



HAL
open science

Use of PCR-restriction fragment length polymorphism of *inlA* for rapid screening of *Listeria monocytogenes* strains deficient in the ability to invade Caco-2 cells

Sandrine Rousseaux, Maïwenn Olier, Jean-Paul J.-P. Lemaître, Pascal Piveteau, Jean Guzzo

► To cite this version:

Sandrine Rousseaux, Maïwenn Olier, Jean-Paul J.-P. Lemaître, Pascal Piveteau, Jean Guzzo. Use of PCR-restriction fragment length polymorphism of *inlA* for rapid screening of *Listeria monocytogenes* strains deficient in the ability to invade Caco-2 cells. *Applied and Environmental Microbiology*, 2004, 70 (4), pp.2180-2185. 10.1128/70.4.2180-2185.2004 . hal-02674137

HAL Id: hal-02674137

<https://hal.inrae.fr/hal-02674137>

Submitted on 31 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Use of PCR-Restriction Fragment Length Polymorphism of *inlA* for Rapid Screening of *Listeria monocytogenes* Strains Deficient in the Ability To Invade Caco-2 Cells

S. Rousseaux, M. Olier, J. P. Lemaître, P. Piveteau, and J. Guzzo*

Laboratoire de Microbiologie, UMR INRA 1232, ENSBANA, 21000 Dijon, France

Received 9 June 2003/Accepted 5 January 2004

A PCR-restriction fragment length polymorphism (RFLP) method was developed in order to screen a large number of strains for impaired adhesion to epithelial cells due to expression of truncated InlA. *inlA* polymorphism was analyzed by PCR-RFLP in order to correlate *inlA* PCR-RFLP profiles and production of truncated InlA. Thirty-seven *Listeria monocytogenes* strains isolated from various sources, including five noninvasive and two invasive reference strains, were screened. Two endonucleases (AluI and Tsp509I) were used, and they generated five composite profiles. Thirteen *L. monocytogenes* isolates were characterized by two specific PCR-RFLP profiles similar to PCR-RFLP profiles of noninvasive reference strains previously described as strains that produce truncated InlA. Ten of the 13 isolates showed low abilities to invade human epithelial Caco-2 cells. However, 4 of the 13 isolates were able to invade Caco-2 cells like reference strains containing complete InlA. Sequencing of *inlA* and Western blot analysis confirmed that truncated InlA was expressed in the 10 *L. monocytogenes* strains which were isolated from food. This PCR-RFLP method allowed us to identify 10 new strains expressing a truncated internalin. Based on the results obtained in this study, the PCR-RFLP method seems to be an interesting method for rapidly screening *L. monocytogenes* strains deficient in the ability to invade Caco-2 cells when a sizeable number of strains are studied.

Listeria monocytogenes, a facultative intracellular pathogen, is widespread in the environment (1, 24, 25). This bacterium can contaminate processed foods, and several studies have established that listeriosis is an important food-borne infection (14, 21). Moreover, *L. monocytogenes* can be isolated from the gastrointestinal tracts of healthy persons (9, 23). It has been estimated that between 1 to 6% of the general population carry this bacterium (4, 11, 13, 22). Recently, variable capacities of human carriage isolates of *L. monocytogenes* to invade human cell cultures were observed. In fact, Jonquières et al. (10) reported that *L. monocytogenes* human carriage isolate LO28 entered a fibroblast line expressing L-CAM (the chicken homolog of E-cadherin) poorly and produced a truncated form of the protein InlA (63 kDa), an internalin implicated in entry into host cells, while virulent and invasive strains of *L. monocytogenes* produced an 80-kDa InlA. In the same study, Jonquières et al. (10) also described one clinical and three food *L. monocytogenes* isolates expressing a truncated InlA. In a recent study, Olier et al. (18, 19) reported that several *L. monocytogenes* human carriage isolates were attenuated for virulence, were affected in the ability to invade Caco-2 cells, and also produced truncated InlA (47 kDa). Sequence analysis of *inlA* revealed that point mutations were responsible of production of the truncated InlA and that there were polymorphisms in *inlA* (10, 19, 20). In this paper, we describe a PCR-restriction fragment length polymorphism (RFLP) method based on *inlA* polymorphism for rapidly screening potentially noninvasive *L. monocytogenes* strains when a sizeable number of strains are

examined. Furthermore, we present evidence concerning the occurrence of potentially noninvasive *L. monocytogenes*.

