We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists



186,000

200M



Our authors are among the

TOP 1% most cited scientists





WEB OF SCIENCE

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us? Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected. For more information visit www.intechopen.com



The Use of Pulsed Field Gel Electrophoresis in *Listeria monocytogenes* Sub-Typing – Comparison with MLVA Method Coupled with Gel Electrophoresis

Sophie Roussel et al.* ANSES: Maisons-Alfort Laboratory for Food Safety, Paris, France

1. Introduction

Out of the several molecular methods currently available, pulsed field gel electrophoresis (PFGE) is one of the most discriminatory and reproducible methods for the sub-typing of *Listeria monocytogenes (L. monocytogenes)* (Kerouanton *et al.*, 1998; Brosch *et al.*, 1996). The combination of restriction endonucleases *AscI* and *ApaI* has shown excellent discrimination for *L. monocytogenes* (Brosch et al., 1996). Thus, the PFGE method, using these two enzymes, is considered to be the international standard for sub-typing (Graves and Swamminathan, 2001). However, although the protocol has been shortened to 30 hours from the time a pure culture of the bacteria has been obtained (Graves and Swamminathan, 2001), PFGE remains a manual, time-consuming and labor intensive subtyping method. It also requires highly skilled operators and does not offer standardized reagents.

ANSES Maisons-Alfort Laboratory for Food Safety has been the European Union Reference Laboratory (EURL) for *L. monocytogenes* in the food chain since 2006. One of the main EURL activities is to develop relevant subtyping methods that are faster than the reference subtyping method, PFGE and that can be easily be implemented in the National Reference Laboratories (NRLs) of European countries.

Multiple-locus variable-number tandem-repeat analysis (MLVA) is a rapid subtyping method based on (PCR) amplification and size analysis of regions of DNA containing variable numbers of tandem repeats (VNTRs). MLVA has been successfully developed for subtyping various bacterial genera. The amplification products are measured using either a capillary electrophoresis system (CE) or a simple agarose gel electrophoresis system. However, with the latter, it is necessary to select VNTR loci with repeat sizes large enough

Eva Moller Nielsen² and Anne Brisabois¹

^{*} Marie-Léone Vignaud¹, Jonass T Larsson², Benjamin Félix¹, Aurore Rossignol¹,

¹ANSES: Maisons-Alfort Laboratory for Food Safety, Paris, France

²Statens Serum Institut (SSI), Department of Microbiological Surveillance and Research, Copenhagen, Denmark

(greater than or equal to 9 bp) that the difference between two alleles is clearly visible on the gel (Vergnaud and Pourcel (2006)).

For L. monocytogenes, a standardized PulseNet USA MLVA protocol has recently been developed based on the detection of nine VNTR loci, with a VNTR size between 6 and 15 bp. The panel of strains was composed of 250 epidemiologically unrelated strains and most of the tested isolates were of human origin. The clusters obtained correlate with isolate serotypes (Hyytia-Trees, 2010; Sperry et al., 2008). At the Serum Statens Institute (SSI) in Copenhagen, Larsson et al. (2010) developed another scheme using 10 VNTR loci based on the analysis of 20 genome sequences. Seven loci were common to the PulseNet protocol. For five of these loci, degenerate primers were designed to match genome diversity. The results demonstrated better discriminatory power for MLVA compared to combined ApaI/AscI PFGE. This scheme has been successfully used for the surveillance of L. monocytogenes in Denmark (Larsson et al., 2010). Of the nine loci used by PulseNet, four have been previously described: Lindstedt et al. (2008) developed an MLVA scheme based on the use of five VNTR loci to characterize 140 isolates, mainly from human and environmental sources and belonging to various serotypes. The discriminatory power of MLVA is similar to that of AscI-PFGE. Another MLVA scheme using only three described VNTR loci has been developed to type 60 serotype 4b isolates from various sources. Simpson's diversity index has been shown to be higher than that of ApaI-PFGE, MLST, and EcoRI ribotyping (Miya et al., 2008).

In the MLVA schemes developed so far, fragment detection is performed by CE. Nevertheless, Murphy et al (2007) demonstrated that it is possible to detect VNTR loci on agarose gels. However, most of the 45 isolates tested in this study had the same origin (food origin) and the same serotype (1/2a). Moreover, out of the six VNTR loci described by Murphy et al. (2007), four were excluded from the PulseNet USA MLVA protocol because two loci have low diversity and two others display sequence variability in flanking regions. The purpose of the present study was to evaluate the feasibility of a MLVA protocol coupled with conventional gel electrophoresis. The results were compared with those obtained by PFGE.

2. Materials and methods

2.1 Strain panel

This study was conducted on 72 strains (Figure 1): 45 isolated from clinical samples and 18 isolated from different food products. This panel included also nine reference strains with six CLIP strains (CLIP 74903 (1/2b), CLIP 74904 (1/2c), CLIP 74905 (3a), CLIP 74906 (3b), CLIP 74907 (3c), CLIP 74912 (4d)) and three fully sequenced strains: EGDe (1/2a), F2365 (4b) and CLIP 80459 (4b). Twenty human strains came from SSI. Twelve non-human field isolates came from the EURL and were collected from French food analysis laboratories, as part of their monitoring, surveillance sampling activities or research projects. Thirty-three strains had previously been used in the WHO international multicenter *L. monocytogenes* subtyping study (Bille & Rocourt, 1996). These strains were labeled TS ("Test study") (Schönberg et al. (1996). Twenty of the 72 strains were related to nine different epidemiological groups (02, 03, 05, 11, 15, 16, 19, 21, 22) (Bille & Rocourt, 1996). Four strains

were represented by two duplicates each (TS32,TS72;TS56,TS77;TS35,TS75;TS63,TS73). A panel of 40 strains (20 SSI human strains, 8 ANSES food strains and 12 TS strains) was typed both at ANSES and SSI.

