MOLECULAR IDENTIFICATION AND TYPING

Molecular grouping of *Listeria monocytogenes* based on the sequence of the *inlB* gene

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The major part of the gene *inlB* was sequenced in 24 strains of *Listeria monocytogenes* belonging to serovars 1/2a, 1/2b, 1/2c, 3b and 4b. A phylogenetic analysis based on the *inlB* nucleotide sequences showed that strains of serovars 1/2a and 1/2c were closely related, as well as those of serovars 1/2b and 3b. Strains sharing serovar 4b could be divided into two distinct groups. There were differences in amino-acid sequence between all serovars except between serovars 1/2b and 3b. Differences in amino-acid sequence were also seen within each of the serovars 1/2a and 4b. The data presented indicate that the *inlB* gene may be useful for typing purposes as an alternative or complement to serotyping.

Introduction

Listeria monocytogenes is the causative organism of listeriosis, which primarily affects immunocompromised individuals, including pregnant women. Listeriosis is manifested mainly as meningitis in adult patients and as premature labour or abortion in pregnant women [1].

L. monocytogenes is considered to have an oral route of infection, i.e., the patients who fall ill with listeriosis have ingested food that is contaminated by the bacterium [2].

The uptake of *L. monocytogenes* from the gastrointestinal tract is induced by the bacterium itself by the protein internalin, which is encoded by the gene *inlA* [3]. Internalin induces uptake of the bacterium by normally non-phagocytic cells, e.g., the epithelial cells in the gastrointestinal mucosa. From the epithelial cells, the bacteria spread into adjacent cells and further into the bloodstream. An important site for the replication of *L. monocytogenes* is the liver [4]. The product of the gene *inlB* is essential for the bacteria to enter the hepatocytes [5]. This gene is situated downstream from *inlA* and belongs to the same gene family [3].

An earlier study characterised a gene segment containing the downstream part of *inlA* and most of *inlB* by PCR combined with restriction enzyme analysis (PCR-REA) [6]. It was found that *L. monocytogenes* serovar 4b strains could be divided into two groups according to the PCR-REA pattern: 4b-I and 4b-II. Poyart et al. [7] showed that the 3' end of *inlA* exhibits a low degree of polymorphism. Thus, it is likely that the observed differences between the two groups in the PCR-REA study emanate from *inlB*. To test this hypothesis, the present study sequenced the major part of the *inlB* gene from serovar 4b strains belonging to the two PCR-REA groups. The same segment of *inlB* from strains belonging to serovars 1/2a, 1/2b, 1/2c and 3b, which are the serovars most commonly found, were also sequenced and a phylogenetic analysis of the sequences was made. Furthermore, the deduced amino-acid sequences from the *inlB* gene products of strains representing the two serovar 4b PCR-REA groups, as well as other serovars, were compared. To investigate the genetic diversity, all strains were typed by restriction endonuclease analysis (REA) and pulsed-field gel electrophoresis (PFGE).

Materials and methods

Bacterial strain

The strains were chosen from different sources, countries and years of isolation to obtain heterogeneous material (Table 1).

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Table 1. Serovar, source, country and year of isolation of *L. monocytogenes* strains for which the *inlB* gene was sequenced

Strain no.	Serovar	Source	Country	Year	PFGE type
77	1/2a	Human	Sweden	1988	A
83	1/2a	Goat faeces	Sweden	1987	B
125	1/2a	Human	Sweden	1988	C-1
465 (ATCC 19111)	1/2a	Guinea-pig	England	1926	C-2
1065	1/2b	Human	Sweden	1980	E-1
1419	1/2b	Human	Sweden	1958	E-2
2324	1/2b	Cheese	Canada	1989	E-3
2393	1/2b	Human	Sweden	1992	F-1
524	1/2c	Human	Sweden	1989	D-1
525	1/2c	Human	England	1988	D-1
1929	1/2c	Medwurst	Sweden	1993	D-2
2551	1/2c	Cheese	Denmark	1997	D-2
594	3b	Human	Germany	NK	F-2
1181	3b	Salami	USA	1989	E-3
1513	3b	Mackerel	Sweden	1993	E-4
1805	3b	Trout	Sweden	1994	E-3
122	4b-I	Human	Sweden	1988	G-1
123	4b-I	Human	Sweden	1988	G-1
501	4b-I	Human	Sweden	1976	G-1
520	4b-I	Human	Sweden	1989	G-2
498	4b-II	Human	Sweden	1974	H-1
502	4b-II	Human	Sweden	1977	H-1
526	4b-II	Human	Sweden	1990	H-2
559 (NCTC 11994)	4b-II	Cheese	England	1986	H-1

NK, not known; 4b-I and 4b-II are the two PCR-REA groups obtained in [6]. The results of PFGE analysis of the strains are also included in the table.