MATERIALS AND METHODS

Bacterial strains. The *L. monocytogenes* isolates used are listed in Table 1. Nine *L. monocytogenes* human fecal carriage isolates (H2, H6, H11, H12, H27, H28, H31, H35, and H38), three isolates from sporadic human listeriosis (H4, H21, and H22), three isolates from food-processing facilities (1E, 3E, and 6E), an isolate from compost (C9), six food isolates from brine (1S, 2S and 3S) and cheese (1F, 3F, and 7F), and four rook fecal carriage isolates (23, 38, 81, and 97) were obtained from the strain collection of Laboratoire de Microbiologie UMR 1232, Dijon, France. Four isolates from meat (NV4, NV5, NV7, and NV8) were provided by the Laboratoire départemental de la Haute Vienne, Limoges, France. Strain Scott A was obtained from the collection of Institut Pasteur, Paris, France, and strains LO28 and EGD-e were kindly provided by P. Cossart, Institut Pasteur, Paris, France. Strains Scott A and EGD-e were used as virulent reference strains for comparative analysis. Human fecal carriage isolates H1, H17, H32, and H34 (18, 19), as well as LO28 (10), were recently described as strains that produce a truncated InlA; thus, the virulence potential of these strains was affected. These five isolates were used as noninvasive reference strains for development of the PCR-RFLP method.

Restriction analysis (PCR-RFLP) of *inlA*. A 733-bp *inlA* fragment was amplified with primers seq01 (5'-AATCTAGCACCCTGTCTGGG-3') and seq02 (5'-TGTGACCTTCTTTTACGGG-3'). This fragment encodes a region between repeat A10 and part of repeat B1 of InlA (Fig. 1). This *inlA* fragment was selected for the polymorphism study because of its genetic heterogeneity due to point mutations (18, 19, 20). PCRs were performed with 50- μ l (total volume) reaction mixtures containing 5 μ l of 10 \times Taq polymerase buffer (Appligene-Oncor, Illkirch, France), each deoxynucleoside triphosphate at a concentration of 200 μ M, 1.5 mM MgCl₂, each primer at a concentration of 0.5 μ M, 1.25 U of Taq DNA polymerase (Appligene-Oncor), and 25 ng of DNA template. The following program was used: denaturation for 4 min at 94°C, followed by 30 cycles consisting of 94°C for 30 s, 52°C for 1 min, and 72°C for 2.5 min, and a final extension step consisting of 72°C for 7 min.

Restriction endonucleases AluI and Tsp509I were selected on the basis of a partial sequence analysis of *inlA* of invasive reference strain EGD-e (accession number LMO00433), noninvasive reference strains H1, H17, H32, H34, and LO28 (accession numbers AF468816, AY126441, AY126442, AY126443, and AY166686, respectively), and invasive reference strain Scott A (accession num-

* Corresponding author. Mailing address: Laboratoire de Microbiologie, UMR INRA 1232, ENSBANA, 1 Esplanade Erasme, 21000 Dijon, France. Phone: 33 3 80 39 66 75. Fax: 33 3 80 39 39 55. E-mail: juzzo@u-bourgogne.fr.

TABLE 1. PCR-RFLP profiles of *L. monocytogenes* strains and entry percentages with Caco-2 cells