2.2 Methods

2.2.1 Serotyping

Species identification was performed using agar Listeria according to Ottaviani & Agosti (ALOA) plates (AES, Combourg, France) and the CAMP Test (McKellar 1994). Each strain was serotyped by agglutination using commercially available antisera (Denka, Eurobio, Les Ulis, France), after adapting the manufacturer's instructions and using the procedures outlined by Seeliger & Hohne (1979). Our laboratory has been certified by the French Accreditation Committee (COFRAC) for this serotyping method as an internal method (accreditation no. 1-22465, Section Laboratories, www.cofrac.fr). Determination of the O-antigen was performed from a pure culture [instead of a bacterial suspension]. Determination of the H-antigen was performed using semi-liquid brain heart infusion (BHI) media with 0.5% agar [instead of 0.2%].

2.2.2 Molecular serotyping

Molecular serotyping was performed using the protocol developed by Kerouanton et al. (2010).

2.2.3 PFGE

PFGE was performed using the standard CDC PulseNet protocol (Graves & Swamminathan, 2001) with minor modifications. Each strain was grown overnight on tryptone soya agar with yeast extract (TSAYE) plates instead of BHI. For the DNA digestion step in agarose plugs using ApaI and AscI enzymes, 10 units of enzyme were used per plug [instead of 25 units of enzyme per plug for AscI] and 160-200 units of enzyme per plug for ApaI in the PulseNet protocol. Plugs were incubated with restriction enzymes for 4 h [instead of 5 h]. Gels were then stained with ethidium bromide and banding patterns were visualized under UV light, using the Gel Doc EQ system and Quantity One software (Bio-Rad). DNA patterns were analyzed with BioNumerics software (ver. 6.5, Applied Maths, Kortrijk, Belgium). The recommendations of Barrett et al. (2006) were followed for gel analysis: gels including partial digestions, or unclear bands were not analyzed. All bands with sizes lower than 33 kb were systematically removed. A similarity value of 97.0% was established as a cut-off to consider two profiles as indistinguishable in UPGMA dendrograms using the Dice coefficient, with a 1% tolerance limit and 1% optimization. If the similarity value was strictly less than 97%, the two profiles were considered as different. The dendrogram settings used were chosen according to PulseNet Europe recommendations (Martin et al., 2006). The similarity value taken as the cut-off was established according to the EURL database settings. Each PFGE profile was arbitrarily assigned a number. Our laboratory has been certified by COFRAC for PFGE analyses (Accreditation no. 1-22465, Section Laboratories, www.cofrac.fr).

2.2.4 MLVA

2.2.4.1. Strain isolation and DNA extraction

Bacterial cultures were revived by plating onto TSAYE plates (Humeau, La Chapelle-sur-Erdre, France). Species confirmation was performed by isolation on ALOA plates (AES, Combourg, France). DNA extraction was performed using the InstaGene kit (Bio-Rad, Marnes-la-Coquette, France) according to the manufacturer's recommendations. Extracts were adjusted to approximately 100 ng/ μ l using a spectrophotometer (Biophotometer, Eppendorf, ville, France).

2.2.4.2 Locus selection

VNTR loci found in the literature with a repeat size greater than or equal to 9 bp were selected. New VNTR loci were selected from the complete genome of the three reference strains. The genomes of strains EGDe (1/2a), F2365 (4b) and CLIP 80459 (4b) were individually screened using the Tandem Repeat Finder (TRF) program (http://tandem.bu.edu/). The tandem repeat databases http://mlva.u-psud.fr and http://www.hpa-bionum.org.uk/VNTRUK/ were then used to compare the genomes.

2.2.4.3 Primer design

The primer sets were either similar to those described in the literature (Table 2), or designed in regions flanking the VNTR locus, (Table 3), using AlleleID® software (Premier Biosoft International, USA). All the primers were synthesized by Eurogentec (France).

2.2.4.4 Amplification of VNTR loci

The VNTR loci were amplified on DNA from strains EGDe and F2365. The amplification products were electrophoresed on two gels run independently.

For each primer set, the final mix contained 1 U HotStart Taq Polymerase (Roche), 2 or 3 mM MgCl₂, 0.2 mM desoxynucleotide triphosphate, 1X PCR buffer, PCR grade water, 0.3 μ M each primer, and 1 μ l of DNA in a 25 μ l reaction mixture. PCR was performed on a thermal cycler (GeneAmp PCR System, 9700, PE, Applied Biosystems). For Lm-8, the parameters used were those described by Sperry et al. (2008): initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 20 s, annealing at 50°C for 20 s, extension at 72°C for 20 s and a final extension at 72°C for 5 min. For LMCEB 02, 06, 12, 14 and Lm-26: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 72°C for 30 s and a final extension at 72°C for 30 s and a final extension at 72°C for 7 min. For LMCEB 05 the annealing was performed at 54°C. For JLR-4, the parameters used were those described by Larsson et al. (2010). At SSI, amplification for JLR-4 and Lm-8 was performed according to Larson et al. (2010).

