PulseNet protocol [3, 4] with a little modification for the 8 PCR serogroup IVb-v1 strains. PFGE was performed using CHEF Mapper systems (BIO RAD, Hercules, CA, U.S.A.) with the following conditions: angle, 120°; 6V/ cm; temperature, 12°C; switch time, 4 sec−40 sec, linear; run time, 18 hr for *Asc*I; switch time, 0.35 sec−30.82 sec, linear; and run time, 18.5 hr for *Apa*I. Although the PFGE *Asc*I/*Apa*I combined profiles of the 8 strains had four distinguishable patterns, the patterns were similar to one another (data not shown). These results are consistent with a previous study by Leclercq *et al*. [6], suggesting that the PCR serogroup IVb-v1 may not be a recently developed clone.

One serotype 4d isolate recovered from domestic beef in 2010 showed a new PCR profile, with amplified *prs*, *prfA*, *orf2819* DNA fragments and an unexpected band (approxi-

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Table 1. Source and origin of food samples used in this study

Source	Beef	Pork	Poultry	Meat products	Fish and Fish products	Natural cheese	Others	Total
Japan	15	6	23	1	24		6[a)]	75
Brazil			26					26
Australia	6	1		1				8
U.S.A.	6				1			7
Thailand			4					4
Canada	1	1						2
China		1	1					2
Philippines			2					2
Chile		1	1					2
Mexico	2							2
Spain				1				1
Hungary				1				1
France			1					1
Unknown	5	8	7	1		4	1[b)]	26
Total	35	18	65	5	25	4	7	159

a) Vegetables (4 samples) and Raw milk (2 samples), b) Venison.

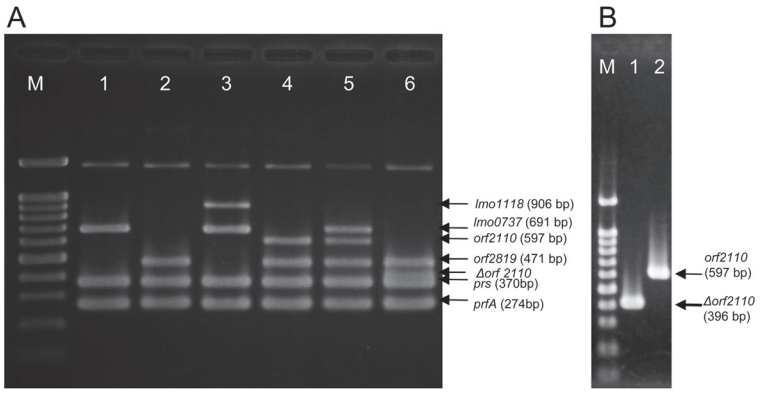

Fig. 1. A: PCR patterns of multiplex PCR assay. Lane M;100 bp ladder (100–1,000, 1,500 bp), lanes 1-5; PCR serogroup IIa, IIb, IIc, IVb, IVb-v1 strains, respectively, lane 6; the strain which had a new PCR profile, IVb with Δ*orf2110*. B: Simplex-PCR assay with *orf2110* primers. Lane M;100 bp ladder, lane 1; the strain which had a new PCR profile, IVb with Δ*orf2110*, lane 2; PCR serogroup IVb strain.

Table 2. Correlation of PCR serogroup and conventional serotype

PCR serogroup	Number of strains	Serotype							
		1/2a	3a	1/2b	3b	1/2c	4ab	4b or 4e	4d
IIa	52	45[a)]	7						
IIb	27			25	2				
IIc	36					36[b)]			
IVb	63						1	57	5
IVb-v1	8							8[c)]	
IVb with Δ*orf2110*	1								1[d)]
Total	187	45	7	25	2	36	1	65	6

a) Containing 1 isolate, IIc profile arranged by *flaA* positive. b) Containing 1 isolate, IIa profile arranged by *flaA* negative. c) 7 isolates: Chicken from Brazil between 1998 and 2007, 1 isolate: Beef from Australia in 2012. d) Isolate from domestic beef in 2010.

mate 400 bp) instead of *orf2110* (597 bp, Fig. 1). The sequence of the fragment was examined, because the unexpected fragment was also amplified by simplex-PCR assay with the *orf2110* primers (Fig. 1). The sequence was identical to the 597-nt fragment of *orf2110*, except for a 201-nt deletion at position 205–405 (Gene bank accession no. AB890369).