Strain	Origin	Serotype	AluI profile ^a	Tsp509I profile ^a	Composite profile ^b	Internalin theoretical molecular mass (kDa)	Entry %	Reference
H2	Healthy 3-year-old child (Beaune, France, 1992)	4b	2	1	B	80	9.74 ± 1.52	18
H4	Sporadic patient isolate, blood culture (Beaune, France, 1992)	ND ^c	3	2	C	80	17.54 ± 0.75	This study
H6	Healthy 30-year-old woman (Beaune, France, 1991)	3 (immobile)	2	1	B	80	6.73 ± 0.86	19
H11	Healthy 35-year-old man (Beaune, France, 1992)	1/2a	3	2	C	80	16.70 ± 1.69	19
H12	Healthy 39-year-old man, oral isolate (Beaune, France, 1992)	4b	2	1	B	80	14.29 ± 1.08	19
H21	Sporadic patient isolate, blood culture (Strasbourg, France, 1992)	ND	2	1	B	80	20.18 ± 1.38	19
H22	Sporadic patient isolate, blood culture (Strasbourg, France, 1992)	4b	2	1	B	80	ND	19
H27	Healthy 2-year-old child (Beaune, France, 1994)	4b	2	1	B	80	12.54 ± 4.27	19
H28	Healthy 7-year-old child (Beaune, France, 1994)	1/2b	2	1	B	80	10.86 ± 0.47	19
H31	Healthy 55-year-old man (Beaune, France, 1995)	1/2a	3	2	C	80	10.41 ± 2.75	19
H35	Healthy 11-year-old child (Beaune, France, 1997)	1/2b	2	1	B	80	10.80 ± 2.53	19
H38	Healthy carrier (Beaune, France, date not communicated)	1/2a	1	3	A	80	16.55 ± 0.86	This study
NV4 ^d	Minced beef	1/2a	4	2	D	67.5	0.124 ± 0.007	This study
NV5 ^d	Minced beef	1/2c	4	2	D	68	0.131 ± 0.006	This study
NV7 ^d	Bovine carcass	1/2c	4	2	D	50	0.32 ± 0.03	This study
NV8 ^d	Bovine carcass	1/2a	4	2	D	43	0.48 ± 0.03	This study
1E ^d	Machine at an industrial cheese-making plant (Dijon, France, 1990)	1/2b	2	1	B	80	6.62 ± 1.44	This study
3E ^d	Sink at an industrial cheese-making plant (Dijon, France, 1990)	1/2b	2	1	B	80	6.83 ± 2.90	This study
6E ^d	Wall at an industrial cheese-making plant (Dijon, France, 1990)	1/2a	5	3	E	80	24.21 ± 2.06	This study
1S	Brine (Dijon, France, 1990)	1/2a	1	3	A	47	0.36 ± 0.09	This study
2S ^d	Brine (Dijon, France, 1990)	1/2a	1	3	A	47	0.48 ± 0.38	This study
3S	Brine (Dijon, France, 1990)	1/2a	1	3	A	47	0.31 ± 0.01	This study
1F ^d	Cheese (Dijon, France, 1990)	1/2a	1	3	A	47	0.93 ± 0.19	This study
2F ^d	Cheese (Dijon, France, 1990)	1/2a	1	3	A	47	0.38 ± 0.12	This study
7F ^d	Cheese (Dijon, France, 1990)	3b	1	3	A	47	0.51 ± 0.16	This study
C9	Compost (Dijon, France, 2002)	ND	2	1	B	80	17.22 ± 1.99	This study
23 ^d	Rook feces (Besançon, France, 1995)	ND	2	1	B	80	24.86 ± 0.81	1
38 ^d	Rook feces (Besançon, France, 1995)	ND	1	3	A	80	14.54 ± 1.32	1
81 ^d	Rook feces (Besançon, France, 1995)	ND	4	2	D	80	15.81 ± 0.53	1
97 ^d	Rook feces (Besançon, France, 1995)	ND	3	2	C	80	8.71 ± 0.54	1
Reference strains								
Scott A	Massachusetts milk outbreak (1983)	4b	2	1	B	80	9.55 ± 1.70	5
EGD-e	Laboratory strain	1/2a	4	2	D	80	22.86 ± 1.60	16
LO28	Healthy pregnant carrier (Spain)	1/2c	4	2	D	63	0.89 ± 0.28	10
H1	Healthy pregnant carrier (Beaune, France, 1991)	1/2a	1	3	A	47	0.95 ± 0.42	18
H17	Healthy 10-year-old child (Beaune, France, 1992)	3a	1	3	A	47	0.024 ± 0.006	19
H32	Healthy 19-year-old women (Beaune, France, 1996)	1/2a	1	3	A	47	0.36 ± 0.15	19
H34	Healthy 8-year-old child (Beaune, France, 1997)	1/2a	1	3	A	47	0.39 ± 0.04	19

^a Each different set of banding profiles for each restriction endonuclease was given an arbitrary number.

^b Reflects the total differences or similarities in the banding patterns with the two restriction endonucleases.

^c ND, not determined.

^d Isolates from the same source whose lysotypes were different.

ber AY166685). Ten (EGD-e and LO28), nine (H1, H17, H32, and H34), and seven (Scott A) AluI (AG/CT) restriction sites were detected. Ten (Scott A), seven (EGD-e and LO28), and six (H1, H17, H32, and H34) Tsp509I restriction sites were detected. These two restriction endonucleases were used independently. PCR-RFLP fragments were separated by electrophoresis on a 3.5%

agarose gel (type 05 DNA grade; Euromedex, Mundolsheim, France). Gels were stained with ethidium bromide and were recorded with Bio-Rad gel doc 2000.