PFGE

REA by PFGE was performed according to the procedure described by Unnerstad et al. [8], with the following running conditions: initial pulse time 4.0 s interpolated up to 40 s over 24 h, with a voltage of 6 V/cm. The enzyme used was *AscI*.

PCR

One colony of each strain was inoculated into 20 ml of brain-heart infusion broth. After incubation at 37°C for 24 h, 15 µl of each culture were mixed with 140 µl of sterile water and denatured with 14 µl of 0.8 M NaOH in an Eppendorf tube. The tubes were heated at 70°C for 10 min, cooled on ice, and the sample was neutralised with 18 µl of 1 M Tris (pH 8.0) and 12 µl of 0.8 M HCl. The pH of the suspension was checked and considered acceptable if it was between 7 and 9. Five µl (c. 100 000 bacteria) were used for PCR in combination with the primers Lmp 23 (5' ATTAG-CTGCTTTCGTCCAACCAATGAAAG 3', positions 5893–5865) and Lmp 24 (5' ACAACTGGCGATAGC-GATAATGCGC 3', positions 3836–3860). The sequence data used for construction of PCR primers and the position numbers were those published previously by Gaillard et al. [3]. The PCR was performed essentially as described by Saiki et al. [9]. The PCR mixture (50 µl) contained 30 mM Tricine, pH 8.4 (Sigma), 2.0 mM MgCl₂, Thesit (Sigma) 0.1%, 200 µM of each deoxynucleoside triphosphate (dATP, dTTP, dCTP, dGTP; Boehringer Mannheim), 0.2 µM of each primer and 1.0 unit of AmpliTaq DNA polymerase (Perkin Elmer). PCR was performed in a Perkin Elmer thermocycler (P13480) and run for 40 cycles

(94°C for 1.25 min; 57°C for 1.25 min; 72°C for 2 min).

Amplified samples (5 µl) were analysed by electrophoresis in agarose 2% gel and the PCR products were visualised by ethidium bromide staining (1.5 µg/ml) for 15 min.

Sequencing

Nucleotide sequences were determined with an ABI PRISM 377 DNA sequencer. The primers used for sequencing are listed in Table 2. The primers were designed from a previously published sequence [3] as well as from data obtained in this study.

The sequence data were analysed with the aid of the CAP Sequence Assembly Machine (<http://gcg.tigem.it/ASSEMBLY>), Clustal V and DNAid. Phylogenetic analysis was conducted with the phylogenetic inference package (PHYLIP).

Results

The results of the REA-PFGE analyses are shown in Table 1. According to their restriction profile, strains were classified with capital letters. Strains with a different capital letter differed in more than three restriction fragments, corresponding to more than a single genetic event [10]. If strains differed in three or fewer restriction fragments, corresponding to a single genetic event [10], they were designated, e.g., D-1 and D-2.

Table 2. Primers used for sequencing of *inlB* in *L. monocytogenes*

Primer	Nucleotide sequence (5'–3')	Position	Reference
Lmp 27	GACCTTCGATGGTTGCTTGA	5834–5815	[3]
Lmp 28	CTCGCACCGCTGTAAAGCTC	5302–5321	[3]
Lmp 29	CCATTTTCGGGCTTCTCTATC	5349–5330	[3]
Lmp 30	GGTCGTTCCGAATACAGTGA	4777–4796	[3]
Lmp 31	CATAATCGCCATCATCACTT	4853–4834	[3]
Lmp 32	GACCTAAGTTCGCTCAAGGA	4418–4437	[3]
Lmp 33	CCAAACTGCATTCCCTGGCGC	5470–5451	This study
Lmp 34	GTAAGGACAGGACGCGAAT	5029–5048	This study
Lmp 36	CGTCCAACCAATGAAAGTGG	5862–5881	This study
Lmp 37	GCGCCAGGGAATGCAGTTTGG	5470–5489	This study

