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# *Listeria monocytogenes* Subgroups IIIA, IIIB, and IIIC Delineate Genetically Distinct Populations with Varied Pathogenic Potential▽

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***Listeria monocytogenes*** **lineage III strains belonging to subgroups IIIA (*n* = 8), IIIB (*n* = 5), and IIIC (*n* = 6) were examined along with other known serotype strains (*n* = 11) by PCR and Southern hybridization using several recently described species-, virulence-, and serotype-specific primers and probes. The virulence of seven representative lineage III strains was then evaluated in mice via the intraperitoneal route. The results suggest that subgroup IIIA consists of typical rhamnose-positive avirulent serotype 4a and virulent serotype 4c strains, subgroup IIIC consists of atypical rhamnose-negative virulent serotype 4c strains, and subgroup IIIB consists of atypical rhamnose-negative virulent non-serotype 4a and non-serotype 4c strains, some of which may be related to serotype 7. It is possible that subgroup IIIB (including serotype 7) may represent a novel subspecies within *L. monocytogenes*.**

*Listeria monocytogenes* is a facultative intracellular bacterium that displays considerable variations in virulence and pathogenicity (5, 6, 8, 19). On the basis of the serological reactions between *Listeria* somatic (O)/flagellar (H) antigens and their corresponding antisera, *L. monocytogenes* is divided into at least 12 serotypes (i.e., 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e, and 7) (17). By using various genetic typing techniques, *L. monocytogenes* can be further classified into three lineages, of which lineage I encompasses serotypes 1/2b, 3b, 4b, 4d, and 4e; lineage II includes serotypes 1/2a, 1/2c, 3a, and 3c; and lineage III comprises serotypes 4a and 4c (2, 10, 13, 20). However, the lineage status of serotype 7 remains unclear due to limited availability of such strains. The clinical relevance of the serological and genetic typing schemes is demonstrated by the observations that serotypes 1/2a, 1/2c, 1/2b, and 4b (of lineages I and II) are involved in over 98% of the documented human listeriosis cases and that serotypes 4a and 4c (of lineage III) are rarely associated with outbreaks of the disease despite their frequent isolation from a variety of food and environmental specimens (20).

Although a general consensus on the compositions and divisions of lineages I and II exists, some uncertainty remains concerning the phenotypic characteristics and taxonomic status of lineage III. Within lineage III, three genetically distinct subgroups (i.e., IIIA, IIIB, and IIIC) have been identified after a recent comparative analysis of *actA* and *sigB* gene sequences from 46 strains (14). Phenotypically, lineage IIIA strains behave like typical *L. monocytogenes* in their ability to ferment rhamnose, whereas lineage IIIB and IIIC strains are notably deficient in rhamnose utilization (14). Given that positive rhamnose activity has been incorporated in various rapid identification kits (e.g., API Listeria [BioMerieux] and Micro-ID [Organon Technika]) for differentiation of *L. monocytogenes* from other nonpathogenic *Listeria* species (16), the identification of several unusual, rhamnose-negative lineage III strains (e.g., FSL-J1-158) has led to the suggestion of the possible existence of novel *Listeria* species or *L. monocytogenes* subspecies (20).

In the current study, we conducted further genetic characterization of *L. monocytogenes* lineage III strains by using several recently reported species-specific (lmo0733) (7), virulence-specific (lmo2821, lmo2672, and lmo1134) (5), and serotype-specific (ORF2819) (3) primers and probes in PCR and Southern hybridization. This was followed by in vivo assessment of the virulence of selective lineage III strains in a mouse model. The value of the aforementioned primers and probes (in particular lmo0733, lmo2821, and lmo1134) in the molecular discrimination of lineage III from other lineages has been shown previously, as *L. monocytogenes* serotype 4a and 4c strains belonging to lineage III reacted differently with these primers and probes in PCR and Southern blotting in comparison with other serotypes belonging to lineages I and II (9). In addition, amplified rRNA gene restriction analysis (ARDRA) (18) and an internalin A gene (*inlA*)-based PCR (4, 12) were performed to verify the species identity of *L. monocytogenes* lineage III strains under investigation.