2.2.4.5 Detection of VNTR loci

Aliquots (5 µl) of amplified products were electrophoresed on 2% agarose gels (Resophor, Eurobio, France) in 1X TBE buffer (0.45 mM Tris-HCl, 0.45 mM boric acid, 1 mM EDTA, pH 8). Electrophoresis was performed in 12 cm long gels and run at 80 V for 30 min followed by 90 V for 4 h. In each run, the 20 bp DNA Ladder (Bio-Rad, France) and the PCR products from the two strains EGDe and F2365 were systematically included at least twice to facilitate the sizing of amplified DNA fragments. Each run included a negative/water control to ensure the absence of contamination.

302

The gels were stained in $2 \mu g/ml$ ethidium bromide for 90 min and photographed under UV illumination (Gel Doc EQ^R Bio-Rad, France). The length of each amplified VNTR locus was measured using Quantity One software (Bio-Rad, France). An allele number string based on the estimated number of tandem repeats at each locus was assigned to the amplified DNA fragments from each isolate. Detection of PCR products by capillary electrophoresis was performed according to Larsson et al. (2010).

2.2.4.6 Data analysis

The allele strings were imported into BioNumerics software. Dendrograms were constructed using a categorical coefficient and UPGMA clustering. Allele nomenclature was that recommended by PulseNet USA. No amplification was coded as negative (-1). Efficient amplification with no VNTR detected was coded as "zero" (0). Partial repeats were rounded down to the closest whole number.

2.2.4.7 Sequence verification

The loci and flanking regions were amplified in both directions with high-fidelity HotStart Taq Polymerase (Roche). Amplification products were sequenced by Eurofins (MWG Operon, France). The sequence analysis was performed with the CodonCode Aligner software (CodonCode Corporation, USA).

2.2.4.8 Stability determination

The stability test was performed according to Sperry et al. (2008): the strains EGDe, F2365 and CLIP 80459 were tested 45 times. All DNA were tested for MLVA.

2.2.4.9 Reproducibility

The reproducibility of the MLVA method was determined from the results obtained from the two reference strains included in each run, and with the four TS strains represented in duplicate and the epidemiologically related strains included in this study. Moreover, amplification products were systematically run on two independent gels. At least two independent PCRs were performed from a given DNA extract from the reference strains.

3. Results

3.1 Serotyping data

The agglutination serotyping distribution was as follows: 27 serotype 1/2a strains, 10 serotype 1/2b strains, 5 serotype 1/2c strains, 25 serotype 4b strains, 1 strain of each serotype 3a, 3b, 3c, 4d and 1 autoagglutinable strain.

3.2 Subtyping data

3.2.1 Development of an MLVA assay

3.2.1.1 Selection of VNTR loci from the literature

A total of 16 VNTRs have been described in the literature (Table 1). Although some VNTRs are common to different MLVA schemes, their nomenclature is different. Moreover, the primer pairs used for the amplification of a given locus can differ among studies.

The number in brackets indicates the size of the tandem repeat motif in the VNTR locus; ¹ Excluded due to low diversity (Hyytia-Trees, 2010); ² Excluded due to sequence variability in flanking region (Hyytia-Trees, 2010); ³ Excluded due to short repeat unit length (3 bp) and low diversity (Hyytia-Trees, 2010); ⁴ Degenerate primers.

PulseNet USA (Hyttia-Trees, 2010)	Sperry et al. (2008)	Larsson et al. (2010)	Lindstedt et al. (2008)	Murphy et al. (2007)	Miya et al. (2008)
LM-2 (6 bp)	Lm-2	LMV1-JLR ⁴	LMV1		
LM-3 (9 bp)	Lm-3	LMV7-JLR ⁴	LMV7	LMTR-1	
LM-8 (15 bp)	Lm-8				
LM-10 (12 bp)	Lm-10			LM-TR-4	
LM-11 (12 bp)	Lm-11	LM11-LR			
LM-15 (12 bp)	Lm-15	JLR2 ⁴			
LM-23 (6 bp)	Lm-23	JLR1			TR2
LM-32 (6 bp)	Lm-32	JLR34			
LMV09 (9 bp)		LMV9-JLR ⁴	LMV9		
				LM-TR-2 ¹ (18 bp)	
				LM-TR-3 ² (9 bp)	TR1 ²
		LMV2-JLR (9 bp)	LMV2	LM-TR-5 ²	
				LM-TR-6 ¹ (12 bp)	TR3 ³ (3 bp)
		LMV6-JLR (15 bp)	LMV6		
		JLR4 (9 bp)			

Table 1. Comparison of 16 MLVA VNTR loci described in the literature and used for subtyping *L. monocytogenes*.

In this study, VNTR loci were selected according to the following criteria: (1) a repeat size greater than or equal to 9 bp (2) diversity and (3) no sequence variability in flanking regions. For this reason, four loci used by Murphy et al. (2007), LM-TR-2, LM-TR6, LM-TR-3 and LM-

TR-5, were excluded from this study because Hyytia-Trees (2010) demonstrated low diversity in LM-TR-2 and LM-TR6 and sequence variability in flanking regions in LM-TR-3 and LM-TR-5.

Eight loci, Lm-3 (=LmTR-1 for Murphy et al. (2007)), Lm-8, Lm-10 (=LmTR-4 for Murphy et al. (2007)), Lm-11, Lm-15, JLR4, LMV6-JLR and LMV9-JLR (Table 1) were thus selected. The primers used in the present are shown in Table 2.