For each strain, the sequence of c. 1500 bp of the *inlB* gene was determined. The sequences are deposited in Genbank, accession nos. AF 121024–AF121047. A short segment is shown in Table 3. The DNA sequence similarity was highest (99.8–100%) among strains within serovars 1/2b, 1/2c and 3b. The similarity among strains within serovar 1/2a was 99.5–99.9% and within serovar 4b it was 98.3–100%. Sequence similarities between strains of different serovars varied from 89.2% (serovars 1/2a–4b) to 100% (serovars 1/2b–3b). The sequencing primers initially designed functioned well when used on PCR fragments from serovars 1/2a, 1/2b, 1/2c and 3b strains. However, when the sequencing primers Lmp 27–30 were used with PCR products from strains of serovar 4b, most sequencing reactions yielded no data. The number of mismatches ranged from 1 to 6 bases between primers Lmp 27 and 30 and the serovar 4b strains. Therefore, these primers were replaced by a set of newly designed primers, Lmp 33, Lmp 34, Lmp 36 and Lmp 37. The polymorphism in the *inlB* gene of the various strains

was converted into a phylogenetic tree (Fig. 1) by a comparative analysis. In this analysis, the strains formed clusters in agreement with their serovars. A comparison of the deduced amino-acid sequences demonstrated that amino-acid substitutions were present between all serovars except between serovars 1/2b and 3b. There were also amino-acid substitutions between *inlB* genes within each of the serovars 1/2a and 4b (Table 4). The amino-acid sequence similarities between serovars were between 91.9% (serovars 1/2a and 4b) and 100% (serovars 1/2b and 3b).

Strains within serovar 1/2a displayed amino-acid similarities of 99.4–100% and strains within serovar 4b showed 98.6–100% similarity.

Discussion

In an earlier study, 133 *L. monocytogenes* strains sharing serovar 4b could be divided into two groups (4b-I and 4b-II) based on a PCR-REA of the 3' end of *inlA* and nearly the entire *inlB* gene [6]. The present study, based on the amplification and sequencing of c. 1500 bp (out of 1890 bp) of *inlB* obtained the same grouping as above, i.e., strains belonging to serovar 4b could be divided into two groups, identical with 4b-I and 4b-II. Thus, it seems likely that these two PCR-REA groups solely reflect differences in the *inlB* sequences. This is also in agreement with Poyart et al. [7] who showed that the variability in the 3' end of the *inlA* gene is very low. The *inlB* sequence data obtained not only support a division of strains belonging to serovar 4b, but also of strains of serovar 1/2a (Fig. 1). This has also been shown recently by PCR-REA [11]. At the amino-acid level, strains of groups 4b-I and 4b-II differ in six amino acids, whereas there are differences in three amino acids between the strains representing the two 1/2a groups. In contrast, the strains of serovar 1/2b and 3b differ to some extent in their *inlB* DNA sequences, but the corresponding amino-acid sequences are identical.

The fact that primers Lmp 27–30 did not function well when used on PCR products from serovar 4b strains is not surprising, as the sequence data published by Gaillard et al. [3] and upon which primers Lmp 27–30

Table 3. Partial nucleotide sequences of *inlB* in different serovars of *L. monocytogenes*

Strain no.	Serovar	Nucleotide sequence
77	1/2a	CAATTACTACATGGTATCAAT
83	1/2a	CAATTACTACATGGTATCAAT
125	1/2a	CAATTACTACATGGTATCAAT
465	1/2a	CAATTACTACATGGTATCAAT
1065	1/2b	CAATTACTACTTGGTATCAAT
1419	1/2b	CAATTACTACTTGGTATCAAT
2324	1/2b	CAATTACTACTTGGTATCAAT
2393	1/2b	CAATTACTACTTGGTATCAAT
524	1/2c	CAATTACTACATGGTATCAAT
525	1/2c	CAATTACTACATGGTATCAAT
1929	1/2c	CAATTACTACATGGTATCAAT
2551	1/2c	CAATTACTACATGGTATCAAT
594	3b	CAATTACTACTTGGTATCAAT
1181	3b	CAATTACTACTTGGTATCAAT
1513	3b	CAATTACTACTTGGTATCAAT
1805	3b	CAATTACTACTTGGTATCAAT
122	4b-I	CAATTACCACGTGGTATCAAT
123	4b-I	CAATTACCACGTGGTATCAAT
501	4b-I	CAATTACCACGTGGTATCAAT
520	4b-I	CAATTACCACGTGGTATCAAT
498	4b-II	CAATTACTACGTGGTATCAAT
502	4b-II	CAATTACTACGTGGTATCAAT
526	4b-II	CAATTACTACGTGGTATCAAT
559	4b-II	CAATTACTACGTGGTATCAAT
		***** ** *****
Position	5586	5606