A total of 30 *L. monocytogenes* strains were examined (Table 1). These included 11 strains representing serotypes 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, and 4e, which were typed in a prior study by agglutination and enzyme-linked immunosorbent assay (11), and 19 lineage III strains representing subgroups IIIA (*n* = 8), IIIB (*n* = 6), and IIIC (*n* = 5), which were characterized through phylogenetic study of partial *sigB* and *actA* sequences along with phenotypic evaluation (14). Further, one strain each of *Listeria ivanovii* (ATCC 19119), *Listeria seeligeri* (ATCC 35967), *Listeria innocua* (ATCC 25400), *Listeria welshimeri* (ATCC 43550), and *Listeria grayi* (ATCC

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TABLE 1. PCR and Southern blot analyses of *L. monocytogenes* strains[a]

| Strain | Origin | Serotype or subgroup[b] | Ribotype[c] | Rhamnose usage[d] | PCR amplification result[e] | | | | | | Size of band(s) (kb) by Southern hybridization with the following probe: | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 800 bp (inlA) | 453 bp (lmo0733) | 611 bp (lmo2821) | 481 bp (lmo2672) | 367 bp (lmo1134) | 471 bp (ORF2819) | lmo0733 | lmo2821 | lmo2672 |
| RM2989 | Milk | 1/2a | ND | ND | + | + | + | + | + | − | 5.0 | 5.0 | 2.5 |
| RM3158 | Human | 1/2b | ND | ND | + | + | + | + | + | + | 6.0 | 5.0 | 2.5 |
| RM3367 | Environment | 1/2c | ND | ND | + | + | + | + | + | − | 5.0 | 5.0 | 2.5 |
| RM3162 | Human | 3a | ND | ND | + | + | + | + | + | − | 1.5 | 4.0, 1.5 | 2.5 |
| RM3836 | Beef frank | 3b | ND | ND | + | + | + | + | + | + | 6.0 | 5.0 | 2.5 |
| RM3027 | Human | 3c | ND | ND | + | + | + | + | + | − | 5.0 | 5.0 | 2.5 |
| HCC23 | Catfish | 4a | ND | ND | + | + | − | − | − | − | 1.5 | | |
| ATCC 19115 | Human | 4b | ND | ND | + | + | + | + | + | + | 6.0 | 5.0 | 2.5 |
| ATCC 19116 | Chicken | 4c | ND | ND | + | + | + | − | − | − | 3.0, 1.5 | 1.5 | |
| ATCC 19117 | Sheep | 4d | ND | ND | + | + | + | + | + | + | 6.0 | 5.0 | 2.5 |
| ATCC 19118 | Chicken | 4e | ND | ND | + | + | + | + | + | + | 5.0 | 5.0 | 2.5 |
| F2-458 | Human | IIIA | DUP-1061A | + | + | + | + | − | − | − | 1.5 | 1.5, 1.0 | |
| J1-031 | Human | IIIA | DUP-1059A | + | + | + | + | − | − | − | 3.0, 1.5 | 2.0 | |
| J1-168 | Human | IIIA | DUP-18606 | + | + | + | + | − | − | − | 3.0 | 4.0, 2.0 | |
| J2-071 | Animal | IIIA | DUP-1061A | + | + | + | + | − | − | − | 1.5 | 3.0 | |
| J2-074 | Animal | IIIA | DUP-10146 | + | + | + | + | − | − | − | 3.0 | 5.0 | |
| R2-128 | Food | IIIA | DUP-1061A | + | + | + | + | − | − | − | 1.5 | 1.5, 1.0 | |
| X1-002 | Food | IIIA | DUP-1059B | + | + | + | − | − | − | − | 1.5 | | |
| X1-011 | Food | IIIA | DUP-1036A | + | + | + | − | − | − | − | 5.0 | | |
| F2-086 | Human | IIIB | DUP-10142 | − | + | − | − | + | − | − | | | 5.0 |
| F2-407 | Human | IIIB | DUP-18036 | − | + | − | − | + | − | − | | | 5.0 |
| J1-158 | Animal | IIIB | DUP10142 | − | + | − | − | + | − | − | | | 5.0 |
| M2-030 | Unknown | IIIB | DUP10142 | − | + | − | − | + | − | − | | | 5.0 |
| R2-142 | Food | IIIB | DUP-18036 | − | + | − | − | + | − | + | | | 5.0 |
| W1-112 | Unknown | IIIB | DUP-1033A | − | + | − | − | + | − | + | | | 5.0 |
| F2-208 | Human | IIIC | DUP-10148 | − | + | + | + | + | − | − | 1.5 | 1.5, 1.0 | 5.0 |
| F2-270 | Human | IIIC | DUP-18007A | − | + | + | + | + | − | − | 1.5 | 1.5, 1.0 | 5.0 |
| F2-595 | Human | IIIC | DUP-18007A | − | + | + | + | + | − | − | 1.5 | 1.5, 1.0 | 5.0 |
| M1-003 | Animal | IIIC | DUP-10148 | − | + | + | + | + | − | − | 1.5 | 1.5, 1.0 | 5.0 |
| W1-110 | Unknown | IIIC | DUP-1055A | − | + | + | + | + | − | − | 3.0 | 1.5, 1.0 | 5.0 |