Locus name	Primer names	References
Lm-10	Lm-10F-Lm-10R LM-TR-4F-LM-TR-4R	Sperry et al. (2008) Murphy et al. (2007)
Lm-11	Lm-11F-Lm-11R LM11-LR F-LM11-LR-R	Sperry et al. (2008) Larsson et al. (2010)
Lm-3	LMV7-F ; LMV7-R LM-TR-1-F ; LM-TR-1-R Lm-3 F; Lm-3 R LMV7-JLR F ; LMV7-JLR R	Lindstedt et al. (2008) Murphy et al. (2007) Sperry et al. (2008) Larsson et al. (2010)
Lm-8	Lm-8F ; Lm-8R	Sperry et al. (2008)
LMV6-JLR	LMV6-JLR LMV6-F ; LMV6-R	Larsson et al. (2010) Lindstedt et al. (2008)
LMV9-JLR	LMV9-JLR LMV9-F – LMV9-R	Larsson et al. (2010) Lindstedt et al. (2008)
Lm-15	Lm-15F-Lm-15R JLR2 F-JLR2R	Sperry et al. (2008) Larsson et al. (2010)
JLR-4	JLR4 F-JLR4R	Larsson et al. (2010)

Table 2. Primers used for amplification of the eight VNTR loci selected from the literature.

3.2.1.2 Selection of VNTR loci from a bioinformatics-based search

Following a search using TRF in MLVA databases, nine VNTR loci (LMCEB01,02,03,04,05,06,12,14, and Lm-26) were selected (Table 3).

The locus Lm-26 had already been published but has not been used previously due to its low diversity (Sperry et al., 2008). For each of the nine VNTR loci, primers were designed in the regions flanking the locus (Table 4).

3.2.1.3 Amplification and detection of the selected VNTR loci from the two strains EGDe and F2365

For Lm-3, Lm-10, Lm-11, Lm-15, LMV6-JLR and LMV9-JLR, the size of the amplification products obtained with all the primer pairs tested, observed in the same run and in two different runs differed from the true length by up to 18 bp (data not shown). For this reason, other primer pairs were designed and tested. Sizing discrepancies were nevertheless observed (data not shown).

VNTR		Repeat motif	Identification in EGDe		Identification in F2365		Locus tag and protein description	
locus name	Repeat	length (bp)	Location (nt)	Number of repeats	Location (nt)	Number of repeats	<u> </u>	
LMCEB01	TACAGGGTCA ACCGGATCAA CCGGATT	27	173484- 173534	1.8	178831- 178897	2.4	lmo0175: peptidoglycan binding protein	
LMCEB02	GGAGTTGCTG GATCTGTTGGT GTAGATGGTT CGTCAGGTGT T	42	345133- 345213	2.4	358155- 358297	3.4	lmo0320: similar to surface protein (peptidoglycan bound, LPXTG motif)	
LMCEB03	GATCCAGACC CAGTAAATCC AGATCCAACT	30	589559- 589651	2.2	596161- 596226	2.2	lmo0551	
LMCEB04	ACAGGACTTG ATCAAATAGA A	21	1251475- 1251565	2.7	1228575- 1228632	2.7	lmo1226	
LMCEB05	TAAAGTGACT AATACTTGTTC ATTT	25	1808069- 1808118	2.0	1787712- 1787762	2.0	lmo1738: similar to amino acid ABC transporter	
LMCEB06	TTCGAATTTCC ACCACCACCT ACGGATGAAG AGTTAAGACT TGCTTTGCCA GAGACACCAA TGCTTCTTGGT TTTAATGCTCC TGCTACATCA GAACCGAGCT CA	105	210255- 210498	2.3	215616- 215754	1.3	ActA: actin- assembly inducing protein precursor	
LMCEB12	CTTCTGGTGTT TCAGGAGTTT CTGGTA	27	695517- 695569	2.1	701866- 701977	4.2	lmo0652 and lmo0653	
LMCEB14	AGAACTTTCA AAATGTACTT TATTTTGATTT AGTTCTTCAAT ATAAATCTGA GCAAAGCGAT GATTTAATCCT TCCC	77	2779641- 2779814	2.3	2732284- 2732373	1.2	dnaX: highly similar to DNA polymerase III and lmo2705	
Lm-26 Sperry et al. (2008)	AATGTATTTTT ATTTAAA	18	2169160- 2169208	2.7	2157678- 2157744	3.7	argG: argininosuccinate synthase	

Table 3. Characteristics of the nine VNTR loci selected through a bioinformatics-based search.

Conversely, for Lm-8, Lm-26, JLR4, LMCEB 01,02,03,04,05,06,12,14, the size of the amplification products observed in the same run and in two different runs remained the same. Moreover, the sizes (Table 4) were identical to those predicted by genome sequence analysis. Sequencing of the amplification products demonstrated that the size differences