Fig. 1. The relationship between different strains of *L. monocytogenes* inferred from nucleotide sequence polymorphism of the *inlB* gene. The tree was assembled with the aid of the average linkage clustering method in the NEIGHBOR program of PHYLIP.

are based, emanate from a serovar 1/2a strain. The strains of serovar 4b showed the lowest sequence similarity with serovar 1/2a and, therefore, may need specially designed primers for certain genomic regions. This was also the case in the present study.

It is noteworthy that in this study, as well as in other studies where a division has been made based on polymorphism in virulence-associated genes, serovars 1/2a and 1/2c cluster together [12, 13]. In the study by Vines et al. [13], strains of serovars 1/2b, 3b and 4b

were also grouped together, whereas in the present study the members of serovar 4b formed their own group, but from the same branch as the 1/2b–3b strains. Serotyping, based on O and H antigens, is probably the oldest and most established typing method for *L. monocytogenes*. It is an interesting observation that there seems to be a correlation between the serovars and the structure of virulence-associated genes. This indicates that the evolution of the genes encoding the O and H antigens has been parallel to the evolution of virulence-associated genes. This has also

Table 4. Deduced amino-acid sequences from the major part of inlB in different serovars of *L. monocytogenes*

Strain no.	Serovar	Amino-acid sequence
77, 465	1/2a	KPLTNLKNLWFLDENKIKDLSSSLKDLKKLKSLSLEHNGISDINGLVHL
83	1/2a	KPLTNLKNLWFLDENKIKDLSSSLKDLKKLKSLSLEHNGISDINGLVHL
125	1/2a	KPLTNLKNLWFLDENKIKDLSSSLKDLKKLKSLSLEHNGISDINGLVHL
1065, 1419,	1/2b	KPLANLKNLWFLDENKIKDLSSSLKDLKKLKSLSLEHNGISDINGLVHL
2324, 2393,	1/2b	
594, 1181,	3b	
1513, 1805	3b	
524, 525,	1/2c	KPLANLKNLWFLDENKVKDLSSSLKDLKKLKSLSLEHNGISDINGLVHL
1929, 2551	1/2c	
122, 501,	4b-I	KPLANLKNLWFLDENKIKDLSSIKDLKKLKSLSLEHNGISDINGLVHL
520	4b-I	
123	4b-I	KPLANLKNLWFLDENTIKDLSSIKDLKKLKSLSLEHNGISDINGLVHL
498, 502,	4b-II	KPLANLKNLWFLDENKIKDLSSSLKDLKKLKSLSLEHNGISDINGLVHL
526, 559	4b-II	
		*** *****
77, 465	1/2a	PQLESYLGNKKITDITVLSRLTKLDTLSLEDNQISDIVPLAGLTKLQNL
83	1/2a	PQLESYLGNKKITDITVLSRLTKLDTLSLEDNQISDIVPLAGLTKLQNL
125	1/2a	PQLESYLGNKKITDITVLSRLTKLDTLSLEDNQISDIVPLAGLTKLQNL
1065, 1419,	1/2b	LQLESYLGNKKITDITILSRLTKLDTLSLEDNEISDIVPLSGLTKLQNL
2324, 2393,	1/2b	
594, 1181,	3b	
1513, 1805	3b	
524, 525,	1/2c	PQLESYLGNKKITDITVLSRLTKLDTLSLEDNQISDIVPLAGLTKLQNL
1929, 2551	1/2c	
122, 501,	4b-I	PQLESYLGNKKITDITILSRLTKLDTLSLEDNEISDIVPLSGLTKLQNL
520	4b-I	
123	4b-I	PQLESYLGNKKITDITILSRLTKLDTLSLEDNEISDIVPLSGLTKLQNL
498, 502,	4b-II	LQLESYLGNKKITDITILSRLTKLDTLSLEDNEISDIVPLSGLTKLQNL
526, 559	4b-II	