[a] Amplified rRNA gene restriction analysis was performed on all 30 strains by PCR using primers derived from the *E. coli* 16S rRNA gene followed by digestion with restriction enzyme AluI. All 30 strains tested showed pattern 1, which indicates *L. monocytogenes* (18).

[b] The serotype (serotypes 1/2a to 4e) was determined by agglutination and enzyme-linked immunosorbent assay (11), and the subgroup (subgroups IIIA, IIIB, and IIIC) was ascertained by phylogenetic examination of *actA* and *sigB* genes as well as phenotypic characterization (14).

[c] The ribotype was based on the EcoRI ribotype pattern generated with the RiboPrinter (14). ND, not done.

[d] The rhamnose usage was evaluated as described elsewhere (14). +, used rhamnose; −, did not use rhamnose; ND, not done.

[e] The presence (+) or absence (−) of a band of the indicated size by PCR amplification with the specific primers shown.

33090) were also included to verify the specificity of *Listeria* primers and probes.

*Listeria* strains were retrieved from glycerol stocks maintained at −80°C and grown on plates containing Trypticase soy agar with 5% sheep blood (TSA II; Becton Dickinson Microbiology Systems, Cockeysville, MD) overnight at 37°C. A few colonies from each strain were then propagated in 50-ml Falcon tubes containing 10 ml of brain heart infusion (BHI) broth (Difco Laboratories, Detroit, MI) at 37°C for 18 h with aeration to an optical density at 540 nm of 1.2. Genomic DNA was purified from *Listeria* broth cultures after treatment with lysozyme and proteinase K and extracted with phenol-chloroform (7). The purified DNA was resuspended in 1× TE (10 mM Tris-HCl [pH 8.0] and 1 mM EDTA [pH 8.0]), and the DNA concentrations were determined spectrophotometrically at 260/280 nm UV in a GeneSpec I system (Miraibio, Alameda, CA). An aliquot of DNA from each *Listeria* strain was diluted in distilled water to 10 ng/μl for PCR amplification.

The identities and nucleotide sequences of PCR primers employed in this study are summarized in Table 2. For the ARDRA, primers from the 16S rRNA gene of *Escherichia coli* (at positions 10 to 27 and 1507 to 1485) were used in PCR to generate a 1,498-bp fragment (18). Upon digestion with restriction enzyme AluI, the 1,498-bp amplicon produces band pattern type 1 for *L. monocytogenes*; band pattern type 2 for *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. ivanovii*; and band pattern type 3 for *L. grayi* (18). As the *inlA* gene is uniquely present in *L. monocytogenes* species (4, 12), a set of *inlA* primers was designed and applied to further confirm the species identity of lineage III strains (Table 2). The species-, virulence-, and serotype-specific primers from lmo0733, lmo2821, lmo2672, lmo1134, and ORF2819 genes have been reported recently (3, 5, 7) (Table 2). In particular, lmo0733 is found in all *L. monocytogenes* serotypes tested (7), and lmo2821 is present in all serotypes but serotype 4a; lmo2672 is present in all serotypes but serotype 4a and some serotype 4c strains; lmo1134 in all serotypes but serotypes 4a and 4c (5); and ORF2819 is detected in serotypes 1/2b, 3b, 4b, 4d, 4e, and 7 (3) (Table 2).