Primer	Locus name	Sequence (5'-3')	Amplicon F2365	location in	Amplicon location in EGDe	
name			Position	length (bp)	Position	Length (bp)
LMCEB01F	-LMCEB01	ATT AAA AGA AGC AAK GCT CC	178682	297	173343	279
LMCEB01R	LIVICEDUI	AAA YGC AAC TGG TAC TTT CA	178978		173621	
LMCEB02F	-LMCEB02	TTG ATT CTG GAT TTT CTG G	358114	193	345088	151
LMCEB02R	- LIVICED02	CCA CCA AAA AAC GAT CCA GAA	358306		345239	
LMCEB03F	-LMCEB03	GTA GAA CAG TAA ARG TAA CA	596015	295	589413	295
LMCEB03R	LIVICED05	CCW GAA GAT AAG CTA GAA AC	596290		589707	
LMCEB04F	LACEDOA	AAT CAA GGT ATY CAA CAA CT	1228488	287	1251367	287
LMCEB04R	-LMCEB04	GTT AAR CCA TCT GTT AAT TG	1228774		1251654	
LNCEB05F		TAT AAT GTC TGT TAR CAC TT	1787620	210	1807952	210
LNCEB05R	-LMCEB05	ATT TGG AAT GGW TAT ACT GT	1787829		1808162	
LMCEB06F		AGA AAA RTG AAG AGG TAA ATG	215594	243	210233	348
LMCEB06R	- LMCEB06	TAA TAG CAY TTC TCA AAC TA	215836		210581	
LMCEB12F	265	RAT TTT ATT TTG GTT CAT TGT	701838	320	695492	308
LMCEB12R	-LMCEB12	AAG GYA CTT TTA CAG AAG AA	702100	212	695694	
LMCEB14F		RTG CGA AGT TTT ATT TTG CA	2732111	316	2779467	393
LMCEB14R	-LMCEB14	GAT TTT TGR TTT TTG GTG GTG	2732425		2779620	
LMCEB13F		AAT GGA AGT AGA ATR ATC CC	2157557	251	2169040	233
LMCEB13R	- Lm-26	TTA TAT TAA CAC YGA TGCT T	2157807		2169273	

Table 4. Primers and characteristics of PCR amplification products in the reference strains for each of the nine VNTR loci selected through a bioinformatics-based search.

observed on the gel of strains EGDe and F2365 were solely related to the differences in repeat number, and not nucleotide variation in the flanking regions. For each locus, the repeat number was very similar to that indicated in the databases.

3.2.1.4 Screening of VNTR loci on the total strain panel

The 11 VNTR loci (Lm-8, Lm-26, JLR4, LMCEB01,02,03,04,05,06,12,14) were tested on the total test strain panel to evaluate the polymorphism of each VNTR locus. The loci LMCEB01, 03 and 04 exhibited no diversity (Table 5) and were therefore removed from the study. The eight remaining VNTR loci displayed between two and six alleles. Locus JLR4 had the highest diversity.

VNTR locus name	No. of	No. of repeats	
		Min	Max
LMCEB01	0	2	2
LMCEB02	3	-1	3
LMCEB03	0	2	2
LMCEB04	0	3	3
LMCEB05	2	-1	2
LMCEB06	2	1	2
LMCEB12	3	1	4
LMCEB14	4	-1	4
Lm-26	3	-1	4
Lm-8	2	3	4
JLR4	6	3	12

Table 5. Numbers of alleles and repeats found at each VNTR locus.

3.2.1.5 Comparison of data obtained with conventional electrophoresis and those obtained with CE

Two loci Lm-8 and JLR-4 were tested at Serun Statens Institute on the common panel of 40 strains using CE. Except for two strains, all showed the same repeat number. For Lm-8, one strain from SSI, 20092474, had a real repeat number of 2.7 in CE and 2.4 in agarose gel electrophoresis. For JLR4, one strain from SSI, 20082357, had a real repeat number of 3 in CE and 3.56 in agarose gel electrophoresis.

3.2.1.6 MLVA stability

The stability of each locus was evaluated to determine the effect of laboratory passage. The copy number was determined to be 100% reproducible (data not shown). Each of the eight loci tested on three reference strains were stable.

3.2.1.7 MLVA reproducibility

The MLVA types were indistinguishable for the four duplicate TS strains (TS32,TS72; TS56,TS77; TS35,TS75; TS63,TS73). The MLVA types were correlated with the epidemiological groups for the 17 tested TS strains. Two strains (TS 55 and TS 21) of the

The Use of Pulsed Field Gel Electrophoresis in *Listeria monocytogenes* Sub-Typing – Comparison with MLVA Method Coupled with Gel Electrophoresis

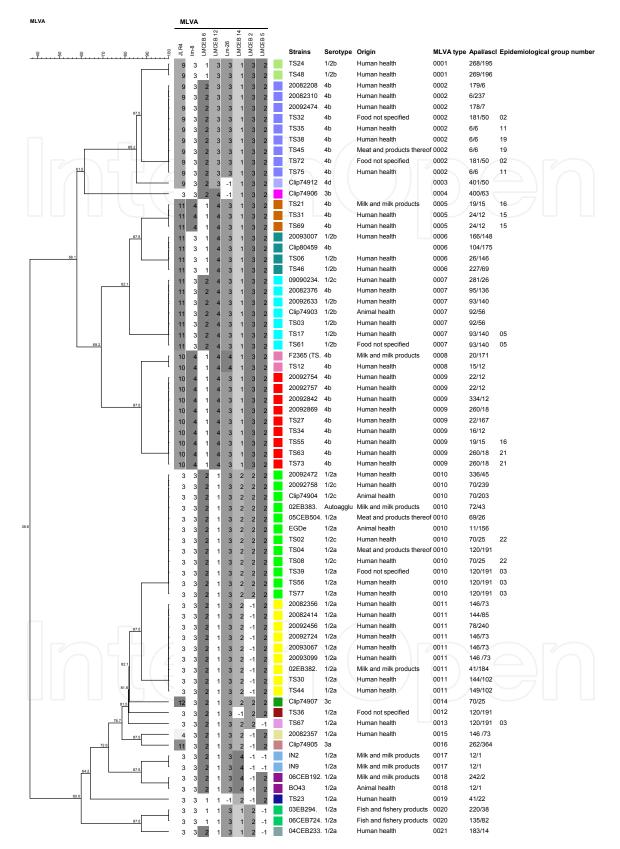


Fig. 1. Cluster analysis of 72 isolates based on MLVA type using the categorical coefficient and UPGMA. Number, origin, serotype, MLVA typing results and combined PFGE results. Each color indicates a distinct MLVA type.