77, 465	1/2a	YLSKNHISDLRALAGLKNLDVLELFSQECLNKPINHQSNNLVVPNTVKNTD
83	1/2a	YLSKNHISDLRALAGLKNLDVLELFSQECLNKPINHQSNNLVVPNTVKNTD
125	1/2a	YLSKNHISDLRALAGLKNLDVLELFSQECLNKPINHQSNNLVVPNTVKNTD
1065, 1419,	1/2b	YLSKNHISDLRALAGLKNLDVLELFSQECLNKSINHQTNNLVVPNTVKNID
2324, 2393,	1/2b	
594, 1181,	3b	
1513, 1805	3b	
524, 525,	1/2c	YLSKNHISDLRALAGLKNLDVLELFSQECLNKPINHQSNNLVVPNTVKNTD
1929, 2551	1/2c	
122, 501,	4b-I	YLSKNHISDLRALAGLKNLDVLELFSQECLNKSINHQTNNLVVPNTVKNID
520	4b-I	
123	4b-I	YLSKNHISDLRALAGLKNLDVLELFSQECLNKSINHQTNNLVVPNTVKNID
498, 502,	4b-II	YLSKNHISDLRALAGLKNLDVLELFSQECLNKSINHQMNNLVVPNTVKNID
526, 559	4b-II	

77, 465	1/2a	GSLVTPEIISDDGDYEKPNVKWHLPEFTNEVSFIFYQPVTIGKAKARFHG
83	1/2a	GSLVTPEIISDDGDYEKPNVKWHLPEFTNEVSFIFYQPVTIGKAKARFHG
125	1/2a	GSLVTPEIISDDGDYEKPNVKWHLPEFTNEVSFIFYQPVTIGKAKARFHG
1065, 1419,	1/2b	GSLVTPEIISDDGDYEKPNVKWHLPEFINEVSFIFYQPVTIGKAKARFHG
2324, 2393,	1/2b	
594, 1181,	3b	
1513, 1805	3b	
524, 525,	1/2c	GSLVTPEIISDDGDYEKPNVKWHLPEFTNEVSFIFYQPVTIGKAKARFHG
1929, 2551	1/2c	
122, 501,	4b-I	GSLVTPEIISDDGDYEKPNVKWHLPEFINEVSFIFYQPVTIGKAKARFHG
520	4b-I	
123	4b-I	GSLVTPEIISDDGDYEKPNVKWHLPEFINEVSFIFYQPVTIGKAKARFHG
498, 502,	4b-II	GSLVTPEIISDDGDYEKPNVKWHLPEFINEVSFIFYQPVTIGKAKARFHG
526, 559	4b-II	

77, 465	1/2a	RVTQPLKEVYTVSYDVGTVIKTKVEAGTRITAPKPPTKQGYVFKGWYTE
83	1/2a	RVTQPLKEVYTVSYDVGTVIKTKVEAGTRITAPKPPTKQGYVFKGWYTE
125	1/2a	RVTQPLKEVYTVSYDVGTVIKTKVEAGTRITAPKPPTKQGYVFKGWYTE
1065, 1419,	1/2b	RVTQPLKEVYTVSYDVGTVIKTKVEAGTRITAPKPPTKQGYVFKGWYTE
2324, 2393,	1/2b	
594, 1181,	3b	
1513, 1805	3b	
524, 525,	1/2c	RVTQPLKEVYTVSYDVGTVIKTKVEAGTRITAPKPPTKQGYVFKGWYTE
1929, 2551	1/2c	
122, 501,	4b-I	RVTQPLKEVYTVSYDVGTVIKTKVEAGTRITAPKPPTKQGYVFKGWYTE
520	4b-I	
123	4b-I	RVTQPLKEVYTVSYDVGTVIKTKVEAGTRITAPKPPTKQGYVFKGWYTE

continued overleaf

Table 4. (continued)