PCR was conducted in a GeneAmp PCR system 9700 (Perkin-Elmer, Boston, MA) in a volume of 25 μl containing 0.5 U *Taq* DNA polymerase (Fisher Scientific, Houston, TX), 1× PCR buffer, 50 μM (each) deoxynucleoside triphosphates, 25 pmol each primer, and 10 ng DNA (7). Reaction mixture with no template DNA was included as a negative control in each run. The cycling programs were run as follows: 94°C for 2 min;

TABLE 2. Identities and specificities of PCR primers

| Gene | Protein | Specificity | Forward and reverse primers (5′–3′)[a] | Size of PCR product (bp) | Reference |
|---|---|---|---|---|---|
| 16S rRNA | | | TGGCTCAGATTGAACGCTG GCGGC<br>TACCTTGTTACGACTTCAC CCCA | 1,498 | 18 |
| *inlA* | Internalin A | *L. monocytogenes* species | ACGAGTAACGGGACAA ATGC<br>CCCGACAGTGGTGCTA GATT | 800 | This study |
| lmo0733 | Putative transcriptional regulator | *L. monocytogenes* species | CGCAAGAAGAAATTGC CATC<br>TCCGCGTTAGAAAAAT TCCA | 453 | 7 |
| lmo2821 | Internalin J | All *L. monocytogenes* serotypes but serotype 4a | TGTAACCCCGCTTACAC AGTT<br>TTACGGCTGGATTGTC TGTG | 611 | 5 |
| lmo2672 | Putative transcriptional regulator | All *L. monocytogenes* serotypes but serotype 4a and some 4c strains | CGGCACACTTGGATTC TCAT<br>AGGGCTAGTGACGGAT GCTA | 481 | 5 |
| lmo1134 | Putative transcriptional regulator | All *L. monocytogenes* serotypes but 4a and 4c | ACCCGATAGCAAGGAG GAAC<br>AACTTCTCTCGATACCCA TCCA | 367 | 5 |
| ORF2819 | Putative transcriptional regulator | *L. monocytogenes* serotypes 1/2b, 3b, 4b, 4d, 4e, and 7 | AGCAAAATGCCAAAAC TCGT<br>CATCACTAAAGCCTCCC ATTG | 471 | 3 |

[a] The forward primer (top) and reverse primer (bottom) is shown for each gene.

30 cycles, with 1 cycle consisting of 94°C for 20 s, 56°C for 20 s, and 72°C for 45 s; and 72°C for 2 min. The resultant PCR products were separated on a 1% agarose gel in the presence of ethidium bromide (0.5 μg/ml), visualized with UV light, and photographed by using a ChemiImager 5500 documentation system (BSI, Stafford, TX). Specific PCR fragments amplified with lmo2821, lmo2672, and lmo0733 primers were excised, purified with a Geneclean kit (MP Biochemicals, Irving, CA), and utilized as probes in Southern blotting.

Southern hybridization was carried out in accordance with a previous protocol (9). Specifically, genomic DNA (20 μg) from each *Listeria* strain was digested with 20 U of HindIII (Promega, Madison, WI) for 3 h at 37°C. Approximately 5 μg of HindIII-digested genomic DNA from each strain was loaded per well and separated by 1.0% agarose gel electrophoresis. After denaturation with NaOH and neutralization with Tris-HCl (pH 8.0), the DNA was transferred onto Hybond N+ membranes (Amersham Pharmacia, Piscataway, NJ), and cross-linked to the membrane in a GS Gene Linker UV chamber (Bio-Rad, Hercules, CA). The bound DNA was then detected with chemically labeled lmo2821, lmo2672, and lmo0733 probes (with corresponding PCR fragments of 611, 481, and 453 bp, respectively) using the ECL direct nucleic acid labeling and detection system (Amersham Pharmacia, Piscataway, NJ).