www.intechopen.com

309

Gel Electrophoresis – Principles and Basics

New problem in the second problem in the	Apal/AsciPPGB	Strains	Serotype	Origin	Apal/Asci	MLVA type	Epidemiological group
1544 1544 154 <td>Asci-Apal</td> <td>TS30</td> <td>1/2a</td> <td>Human health</td> <td>144/102</td> <td>0011</td> <td></td>	Asci-Apal	TS30	1/2a	Human health	144/102	0011	
Image: space of the s	[]						
0 0.00000 0.00000 Nume health 14673 0011 0.000074 15.0 Nume health 14673 0011 0.000074 15.0 Nume health 14673 0011 0.000074 15.0 Nume health 14045 0011 0.000074 15.0 Nume health 105.4 0011 0.000074 15.0 Nume health 105.4 0011 0.0000750 15.0 Nume health 105.0 0011 0.0000750 15.0 Marane health 1018 0011 0.00007	et	20093067	1/2a	Human health	146/73	0011	
0 000227 10-0 Nume Neah 14/7 001 0 000217 10-0 Nume Neah 14/7 001 0 000217 10-0 Nume Neah 14/45 001 0 000218 10-0 Nume Neah 1001 22 0 0002170 10-0 Nume Neah 70.00 000 22 0 0002100 10-0 Nume Neah 70.00 000 22 0 0002100 10-0 Nume Neah 70.00 000 000 0 0002100 10-0 Nume Neah 70.00 000 000 0 0002100 10-0 Nume Neah 70.00 000 000 0 0002172 10-0 Nume Neah 10.19 000 00 0 0002172 10-0 Nume Neah 10.19 000 00 0 0002172 10-0 Nume Neah 10.19 000 00		20093099	1/2a	Human health	146 /73	0011	
2002.14 12.2 Haras hadin 14.466 001 152.2 16.2 Haras hadin 7025 010 22 152.3 16.2 Haras hadin 7025 010 22 000000000000000000000000000000000000	472 ata						
0 0							
1 1							
100 100 100 100 100 100 000000000000000000000000000000000000							22
0 0	P4						
Image: mage:	E2						
0 000000000000000000000000000000000000	79.4	00EB253LM	3c	unknown	70/25	0014	
Image number 2008 2008 2008 Image number 1008 1009 1009 Image number 1009 1009 100 Image number 1009 100 100 Image number 1000 100 100	12	02EB383LM	Autoagglu	Milk and milk products	72/43	0010	
Image in the sector Source in the sector Source in the sector Source in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector Image in the sector	#2 <u>105</u>	05CEB504LM	1/2a	Meat and products thereof	69/26	0010	
0 000 12.4 Almina headh 11156 000 1523 12.4 Human headh 41724 001 1523 12.4 Human headh 41724 001 1523 12.4 Human headh 41724 001 00E8251.LL 13.4 Human headh 1552 000 00E8251.LL 13.4 Human headh 1554 001 00E8251.LL 13.4 Human headh 10191 001 01 1556 12.4 Human headh 10191 001 01 15756 12.4 Human headh 10191 001 01 15757 12.4 Human headh 10191 001 01 15756 12.4 Human headh 121 007 01 15756 12.4 Human headh 12	#8.0 L						
02513621 1/2a Mila ard mk products 4/174 001 0005176 1/2a Human headh 72420 001 0005177 1/2a Human headh 72420 001 0005177 1/2a Human headh 72420 001 0005177 1/2a Human headh 3546 000 0005177 1/2a Human headh 3546 000 0 0005177 1/2a Human headh 100141 000 0 1595 1/2a Human headh 100141 000 0 1595 1/2a Human headh 100141 000 0 1595 1/2a Human headh 100141 001 0 1595 1/2a Malard milgenoduits 1011 007 0 0 1595 1/2a Malard milgenoduits 1011 007 0 0 0 1596 1/2a Malard milgenoduits 1011 0 0 0 0 1596 1/2a Malard milgenoduits 101 0							
1323 10,a Human headt 41/22 0019 0051251 10,a Human headt 20204 0011 0051251 10,a Human headt 20204 0011 0051251 10,a Human headt 3046 0010 0051251 10,a Human headt 15912 0010 0051251 10,a Human headt 1201191 0010 01512524LL 10,a Human headt 1201191 0010 01 17556 10,a Human headt 1201191 0010 01 17577 10,a Human headt 1201191 0010 01 17577 10,a Human headt 1201191 0010 01 17585 10,a Food nic specified 1201191 0010 01 17586 10,a Maran mic products 121 0019 01 10,4 10,3 Maran mic products 121 0019 01 10,4 10,3 10,3 Maran mic products 121 0019 10,4 10,3	542						
Image: space of the s	21.6						
0 00EB321 M 3a unitroin 202047 02a 00EB324 M 12a Hamas heads 39542 002 00EB324 M 12a Hamas heads 39542 003 00EB324 M 12a Hamas heads 29191 001 03 1537 12a Hamas heads 29191 001 03 1567 12a Ma arm mapdacks 211 001 03 1508 12a Ma arm mapdacks 2121 001 03 1504 12a Ma arm mapdacks 211 007 06 1511 12b Hamas heads 256 007 06 1511 12b </td <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>							
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 <td><u> </u></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	<u> </u>						
 							