Plaque formation in Caco-2 cells. The capacity of *L. monocytogenes* isolates to invade and disseminate in Caco-2 cells was evaluated by a plaque formation assay. The human colon carcinoma cell line Caco-2, obtained from the European

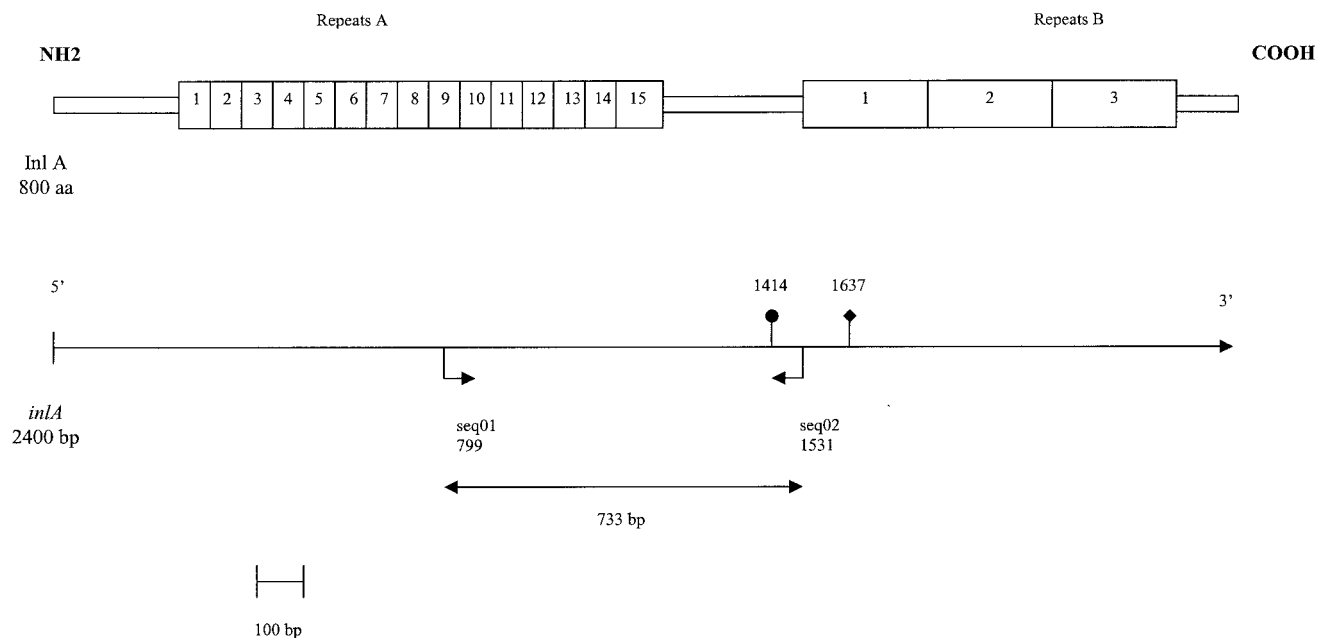


FIG. 1. Structural organization of internalin and partial map of *inlA* (20). The horizontal arrows indicate the positions of primers seq01 and seq02 used to amplify an *inlA* fragment with genetic heterogeneity. The solid circle indicates the position of the mutation which created a nonsense codon in *inlA* of noninvasive reference strains H1, H17, H32, and H34. The solid diamond indicates the position of the deletion which created a nonsense codon in *inlA* of noninvasive reference strain LO28.

Collection of Cell Cultures (ECACC no. 86010202), was used between passages 43 and 50. Cells were routinely grown in 25-cm² plastic tissue culture flasks (Greiner) at 37°C in a humidified atmosphere containing 5% (vol/vol) CO₂ in air. The culture medium used for growth of the cell line was Dulbecco's modified Eagle's minimum essential medium supplemented with 10% (vol/vol) fetal calf serum, 2 mM L-glutamine, 1% (vol/vol) nonessential amino acids, and antibiotics (100 U of penicillin ml⁻¹, 100 µg of streptomycin ml⁻¹). All reagents were purchased from Invitrogen (Life Technologies).