The virulence of seven representative lineage III strains (i.e., subgroup IIIA strains F2-458, J2-074, and X1-002; subgroup IIIB strains F2-086 and R2-142; and subgroup IIIC strains F2-208 and F2-270) (Table 3) was evaluated in a mouse model (6), along with known virulent EGD and avirulent HCC23 strains as controls. Briefly, 6- to 8-week-old female A/J mice

TABLE 3. In vivo assessment of *L. monocytogenes* virulence[a]

| Strain | Serotype or subgroup[b] | CFU[c] | Mortality (no. of dead mice) ($n = 5$)[d] | Relative virulence (%)[e] |
|---|---|---|---|---|
| EGD | 1/2a | $1 \times 10^8$ | 5 | 100 |
| HCC23 | 4a | $8 \times 10^7$ | 0 | 0 |
| F2-458 | IIIA | $1.2 \times 10^8$ | 5 | 100 |
| J2-074 | IIIA | $9 \times 10^7$ | 5 | 100 |
| X1-002 | IIIA | $4 \times 10^7$ | 0 | 0 |
| F2-086 | IIIB | $1.1 \times 10^8$ | 5 | 100 |
| R2-142 | IIIB | $5 \times 10^7$ | 5 | 100 |
| F2-208 | IIIC | $8 \times 10^7$ | 5 | 100 |
| F2-270 | IIIC | $7 \times 10^7$ | 5 | 100 |
| None (saline control) | | | 0 | 0 |

[a] Each group contained five A/J mice. The dose (*L. monocytogenes* suspension) given to each mouse was diluted 1/200.
[b] The serotype (serotype 1/2a or 4a) and subgroup (subgroups IIIA, IIIB, and IIIC) were determined as described in Table 1, footnote *b*.
[c] The number of CFU was assessed by BHI agar plate at $10^{-7}$ dilution.
[d] Mortality was determined at day 5 postinjection, when all mice inoculated with strain EGD died.
[e] Relative virulence (shown as a percentage) was calculated by dividing the number of dead mice with the number of tested and then multiplying by 100 (6).

(Jackson Laboratory, Bar Harbor, ME) were maintained in cages, with five mice per cage. On the day of inoculation, the optical densities (at 540 nm) of *L. monocytogenes* strains in BHI broth cultures were adjusted to 1.35, and for each strain, about 1 ml of the bacterial suspension was pelleted in a benchtop microcentrifuge at 5,000 rpm and washed twice in sterile saline (0.9% NaCl). Dilutions (1/200) of *L. monocytogenes* suspensions were prepared in saline, and each mouse from the testing groups was injected intraperitoneally (i.p.) with 0.1 ml of the diluted bacteria. One group of five mice was injected i.p. with 0.1 ml saline only. Observations were made daily, and mortalities were recorded until all of the mice inoculated with the virulent EGD strain had died. On the 15th day after inoculation, all surviving mice were euthanized.

By using the previously published ARDRA procedure (18), the PCR-amplified 16S rRNA gene fragments from the 30 *L. monocytogenes* strains (including subgroup IIIA, IIIB, and IIIC strains) all formed a distinct restriction band pattern type 1 after digestion with restriction enzyme AluI, confirming their *L. monocytogenes* species identity (Table 1 and data not shown), and those from *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. ivanovii*, and *L. grayi* displayed characteristic restriction band pattern types 2 and 3 (data not shown). In addition, primers from the species-specific *inlA* gene resulted in the amplification of a specific 800-bp band from DNA of all 30 *L. monocytogenes* strains, but not from that of the five other *Listeria* species, reinforcing that the 30 strains under investigation belong to the species of *L. monocytogenes* (Table 1 and data not shown).