0 0 154 10.2 Most and specialist Beredit 120191 0010 00 1557 10.2 Harman headh 120191 0010 00 1557 10.2 Harman headh 120191 0010 00 1557 10.2 Harman headh 120191 0010 00 000EEB102.LL 12.2 Food not specified 120191 0010 00 000EEB102.LL 12.3 Max and mk products 22/1 0019 001 000EEB102.LL 12.3 Max and mk products 1211 0017 001 000EEB102.LL 12.3 Max and mk products 121 0017 001 000EEB102.LL 12.3 Max and mk products 121 0017 001 001 000EB102.LL 12.3 Max and mk products 121 0007 05 1530 120 Harman headh 120140 0007 05 1530 120 Harman headh 1212 0007 05 1531 120 Harman headh 1212 0006 15							
p m		03EB294LM	1/2a	Fish and fishery products	220/38	0020	
p p TS77 10.2 Harras health 120191 0010 03 TS75 10.2 Harras health 120191 0010 03 TS38 10.2 Food nst specified 120191 0012 03 TS38 10.2 Food nst specified 120191 0012 03 P P NZ 12.3 Mik and mik products 2221 0011 D0413 12.3 Mik and mik products 121 0017 06 D0413 12.3 Mik and mik products 121 0017 06 TS36 12.0 Harras health 121 0017 06 TS36 12.0 Harras health 120140 0007 06 TS36 12.0 Harras health 120140 007 06 D05E252LM 30 12.0 Harras health 121 007 06 D05E252LM 12 Harras health 1212 0007 06 15 D05E252LM 12 Harras health 1212 006 15	ett]	TS04	1/2a	Meat and products thereof	120/191	0010	
$ \left(\begin{array}{cccccccccccccccccccccccccccccccccccc$	wa wa #						
r 1539 12a Food not specified 120191 0010 03 r 1538 12a Food not specified 120191 0012 12 r 1539 12a Food not specified 120191 0017 13 r 154 12a Max and mik products 1211 0017 13 r 1604 12a Max and mik products 1211 0017 13 r 1756 12b Haman headh 227148 0006 13 r 1564 12b Haman headh 20769 0007 16 r 1503 12b Haman headh 9740 007 6 r 1503 12b Haman headh 9276 0007 16 r 1503 12b Haman headh 9276 0007 15 r 151 12b Aman headh 9276 0007 15 r 152 4b Haman headh 212 0006 15 r 1521 4b	E	and the second second					
p Field nut products 120191 0012 00CEB192LM 12a Mik and mik products 121 0017 00CEB192LM 12a Mik and mik products 121 0017 00CEB192LM 12a Mik and mik products 121 0017 00CEB192LM 12a Amaid health 1211 0017 00CEB192LM 12a Amaid health 1211 0017 00CEB192LM 12b Human health 1281 0006 0006 17508 12b Human health 1281 0006 0007 12b Human health 198148 0007 05 17513 12b Human health 93140 0007 05 0006E221M 3b unknom 40963 0004 15 1751 12b Human health 1512 0006 15 1751 12b Anna health 2412 0006 15 1751 4b Human health 1512 006 15 1752 4b Human health 2412	<u> </u>	Seat Shere					
0 002E192LM 12a Mik and mik products 2422 0018 12a Mik and mik products 121 0017 0018 10 12a Mik and mik products 121 0017 10 12a Mik and mik products 121 0017 10 12a Mik and mik products 121 0018 10 12a Mik and mik products 121 0017 10 12b Human health 1211 0017 10 12b Human health 1214 0007 05 10 12b Human health 1214 0007 05 100 12b 12b Human health 1214 0077 05 100 12b 12b Human health 1212 0077 05 100 00E124LM 12b Human health 1212 006 15 100 00E124LM 12b Human health 1212 006 15		1.000					03
$ { \ \ \ \ \ \ \ \ \ \ \ \ \$		U(000%)					
$ { \ \ \ \ \ \ \ \ \ \ \ \ $	**						
n N9 1/2a M4 end mik groducts 121 0017 maran health 227146 006 12b Human health 227146 006 17546 1/2b Human health 227146 006 12b 12b 12b Human health 126148 006 17540 1/2b Human health 126148 0006 006 17531 1/2b Human health 92140 0007 06 17517 1/2b Human health 92140 0007 06 000EE9/2LM 12b Anran health 92140 0007 06 000EE9/2LM 12b Anran health 32412 009 15 17512 4b Human health 2212 006 15	60.6	and the second se					
n 1546 12b Human health 22769 0006 1500 Human health 166148 0007 06 1500 1750 Human health 62766 0007 05 1750 172b Human health 631400 0007 05 1751 172b Human health 631400 0007 05 1751 172b Human health 631400 0007 05 006E3521M 3b unknown 40083 0007 15 006E3521M 3b unknown 40083 0007 15 1751 1751 4b Human health 1512 0008 15 1751 1751 4b Human health 2412 0005 15 159 20092757 4b Human health 2412 0005 16 159 20092757 4b Mik and mik products 1915 0009 16 159 20092757 4b Mik and mik products 1915 0009 16 150 1555		A CONTRACTOR OF A CONTRACTOR OFTA CONTRACTOR O					
