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

**NOTE**

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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).

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(2)           | 7      | 75    |
| Brazil       |      |      | 26      |               |                        |                |        | 26    |
| Australia    | 6    | 1    | 1       |               |                        |                |        | 8     |
| U.S.A.       | 6    |      | 1       |               |                        |                |        | 7     |
| Thailand     |      |      | 4       |               |                        |                |        | 4     |
| Canada       | 1    | 1    |         |               |                        |                |        | 2     |
| China        |      |      | 1       |               |                        |                |        | 2     |
| Philippines  | 2    |      |         |               |                        |                |        | 2     |
| Chile        |      |      | 1       |               |                        |                |        | 2     |
| Mexico       | 2    |      |         |               |                        |                |        | 2     |
| Spain        |      |      |         |               |                        |                |        | 1     |
| Hungary      |      |      |         |               |                        |                |        | 1     |
| France       |      |      |         |               |                        |                |        | 1     |
| Unknown      | 5    | 8    | 7       | 1             |                        | 4(1)           |        | 26    |

Total: 159

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

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(2)  | 7    |      |      |      |          |    |
| IIB           | 27     | 25     | 2    |      |      |      |          |    |
| IIC           | 36     |        |      |      | 36(1)|      |          |    |
| IVB           | 63     | 1      | 57   | 5    |      |      |          |    |
| IVB-v1        | 8      |        |      |      |      | 8(1)|          |    |
| IVB with Δorf2110 | 1  |        |      |      |      |      |          |    |

Total: 187

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.

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 Δorf2110. B: Simplex-PCR assay with orf2110 primers. Lane M; 100 bp ladder, lane 1; the strain which had a new PCR profile, IVB with Δorf2110, lane 2; PCR serogroup IVB strain.
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 \textit{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 Δorf2110, a possible variant of serogroup IVb.

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