Early confluent cell monolayers were prepared in six-well tissue culture plates. After overnight growth at 37°C in brain heart infusion broth (Biomerieux), listerial cells were centrifuged (6,000 × *g* for 10 min at room temperature) and serially diluted in phosphate-buffered saline (Dulbecco's phosphate-buffered saline with 1 mg of glucose ml⁻¹ and 36 mg of sodium pyruvate ml⁻¹; Life Technologies). Cells were counted by plating suitable dilutions on brain heart infusion medium. The plates were incubated for 24 to 48 h at 37°C. Caco-2 cells were infected with 0.1-ml portions of the 10⁻⁴ and 10⁻⁵ dilutions. Three replicate wells were used per dilution. After 2 h of contact at 37°C, nonadherent bacteria were removed from the monolayers by washing them three times with phosphate-buffered saline and by overlaying them with 2 ml of Dulbecco's modified Eagle's minimum essential medium containing 0.8% agarose, 20% fetal calf serum, and 2 mM L-glutamine. To kill extracellular adherent bacteria, 10 µg of gentamicin ml⁻¹ was added. After 24 h of incubation at 37°C, 100 µl of trypan blue (0.4%), which stained dead cells, was added to each well, and plaque formation was observed 24 h later. The initial entry was determined by determining the ratio of the number of plaques observed to the initial number of bacteria added, expressed as a percentage. Two independent assays were performed in triplicate for each isolate on separate days with the Caco-2 cell line between passages 43 and 50 (*n* = 6). A statistical analysis (*t* test) was performed.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot analysis. Bacterial surface proteins were extracted by the method of Kochs et al. (12). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting were done as previously described (18). Membrane hybridization was performed with mouse antibodies directed against InlA (L7.7) (15).

DNA sequencing. Sequencing was performed with DNA fragments generated by PCR performed with primers seq01 and seq02. Each PCR product was sequenced in both orientations by Genome Express.

Nucleotide sequence accession numbers. The nucleotide sequences have been deposited in the GenBank/EMBL database, and the accession numbers of partial *inlA* sequences of the isolates are AJ564715 (1S), AJ564716 (2S), AJ564717 (3S),

AJ564712 (1F), AJ564713 (2F), AJ564714 (7F), AJ608706 (NV4), AJ578445 (NV5), AJ564719 (NV7), and AJ564720 (NV8).

RESULTS

Restriction analysis (PCR-RFLP) of *inlA*. The PCR-RFLP method was developed to study polymorphism of an *inlA* region in 37 *L. monocytogenes* isolates (Table 1), which allowed us to screen isolates that produced truncated InlA and thus were deficient in the ability to invade Caco-2 cells.

With primers seq01 and seq02, single DNA fragments of the expected size were obtained for all *L. monocytogenes* isolates. PCR-RFLP profiles of some of the isolates tested are shown Fig. 2.

Restriction of the amplified *inlA* fragment with endonuclease AluI (Fig. 2A and Table 1) generated five different profiles with 8 to 11 bands ranging in size from 15 to 241 bp. Most of the 30 isolates were associated with PCR-RFLP profile 1 or 2. Eight isolates were grouped in PCR-RFLP profile 1 with noninvasive reference strains H1, H17, H32, and H34. Twelve isolates were grouped in PCR-RFLP profile 2 with invasive reference strain Scott A. Four *L. monocytogenes* isolates were associated with PCR-RFLP profile 3, and five isolates were grouped in PCR-RFLP profile 4 with invasive reference strain EGD-e and noninvasive reference strain LO28. Only one isolate (6E) had PCR-RFLP profile 5.

Restriction endonuclease Tsp509I generated three profiles (Fig. 2B and Table 1) with 7 to 11 bands ranging in size from 8 to 281 bp. Twelve *L. monocytogenes* isolates were grouped in PCR-RFLP profile 1 with invasive reference strain Scott A, and nine isolates were grouped in PCR-RFLP profile 2 with noninvasive reference strain LO28 and invasive reference

differences in the entry percentages are statically significant. Western blot analyses showed that the InIA expressed by these 10 noninvasive isolates had a molecular mass that was less than 80 kDa. However, the entry percentages for *L. monocytogenes* isolates H38, 38, and 81 were more than 14%, and an 80-kDa InIA was detected by Western blotting (data not shown).

DNA sequencing. *inlA* sequences of *L. monocytogenes* isolates 1S, 2S, 3S, 1F, 2F, 7F, NV4, NV5, NV7, and NV8 were analyzed to validate the PCR-RFLP method and to determine the reasons for production of the truncated InIA and thus the deficiency in the ability to invade Caco-2 cells.