Strain no.	Serovar	Amino-acid sequence
498, 502, 526, 559	4b-II	RVTQPLKEVYTVSYDVGTVIKTKVEAGTRITAPKPPTKQGYVFKGWYTE *****
77, 465	1/2a	KNGGHEWNFNNTDYMSGNDFTLYAVFKAETTEKAVNLTRYVKYIRGNAGIY
83	1/2a	KNGGHEWNFNNTDYMSGNDFTLYAVFKAETTEKAVNLTRYVKYIRGNAGIY
125	1/2a	KNGGHEWNFNNTDYMSGNDFTLYAVFKAETTEKAVNLTRYVKYIRGNAGIY
1065, 1419, 2324, 2393,	1/2b	KNGGHEWDFSTDYMSGNDFTLYAMFKVETTEKAVNLTRYVKYIRGNAGIY
594, 1181,	3b	
1513, 1805	3b	
524, 525,	1/2c	KNGGHEWNFNNTDYMSGNDFTLYAVFKAETTEKAVNLTRYVKYIRGNAGIY
1929, 2551	1/2c	
122, 501,	4b-I	KNGGHEWNFNSTDYMSGNDFTLYAIFKAETTEKAVNLTRYVKYIRGNAGIY
520	4b-I	
123	4b-I	KNGGHEWNFNSTDYMSGNDFTLYAIFKAETTEKAVNLTRYVKYIRGNAGIY
498, 502, 526, 559	4b-II	KNGGHEWNFNSTDYMSGNDFTLYAMFKAETTEKAVNLTRYVKYIRGNAGIY ***** * ***** ** *****
77, 465	1/2a	KLPRDNLKQGTSLASHRCKALTVDREARNGGKLWYRLKKNIGWTKAENLS
83	1/2a	KLPRDNLKQGTSLASHRCKALTVDREARNGGKLWYRLKKNIGWTKAENLS
125	1/2a	KLPRDNLKQGTSLASHRCKALTVDREARNGGKLWYRLKKNIGWTKAENLS
1065, 1419, 2324, 2393,	1/2b	KLPRDNLKQGTSLASHRCKALTVDREARNGGKLWYRLKKNIGWTKAENLS
594, 1181,	3b	
1513, 1805	3b	
524, 525,	1/2c	KLPRDNLKQGTSLASHRCKALTVDREARNGSKLWYRLKKNIGWTKAENLS
1929, 2551	1/2c	
122, 501,	4b-I	KLPRDNLKQGTSLASHRCKALTVDREARNGSELWYRLKKNIGWTKAENLS
520	4b-I	
123	4b-I	KLPRDNLKQGTSLASHRCKALTVDREARNGSELWYRLKKNIGWTKAENLS
498, 502, 526, 559	4b-II	KLPRDNLKQGTSLASHRCKALTVDREARNGGELWYRLKKNIGWTKAENLS ***** *****
77, 465	1/2a	LDRYDKMEYDKGVTAYARVRNASGNSVWTKPYNTAGAKHVNKL SVYQGKN
83	1/2a	LDRYDKMEYDKGVTAYARVRNASGNSVWTKPYNTAGAKHVNKL SVYQGKN
125	1/2a	LDRYDKMEYDKGVTAYARVRNASGNSVWTKPYNTAGAKHVNKL SVYQGKN
1065, 1419, 2324, 2393,	1/2b	LDRYDKIEYDKGVTAYARVKNALGNAVWTKPYNTAGAKHVNKL SVYQGKN
594, 1181,	3b	
1513, 1805	3b	
524, 525,	1/2c	LDRYDKMEYDKGVTAYARVRNASGNSVWTKPYNTAGAKHVNKL SVYQGKN
1929, 2551	1/2c	
122, 501,	4b-I	LDRYDKIEYDKGVTAYARVKNAPGNAVWTKPYNTAGATLVNKL SVYQGKN
520	4b-I	
123	4b-I	LDRYDKIEYDKGVTAYARVKNAPGNAVWTKPYNTAGATLVNKL SVYQGKN
498, 502, 526, 559	4b-II	LDRYDKIEYDKGVTAYARVKNAPGNAVWTKPYNTAGATLVNKL SVYQGKN ***** *****
77, 465	1/2a	MRILREAKTPITTWYQFSIGGKVGWVDTRALNTFYKQSMEKPTRLTRYV
83	1/2a	MRILREAKTPITTWYQFSIGGKVGWVDTRALNTFYKQSMEKPTRLTRYV
125	1/2a	MRILREAKTPITTWYQFSIGGKVGWVDTRALNTFYKQSMEKPTRLTRYV
1065, 1419, 2324, 2393,	1/2b	MRILREAKTPITTWYQFSIGGKVGWVDTRALNTFYKQSMEKPTRLTRYV
594, 1181,	3b	
1513, 1805	3b	
524, 525,	1/2c	MRILREAKTPITTWYQFSIGGKVGWVDTRALNTFYKQSMEKPTRLTRYV
1929, 2551	1/2c	
122, 501,	4b-I	MRILREAKTPITTWYQFSIDGKVGWVDTRALNTFYKQSMEIPIQLTRYV
520	4b-I	
123	4b-I	MRILREAKTPITTWYQFSIDGKVGWVDTRALNTFYKQSMEIPIQLTRYV
498, 502, 526, 559	4b-II	MRILREAKTPITTWYQFSIDGKVGWVDTRALNTFYKQSMEIPIQLTRYV ***** *****
77, 465	1/2a	SANKAGESYYKVPVADNPVKRGTLAKYKNQKLIVDCQATIEGQLWYRIRT
83	1/2a	SANKAGESYYKVPVADNPVKRGTLAKYKNQKLIVDCQATIEGQLWYRIRT
125	1/2a	SANKAGESYYKVPVADNPVKRGTLAKYKNQKLIVDCQATIEGQWPYRIRA
1065, 1419, 2324, 2393,	1/2b	SASKGNEAYYKVPVADNPVKRGTLAKYKNQKLIVDCQATVEGQLWYRIRT
594, 1181,	3b	
1513, 1805	3b	
524, 525,	1/2c	SANKAGESYYKVPVADNPVKRGTLAKYKNQKLIVDCQATIEGQLWYRIRT
1929, 2551	1/2c	