The species-specific lmo0733 primers generated a specific 453-bp amplicon from 24 of the 30 *L. monocytogenes* strains, with all six subgroup IIIB strains (i.e., F2-086, F2-407, J1-158, M2-030, R2-142, and W1-112) showing no amplification (Table 1). This indicates that subgroup IIIB is somewhat different genetically from other *L. monocytogenes* lineages, including subgroups IIIA and IIIC, although subgroup IIIB strains appear similar to subgroup IIIC strains in rhamnose utilization (Table 1). The fact that the six subgroup IIIB strains together with serotype 4a strain HCC23 and two subgroup IIIA strains (X1-002 and X1-011) were not recognized by the lmo2821 primers suggests some similarities between subgroup IIIB and serotype 4a strains despite their different rhamnose activities (Table 1). The virulence-specific lmo2672 primers amplified this gene in 20 of the 30 strains but failed to react with the 8 subgroup IIIA strains (F2-458, J1-031, J1-168, J2-071, J2-074, J2-128, X1-002, and X1-011) as well as standard serotype 4a (HCC23) and 4c (ATCC 19116) strains, highlighting the genetic difference between subgroup IIIA and the other lineages/subgroups as well as linkage between subgroup IIIA and typical serotypes 4a and 4c (Table 1). Given that the virulence-specific lmo1134 primers are specific for all serotypes but serotypes 4a and 4c (5), their negative reaction with subgroup IIIA, IIIB, and IIIC strains in the current study implies that subgroups IIIA, IIIB, and IIIC are likely serotype 4a or 4c or possibly atypical serotype 4b strains classified into lineage III (9).

Thus, as six of the eight subgroup IIIA strains (F2-458, J1-031, J1-168, J2-071, J2-074, and J2-128) were positive for lmo0733 and lmo2821, they may be typical rhamnose-positive serotype 4c strains. The other two subgroup IIIA strains (X1-002 and X1-011) appear to be typical rhamnose-positive serotype 4a strains. The five subgroup IIIC strains (F2-208, F2-270, F2-595, M1-003, and W1-110) may be atypical rhamnose-negative serotype 4c strains. However, the six subgroup IIIB strains (F2-086, F2-407, J1-158, M2-030, R2-142, and W1-112), which were negative for lmo0733, lmo2821, and lmo1134, may be atypical rhamnose-negative non-serotype 4a and non-serotype 4c strains. In fact, as two of these strains (R2-142 and W1-112) reacted in PCR with ORF2819 primers (which are specific for serotypes 1/2b, 3b, 4b, 4d, 4e, and 7) (3), they may be related to serotype 7 (Table 1 and data not shown). Therefore, it is possible that serotype 7 may constitute part of lineage IIIB. Overall, these observations provide additional genetic evidence for the delineation of three subgroups (i.e., IIIA, IIIB, and IIIC) within lineage III (14).

The results of Southern hybridization with the lmo0733, lmo2821, and lmo2672 probes largely confirmed those of PCR analysis (Table 1 and data not shown). Whereas serotype 3a, 4a, and 4c strains as well as subgroup IIIA and IIIC strains produced smaller HindIII bands of 3.0, 2.0, or 1.5 kb, other serotype strains (with the exception of subgroup IIIB strains) gave larger HindIII bands of 5.0 or 6.0 kb with the lmo0733 probe (Table 1) (9). Similarly, serotype 3a and 4c strains as well as subgroup IIIA and IIIC strains displayed smaller HindIII bands of 4.0, 2.0, 1.5, and/or 1.0 kb, while other serotype strains (excluding serotype 4a strain HCC23 and subgroup IIIB) displayed larger HindIII bands of 5.0 kb with the lmo2821 probe (Table 1) (9). These results highlight the possible genetic relationship between subgroups IIIA and IIIC, which may be distinct from subgroup IIIB. On the other hand, 9 (except serotype 4a and 4c) of the 11 known serotype strains displayed a HindIII band of 2.5 kb with the lmo2672 probe, and 6 subgroup IIIB and 5 subgroup IIIC strains formed a band of 5.0 kb (Table 1), reflecting certain genetic similarities between subgroups IIIB and IIIC. However, DNA from other *Listeria* species (i.e., *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri*, and *L. grayi*) did not react with lmo0733, lmo2821, and lmo2672 probes in Southern blots (9). In short, these findings clearly show that apart from having some genetic resemblance with serotype 3a, lineage III strains are quite different from other lineage I and II strains at the DNA level.