DNA sequencing of *inlA* from strains 1S (accession number AJ564715), 2S (accession number AJ564716), 3S (accession number AJ564717), 1F (accession number AJ564712), 2F (accession number AJ564713), and 7F (accession number AJ564714) with composite profile A showed that there were silent mutations. One single-point mutation consisting of substitution of a cytosine for a thymidine at position 1414 (position based on the first translated codon) was detected. This point mutation created a nonsense codon (TAG) in the coding sequence that led to production of a protein with a theoretical molecular masses of 47 kDa (Table 1). These six isolates produced a truncated InIA (47 kDa) similar to the InIA produced by noninvasive reference strains H1, H17, H32, and H34.

DNA sequencing of *inlA* from NV4 (accession number AJ608706), NV5 (accession number AJ578445), NV7 (accession number AJ564719), and NV8 (accession number AJ564720) with composite profile D also revealed point mutations. For NV4, insertion of a thymidine at position 1901 was observed. This frameshift mutation led to creation of a nonsense codon, TGA, for position 1969, which generated an open reading frame encoding a theoretical 67.5-kDa InIA. For NV5, substitution of a guanine for an adenine at position 1994 (position based on the first translated codon) created a nonsense codon (TAG) in the coding sequence, which led to production of a protein with a theoretical molecular mass of 68 kDa (Table 1). For NV7, deletion of a guanine at position 1480 was observed. This frameshift mutation led to creation of a nonsense codon, TGA, at position 1496, which generated an open reading frame encoding a theoretical 50-kDa InIA. For NV8, substitution of a guanine for an adenine at position 1320 (position based on the first translated codon) created a nonsense codon (TGA) in the coding sequence that led to production of a protein with a theoretical molecular masse of 43 kDa (Table 1).

These results are in agreement with InIA molecular masses determined by Western blot analysis.

DISCUSSION

In this work, a PCR-RFLP method was developed for rapidly screening less invasive *L. monocytogenes* strains expressing truncated InIA proteins. Five PCR-RFLP profiles of the *inlA* region were observed, suggesting that *inlA* genetic heterogeneity was likely due to point mutations, particularly in a region between repeats A10 and B1 of InIA (Fig. 1), as previously described (5, 8, 20, 26). Most of these *inlA* polymorphism studies were carried out with *L. monocytogenes* isolates from food, animals, plants, and the environment (5, 8, 26), and little attention has been paid to clinical isolates (10, 22).

Restriction endonucleases AluI and Tsp509I were good discriminating enzymes for this polymorphism analysis. In other studies workers have also reported using restriction endonuclease AluI to study virulence gene polymorphism (5, 8, 20). In this study, composite profile A was obtained for eight uncharacterized *L. monocytogenes* isolates (H38, 1S, 2S, 3S, 1F, 2F, 7F, and 38) and four noninvasive *L. monocytogenes* reference strains (H1, H17, H32, and H34). Like noninvasive *L. monocytogenes* reference strains H1, H17, H32, and H34, six isolates (1S, 2S, 3S, 1F, 2F, and 7F) produced a truncated InIA. Moreover, five *L. monocytogenes* isolates (NV4, NV5, NV7, NV8, and 81), noninvasive reference strain LO28, and invasive reference strain EGE-e were characterized by composite profile D. Four of these strains (NV4, NV5, NV7, and NV8) produced a truncated InIA. Altogether, 15 *L. monocytogenes* isolates (strains 1S, 2S, 3S, 1F, 2F, 7F, NV4, NV5, NV7, and NV8 and noninvasive reference strains H1, H17, H32, H34, and LO28) that produced a truncated InIA were characterized by two specific composite profiles (profiles A and D), and 10 new *L. monocytogenes* strains expressing truncated forms of InIA were identified by using this method. Production of a truncated InIA was not correlated with the serotype of the isolates. Although all strains that produced a truncated InIA were characterized by these two specific composite profiles, isolates H38, 38, and 81 and reference strain EGD-e, which had a complete internalin, had profiles A and D. Sequence analysis of an *inlA* fragment showed that point mutations responsible for the production of truncated InIA were located at *inlA* position 1302 (NV8), position 1414 (H1, H17, H32, H34, 1S, 2S, 3S, 1F, 2F, and 7F), position 1496 (NV7), position 1637 (reference strain LO28), position 1901 (NV4), and position 1994 (NV5). These point mutations were not detected as they did not correspond to restriction sites (point mutations at *inlA* positions 1302, 1414, and 1496) for endonucleases AluI and Tsp509I or were outside the amplified *inlA* gene fragment (point mutations at *inlA* position 1637 of reference strain LO28, at *inlA* position 1901 of NV4, and at *inlA* position 1994 of NV5). To optimize screening of strains expressing truncated InIA by this rapid method, it would be useful to amplify a longer *inlA* fragment that might include a higher number of point mutations. We could also increase polymorphism analysis by using other restriction endonucleases to detect numerous point mutations responsible for production of different forms of internalin and to obtain more information about the genetic heterogeneity of *inlA*.