Table 4. (continued)

Strain no.	Serovar	Amino-acid sequence
122, 501,	4b-I	NANKGNEAYYKVPVVDSPIKWGTLAKYKNQTLIVDRATATVEGQLWYRIRT
520	4b-I	
123	4b-I	NANKGNEAYYKVPVVDSPIKWGTLAKYKNQTLIVDRATATVEGQLWYRIRT
498, 502,	4b-II	SANKGNEAYYKVPVVDSPIKWGTLAKYKNQTLIVDRATATVEGQLWYRIRT
526, 559	4b-II	
		* * * * *
77, 465	1/2a	SSTF
83	1/2a	SSTF
125	1/2a	SSTF
1065, 1419,	1/2b	SSTF
2324, 2393,	1/2b	
594, 1181,	3b	
1513, 1805	3b	
524, 525,	1/2c	SSFT
1929, 2551	1/2c	
122, 501,	4b-I	SSTF
520	4b-I	
123	4b-I	SSTF
498, 502,	4b-II	SSTF
526, 559	4b-II	

*Consensus between all tested serovars;, same sequence as line above.

been suggested by other authors, e.g., Vines et al. [13]. Similarly, the results of the REA-PFGE analyses in the present study show that the overall genomic variability between most strains seems to correspond to the serovars. The serovars 1/2b and 3b appear to be genetically very closely related, as both the PFGE types and the *inlB* sequences within the strains are almost identical. This has also been reported elsewhere [14].

It should be noted that the *inlB* sequence of serovar 1/2a strain no. 465 (ATCC 19111) isolated in England from a guinea-pig in 1926 is almost identical to the *inlB* sequence of serovar 1/2a strain no. 77 isolated from a human patient in Sweden in 1988, and that in *inlB* sequence of serovar 1/2b strain no. 2324 isolated in 1989 from a cheese in Canada has an identical *inlB* sequence to that of serovar 1/2b strain no. 1419 isolated from a human patient in Sweden 1958. Furthermore, >200 *L. monocytogenes* strains of serovars 4b and 1/2a were analysed in the two above-mentioned PCR-REA studies [6, 11] and only two variants of each serovar were found. These data suggest that the *inlB* gene is stable within the serovars and that it could serve as a genetic marker for serovar identification.

In a paper by Vines et al. [15] three virulence-associated genes (*hlyA*, *plcA* and *inlA*) in two serovar 4b strains and two serovar 1/2b strains were characterised. This set of genes from the analysed strains exhibited a higher degree of conservation than the *inlB* gene segment in the strains of the present study. The similarity in nucleotides, when compared with published sequences from strains of serovars 1/2a and 1/2c, was between 97.3% and 98.8% and at the amino-acid level was between 97.8% and 99.4%. In the present study, the corresponding values between these serovars were 90.7–93.4% and 92.7–95.0%. This

indicates that *inlB* may be better suited than the genes mentioned for differentiation of *L. monocytogenes*, especially between different serovars.