In vivo examination of seven *L. monocytogenes* subgroup IIIA, IIIB, and IIIC strains (i.e., F2-458, J2-074, X1-002, F2-086, R2-142, F2-208, and F2-270) in A/J mice indicates that six of these strains (all but X1-002) are capable of causing mouse mortality, as mice inoculated i.p. with these six strains died by day 5 postinfection, similar to those injected i.p. with the EGD control strain (Table 3). It has been shown previously that while *L. monocytogenes* strains harboring internalin gene lmo2821 tend to be virulent, those without this gene (e.g., serotype 4a strains) are avirulent in the mouse model (5, 6, 15). The results from this study offer further support that lmo2821 may contribute to the enhanced virulence of *L. monocytogenes* strains, with lmo2821-positive subgroup IIIA strains F2-458 and J2-074 being clearly virulent and lmo2821-negative subgroup IIIA strain X1-002 being avirulent. Similarly, the two lmo2821-positive, rhamnose-negative subgroup IIIC strains F2-208 and F2-270 also demonstrate the potential to cause mouse mortality, given the presence of lmo2821 in these strains. However, the two lmo2821-negative, rhamnose-nega-

tive subgroup IIIB strains F2-086 and R2-142 appear to be virulent in spite of having no detectable lmo2821 gene by PCR and Southern blotting, indicating that lmo2821 is not required for *L. monocytogenes* virulence in mice via the intraperitoneal route.

It should be noted that although six out of the seven lineage III strains examined here appear to have the capacity to cause mouse mortality via intraperitoneal inoculation, lineage III strains are not usually responsible for producing diseases in human hosts. Indeed, lineage III strains have seldom been isolated from sporadic human listeriosis cases and from areas where listeriosis is endemic (20). One possible explanation for the negligible number of human listeriosis cases resulting from lineage III strains is that lineage III strains encounter difficulty in crossing the host intestinal barrier. This is supported by the findings that non-serotype 4b strains are much less infective than serotype 4b and 1/2 strains in mice through intragastric inoculation (1). The fact that many lineage III strains do not possess the lmo2821 (or *inlJ*) gene (9) (or contain an altered gene) may also be attributable to their reduced ability to successfully cause intestinal infections, as a *L. monocytogenes* mutant strain with the lmo2821 gene deleted is unable to go past the intestinal phase and is significantly attenuated in virulence (15). Nevertheless, lineage III strains (with the possible exception of serotype 4a) may have the potential to cause disease in patients whose immune functions are compromised, while their ability to establish successfully in immunocompetent hosts is questionable.

Taken together, the experimental results from the present study demonstrate that *L. monocytogenes* subgroups IIIA, IIIB, and IIIC possess characteristic genetic structures, underscoring that they are genetically distinct populations within the species (14). Subgroup IIIA is composed of typical rhamnose-positive avirulent serotype 4a and virulent serotype 4c strains, subgroup IIIC consists of atypical rhamnose-negative virulent serotype 4c strains, and subgroup IIIB contains atypical rhamnose-negative virulent non-serotype 4a and non-serotype 4c strains, some of which may be related to serotype 7. It is possible that subgroup IIIB (including serotype 7) may represent a novel subspecies within *L. monocytogenes*. The species-specific lmo0733 gene has been previously found in all *L. monocytogenes* strains tested (7), but the present study shows that subgroup IIIB strains do not harbor this gene. The fact that subgroup IIIB strains are recognized by the virulence-specific lmo2672 primers indicates the potential of using the lmo2672 gene as a target for specific identification of uncommon, atypical *L. monocytogenes* strains. Therefore, use of the lmo0733 and lmo2672 primers in combination will facilitate detection and identification of all *L. monocytogenes* strains that have the potential to cause mortality in experimental animals.

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