Despite these limitations, this PCR-RFLP method is a useful tool for screening numerous *L. monocytogenes* strains that are deficient in the ability to invade in large strain collections. It permitted us to identify 10 new *L. monocytogenes* strains that produce truncated InIA.

In this study and previous studies (10, 18, 19), 19 strains that produce truncated internalin have been described; it appears that expression of truncated internalin may not be a rare event and not specific to human carriage. In this study, two-thirds of the *L. monocytogenes* strains with a truncated InIA were isolated from food (brine, cheese, or meat). Similarly, in a previous report, Jonquières et al. (10) reported that three of five *L. monocytogenes* isolates that produced truncated internalin were isolated from meat, dairy products, and fish. Epidemiological studies have shown that although the rate of exposure

to *L. monocytogenes* is rather high, the probability of contracting listeriosis is low (7, 17). Several hypotheses to explain this finding have been suggested. Most *L. monocytogenes* strains that occur in food may not be responsible for development of the disease because the levels of strains with attenuated virulence may be higher than the levels previously described. If the high occurrence of food isolates with truncated *InlA* was confirmed, it could partly explain the low occurrence of food-borne listeriosis. Occasional ingestion of food contaminated with attenuated virulence strains could increase the level of human resistance to *Listeria* infections (2). Production of truncated *InlA* may partially explain human carriage. However, several factors may be involved in asymptomatic carriage. In fact, Olier et al. (18, 19) described *L. monocytogenes* with complete *InlA* isolated from healthy carriers. It would be interesting to investigate possible epidemiological links between *L. monocytogenes* strains expressing truncated *InlA* isolated from healthy carriers and attenuated virulence strains producing truncated *InlA* isolated from food. It should be interesting to use molecular typing methods, such as pulsed-field gel electrophoresis or random amplification of polymorphic DNA, to address this hypothesis.

ACKNOWLEDGMENTS

We gratefully acknowledge P. Cossart (Institut Pasteur, Paris, France) for kindly providing antibodies directed against *InlA*.

This work was supported by the Ministère de la Recherche et de l'Enseignement and the Conseil Régional de Bourgogne.