Based on the results of previous PCR-REA study and those of the present study, the *inlB* region may be used for typing purposes as an alternative or complement to serotyping. That could, in the near future, be done by the technique described recently by Ronaghi et al. [16]. This is a sequencing-by-synthesis method based on real-time pyrophosphate detection. The method aims to achieve rapid and automated determination of short DNA sequences. If for example, positions 5893 and 5896 (Table 3) were used, it would be possible to discriminate rapidly between four categories of *L. monocytogenes*: serovar 4b-I, serovar 4b-II, serovars 1/2b and 3b, and serovars 1/2a and 1/2c. This could provide a useful tool for the epidemiological investigation of listeriosis.

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References

1. Nieman RE, Lorber B. Listeriosis in adults: a changing pattern. Report of eight cases and review of the literature, 1968–1978. *Rev Infect Dis* 1980; 2: 207–227.
2. James SM, Fannin SL, Agee BA et al. Listeriosis outbreak associated with Mexican-style cheese – California. *Morbidity Mortal Weekly Rep* 1985; 34: 357–359.
3. Gaillard J-L, Berche P, Frehel C, Gouin E, Cossart P. Entry of *L. monocytogenes* into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from gram-positive cocci. *Cell* 1991; 65: 1127–1141.
4. Gregory SH, Sagnimeni AJ, Wing EJ. Internalin B promotes the replication of *Listeria monocytogenes* in mouse hepatocytes. *Infect Immun* 1997; 65: 5137–5141.
5. Dramsi S, Biswas I, Maguin E, Braun L, Mastroeni P, Cossart P. Entry of *Listeria monocytogenes* into hepatocytes requires expression of *InlB*, a surface protein of the internalin

- multigene family. *Mol Microbiol* 1995; 16: 251–261.
6. Ericsson H, Stålhandske P, Danielsson-Tham M-L et al. Division of *Listeria monocytogenes* serovar 4b strains into two groups by PCR and restriction enzyme analysis. *Appl Environ Microbiol* 1995; 61: 3872–3874.
 7. Poyart C, Trieu-Cuot P, Berche P. The *inlA* gene required for cell invasion is conserved and specific to *Listeria monocytogenes*. *Microbiology* 1996; 142: 173–180.
 8. Unnerstad H, Bannerman E, Bille J, Danielsson-Tham ML, Waak E, Tham W. Prolonged contamination of a dairy with *Listeria monocytogenes*. *Neth Milk Dairy J* 1996; 50: 493–499.
 9. Saiki RK, Gelfand DH, Stoffel S et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988; 239: 487–491.
 10. Tenover FC, Arbeit RD, Goering RV et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; 33: 2233–2239.
 11. Unnerstad H, Nilsson I, Ericsson H et al. Division of *Listeria monocytogenes* serovar 1/2a strains into two groups by PCR and restriction enzyme analysis. *Appl Environ Microbiol* 1999; 65: 2054–2056.
 12. Piffaretti J-C, Kressebuch H, Aeschbacher M et al. Genetic characterization of clones of the bacterium *Listeria monocytogenes* causing epidemic disease. *Proc Natl Acad Sci USA* 1989; 86: 3818–3822.
 13. Vines A, Reeves MW, Hunter S, Swaminathan B. Restriction fragment length polymorphism in four virulence-associated genes of *Listeria monocytogenes*. *Res Microbiol* 1992; 143: 281–294.
 14. Brosch R, Chen J, Luchansky JB. Pulsed-field fingerprinting of *Listeriae*: identification of genomic divisions for *Listeria monocytogenes* and their correlation with serovar. *Appl Environ Microbiol* 1994; 60: 2584–2592.
 15. Vines A, Swaminathan B. Identification and characterization of nucleotide sequence differences in three virulence-associated genes of *Listeria monocytogenes* strains representing clinically important serotypes. *Curr Microbiol* 1998; 36: 309–318.
 16. Ronaghi M, Uhlén M, Nyrén P. A sequencing method based on real-time pyrophosphate. *Science* 1998; 281: 363–365.