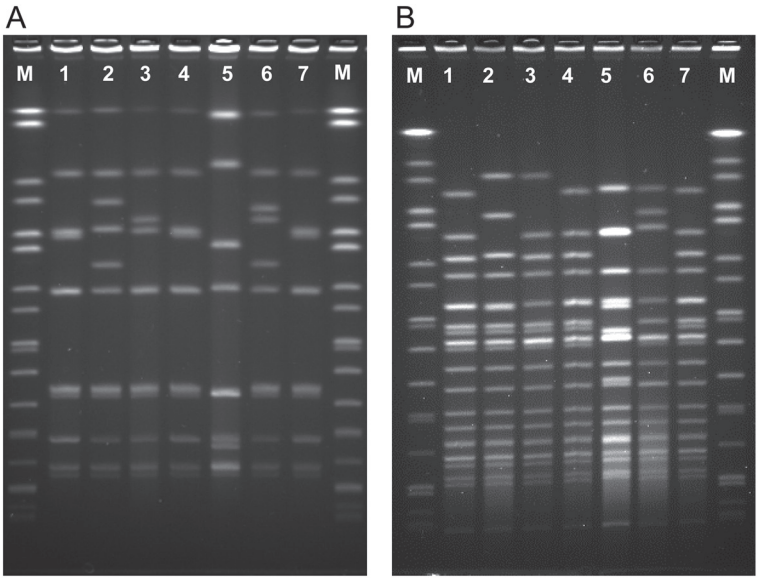

Fig. 2. PFGE patterns of serotype 4d strains digested by *Asc*I (A) and *Apa*I (B). Lane M; *Salmonella Braunderup* H9812 digested with *Xba*I, lane 1; the strain which had a new PCR profile, IVb with Δ*orf2110*, lanes 2, 3 and 7; isolates from food processing environment in Japan between 2009 and 2012, lane 4; isolate from domestic pork in 2009, lane 5; isolate provided by Dr. J. C. Feeley (CDC), lane 6; isolate from domestic meat product in 2012 .

By PFGE analysis, this strain was compared with 5 other serotype 4d, PCR serogroup IVb strains of the present study, as well with the reference strain kindly provided by Dr. J. C. Feeley (CDC, Atlanta, GA, U.S.A.). The PFGE pattern of this strain was indistinguishable from 2 other strains (Fig. 2). The MLST of the strain was assigned to ST1 (data not shown), which had been reported previously in serotype 4b strains [7, 8, 11]. Both serotype 4d and 4b strains belong to PCR serogroup IVb and evolutionary lineage I [9], suggesting this atypical strain may be a variant of PCR serogroup IVb. It is not known whether this atypical strain has spread throughout Japan or was just isolated incidentally.

In conclusion, 99.5% of the 187 *L. monocytogenes* strains isolated in Japan were classified into 4 serogroups by PCR serogrouping methods corresponding to the conventional serotypes. Only 1 isolate showed a new PCR profile: IVb with Δ*orf2110*, a possible variant of serogroup IVb.

REFERENCES

1. Borucki, M. K. and Call, D. R. 2003. *Listeria monocytogenes* serotype identification by PCR. *J. Clin. Microbiol.* **41**: 5537–5540. [Medline] [CrossRef]
2. Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C. and Martin, P. 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol.* **42**: 3819–3822. [Medline] [CrossRef]
3. Graves, L. M. and Swaminathan, B. 2001. PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. *Int. J. Food Microbiol.* **65**: 55–62. [Medline] [CrossRef]
4. Halpin, J. L., Garrett, N. M., Ribot, E. M., Graves, L. M. and Cooper, K. L. 2010. Re-evaluation, optimization, and multilaboratory validation of the PulseNet-standardized pulsed-field gel electrophoresis protocol for *Listeria monocytogenes*. *Foodborne Pathog. Dis.* **7**: 293–298. [Medline] [CrossRef]
5. Kérouanton, A., Marault, M., Petit, L., Grout, J., Dao, T. T. and Brisabois, A. 2010. Evaluation of a multiplex PCR assay as an alternative method for *Listeria monocytogenes* serotyping. *J. Microbiol. Methods* **80**: 134–137. [Medline] [CrossRef]
6. Leclercq, A., Chenal-Francisque, V., Dieye, H., Cantinelli, T., Drali, R., Brisse, S. and Lecuit, M. 2011. Characterization of the novel *Listeria monocytogenes* PCR serogrouping profile IVb-v1. *Int. J. Food Microbiol.* **147**: 74–77. [Medline] [CrossRef]
7. Martín, B., Perich, A., Gómez, D., Yangüela, J., Rodríguez, A., Garriga, M. and Aymerich, T. 2014. Diversity and distribution of *Listeria monocytogenes* in meat processing plants. *Food Microbiol.* **44**: 119–127. [Medline] [CrossRef]
8. Parisi, A., Latorre, L., Normanno, G., Miccolupo, A., Fraccalvieri, R., Lorusso, V. and Santagada, G. 2010. Amplified Fragment Length Polymorphism and Multi-Locus Sequence Typing for high-resolution genotyping of *Listeria monocytogenes* from foods and the environment. *Food Microbiol.* **27**: 101–108. [Medline] [CrossRef]
9. Ragon, M., Wirth, T., Hollandt, F., Lavenir, R., Lecuit, M., Le, M. A. and Brisse, S. 2008. A new perspective on *Listeria monocytogenes* evolution. *PLoS Pathog.* **4**: e1000146. [Medline] [CrossRef]
10. Seeliger, H. P. and Höhne, K. 1979. Serotyping of *Listeria monocytogenes* and related species. pp. 31–49. *In*: Methods in Microbiology, 13 (Bergan, T. and Norris, J. R. eds.), Academic Press, New York.
11. Wang, Y., Zhao, A., Zhu, R., Lan, R., Jin, D., Cui, Z., Wang, Y., Li, Z., Wang, Y., Xu, J. and Ye, C. 2012. Genetic diversity and molecular typing of *Listeria monocytogenes* in China. *BMC Microbiol.* **12**: 119. [Medline] [CrossRef]