REFERENCES

- Bouttefroy, A., J. P. Lemaître, and A. Rousset. 1997. Prevalence of *Listeria* sp. in droppings from urban rooks (*Corvus frugilegus*). *J. Appl. Microbiol.* **82**:641–647.
- Chakraborty, T., F. Ebel, J. Wehland, J. Dufrenne, and S. Notermans. 1994. Naturally occurring virulence-attenuated isolates of *Listeria monocytogenes* capable of inducing long term protection against infection by virulent strains of homologous and heterologous serotypes. *FEMS Immunol. Med. Microbiol.* **10**:1–10.
- Delgado da Silva, M. C., M. T. Destro, E. Hofer, and A. Tibana. 2001. Characterization and evaluation of some virulence markers of *Listeria monocytogenes* strains isolated from Brazilian cheeses using molecular, biochemical and serotyping techniques. *Int. J. Food Microbiol.* **63**:275–280.
- Durst, J., and M. Zimanyi. 1976. The *Listeria monocytogenes* carrier state of the staff of maternity centres in non epidemic periods. *Zentralbl. Bakteriol.* **234**:281–283.
- Ericsson, H., P. Stalhandske, M. L. Danielsson-Tham, E. Bannerman, J. Bille, C. Jacquet, J. Rocourt, and W. Tham. 1995. Division of *Listeria monocytogenes* serovar 4b strains into two groups by PCR and restriction enzyme analysis. *Appl. Environ. Microbiol.* **61**:3872–3874.
- Fleming, D. W. Cochi, S. L. MacDonald K. L. Brondum, J. Hayes, P. S. Plikaytis B. D. Holmes, M. B. Audurier, A. Broome, C. V., and A. L. Reingold. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *N. Engl. J. Med.* **312**:404–407.
- Gellin, B. C., and C. V. Broome. 1989. Listeriosis. *JAMA* **261**:1313–1320.
- Giovannacci, I., C. Ragimbeau, S. Queguiner, G. Salvat, J. L. Vendevre, V. Carlier, and G. Ermel. 1999. *Listeria monocytogenes* in pork slaughtering and cutting plants: use of RAPD, PFGE and PCR-REA for tracing and molecular epidemiology. *Int. J. Food Microbiol.* **53**:127–140.
- Grif, K., I. Hein, M. Wagner, E. Bandl, O. Mpmuguo, J. MacLauchlin, M. P. Dierich, and F. Allerberger. 2001. Prevalence and characterization of *Listeria monocytogenes* in the faeces of healthy Austrians. *Wien Klin. Wochenschr.* **113**:737–742.
- Jonquières, R., H. Bierne, J. Mengaud, and P. Cossart. 1998. The *inlA* gene of *Listeria monocytogenes* LO28 harbors a nonsense mutation resulting in release of internalin. *Infect. Immun.* **66**:3420–3422.
- Kampelmacher, E. H., and L. M. van Noorle Jansen. 1972. Further studies on the isolation of *L. monocytogenes* in clinically healthy individuals. *Zentralbl. Bakteriol.* **221**:70–77.
- Kocks, C., E. Gouin, M. Tabouret, P. Berche, H. Ohayon, and P. Cossart. 1992. *L. monocytogenes*-induced actin assembly requires the *actA* gene product, a surface protein. *Cell* **68**:521–531.
- Macgowan, A. P., R. J. Marshall, I. M. Mackay, and D. S. Reeves. 1991. *Listeria* faecal carriage by renal transplant recipients, haemodialysis patients and patients in general practice: its relation to season, drug therapy, foreign travel, animal exposure and diet. *Epidemiol. Infect.* **106**:157–166.
- McLauchlin, J., N. A. Saunders, A. M. Ridley, and A. G. Taylor. 1988. Listeriosis and food-borne transmission. *Lancet* **i**:177–178.
- Mengaud, J., M. Lecuit, M. Lebrun, F. Nato, J. C. Mazie, and P. Cossart. 1996. Antibodies to the leucine-rich repeat region of internalin block entry of *Listeria monocytogenes* into cells expressing E-cadherin. *Infect. Immun.* **64**:5430–5433.
- Murray, E. G. D. Webb, R. A., and M. B. R. Swann. 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (*n. sp.*) *J. Pathol. Bacteriol.* **29**:407–439.
- Notermans, S., J. Dufrenne, P. Teunis, and T. Chackraborty. 1998. Studies on the risk assessment of *Listeria monocytogenes*. *J. Food Prot.* **61**:244–248.
- Olier, M., F. Pierre, J. P. Lemaître, C. Divies, A. Rousset, and J. Guzzo. 2002. Assessment of the pathogenic potential of two *Listeria monocytogenes* human faecal carriage isolates. *Microbiology* **148**:1855–1862.
- Olier, M., F. Pierre, S. Rousseaux, J. P. Lemaître, A. Rousset, P. Piveteau, and J. Guzzo. 2003. Expression of truncated internalin A is involved in impaired internalization of some *Listeria monocytogenes* human carriage isolates. *Infect. Immun.* **71**:1217–1224.
- Poyart, C., P. Trieu-Cuot, and P. Berche. 1996. The *inlA* gene required for cell invasion is conserved and specific to *Listeria monocytogenes*. *Microbiolology* **142**:173–180.
- Schlech, W. F., 3rd, P. M. Lavigne, R. A. Bortolussi, A. C. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nicholls, and C. V. Broome. 1983. Epidemic listeriosis—evidence for transmission by food. *N. Engl. J. Med.* **308**:203–206.
- Schuchat, A., K. Deaver, P. S. Hayes, L. Graves, L. Mascola, and J. D. Wenger. 1993. Gastrointestinal carriage of *Listeria monocytogenes* in household contacts of patients with listeriosis. *J. Infect. Dis.* **167**:1261–1262.
- Slutsker, L., and A. Schuchat. 1999. Listeriosis in animals, p. 75–95. *In Listeria, listeriosis and food safety*. E. T. Ryser and E. H. Marth (ed.), Marcel Dekker, Inc., New York, N.Y.
- Watkins, J., and K. P. Sleath. 1981. Isolation and enumeration of *Listeria monocytogenes* from sewage, sewage sludge and river water. *J. Appl. Bacteriol.* **50**:1–9.
- Weis, J., and H. P. Seeliger. 1975. Incidence of *Listeria monocytogenes* in nature. *Appl. Microbiol.* **30**:29–32.
- Wiedmann, M., J. L. Bruce, C. Keating, A. E. Johnson, P. L. McDonough, and C. A. Batt. 1997. Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. *Infect. Immun.* **65**:2707–2716.