n TS46 172b Human health 2269 0006 1209 Human health 68/148 0007 05 17540 Human health 68/140 0007 05 17540 Human health 68/140 0007 05 17517 172b Human health 63/140 0007 05 17541 172b Human health 63/140 0007 05 17541 172b Human health 63/140 0007 05 00EE352LM 3b unknown 40063 0047 15 00EE352LM 3b unknown 33/12 0007 15 17512 4b Human health 24/12 0006 15 17512 4b Human health 24/12 0006 16 17512 4b Mak and mik products 1915 0006 16 17512 4b Mak and mik products 1915 0006 16 1753 4b Human health 29116 0006 21 1753 <t< td=""><td>auf</td><td>TS06</td><td>1/2b</td><td>Human health</td><td>227/146</td><td>0006</td><td></td></t<>	auf	TS06	1/2b	Human health	227/146	0006	
n TS03 12b Human health 92/56 0007 17317 172b Human health 92/140 0007 05 20002233 172b Human health 92/140 0007 05 00EB2521M 3b unknown 40083 0007 05 00EB2521M 3b unknown 40083 0007 05 20022842 4b Human health 32/12 0006 15 20022757 4b Human health 24/12 0005 15 TS12 4b Human health 21/12 0006 15 20002757 4b Human health 21/12 0006 15 20002757 4b Human health 21/12 0006 16 20002757 4b Human health 19/15 0006 16 1751 153 4b Human health 19/15 0006 16 1752 4b Human health 20/18 0009 21 1753 4b Human health 10/175 0006<	73	TS46	1/2b	Human health	227/69	0006	
n 1517 12b Human health 93140 0007 05 20092803 1/2b Human health 93140 0007 05 20092803 1/2b Human health 93140 0007 05 00EB3521M 3b urknown 40083 004 00EB241M 1/2b Animal health 9256 007 1531 7531 72b Human health 2412 0005 1531 7512 4b Human health 2412 0005 1531 7599 4b Human health 2412 0005 1599 4b Human health 2412 0005 15 20092757 4b Human health 2412 0065 16 1599 4b Human health 2412 0065 16 1599 4b Human health 2412 0065 16 1509 555 4b Human health 2915 0065 16 159 1535 20082767 4b Human health 20018		20093007	1/2b	Human health	166/148	0006	
n 							
$ \left(\begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\$							05
n 00EB252LM 3b unknown 40083 0004 00EB252LM 12b Anmah heath 9265 0077 20092842 4b Human heath 334/12 0009 2009275 4b Human heath 24/12 0005 15 15 15 15 0009 15 15 20092757 4b Human heath 24/12 0005 15 20092757 4b Human heath 22/12 0009 16 20092757 4b Human heath 19/15 0006 16 15 1555 4b Human heath 19/15 0009 21 15 15 15 15 009 21 15 15 15 15 1009 21 16 15 15 15 1009 21 16 15 15 15 1009 21 16 15 15 15 1009 21 16 15 15 15 1009 <td< td=""><td>114</td><td>the second second</td><td></td><td></td><td></td><td></td><td>05</td></td<>	114	the second second					05
pre 00EB249LM 12b Animal health 92/56 0007 20062842 4b Human health 33/12 0006 TS31 4b Human health 24/12 0006 TS9 4b Human health 21/12 0006 TS9 4b Human health 21/12 0006 TS90 4b Human health 21/12 0006 15 20092757 4b Human health 21/12 0006 15 20092757 4b Human health 21/12 0006 16 20092757 4b Human health 20/18 0006 16 20092757 4b Human health 20/18 0006 16 20092869 4b Human health 260/18 0009 21 17573 4b Human health 260/18 0009 21 17534 Human health 260/18 0009 21 17535 4b Human health 260/18 0002 11 1536 4b Human health </td <td>748</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>05</td>	748						05
n 20092842 4b Human health 33412 0009 1531 4b Human health 24/12 0006 15 1531 4b Human health 24/12 0005 15 1589 4b Human health 24/12 0005 15 20092754 4b Human health 22/12 0009 15 20092754 4b Mik and mik products 19/15 0008 16 1599 4b Mik and mik products 19/15 0005 16 1599 4b Human health 19/15 0006 16 1509 4b Human health 19/15 0009 16 1533 4b Human health 20/18 0009 21 1537 4b Human health 20/18 0009 21 1537 4b Human health 20/18 0009 21 1537 4b Human health 20/18 0009 21 1538 4b Human health 6/6 0002 11							
n n n Fisit 4b Human health 24/12 0005 15 15 TS12 4b Human health 15/12 0008 15 20082754 4b Human health 22/12 0009 15 20082757 4b Human health 22/12 0009 16 20082757 4b Mik and mik products 19/15 0006 16 1555 4b Human health 20/12 0099 16 20082767 4b Mik and mik products 19/15 0006 16 1555 4b Human health 20/18 0009 16 20082860 4b Human health 20/18 0009 21 1537 4b Human health 20/18 0009 21 1537 4b Human health 20/18 0009 11 1538 4b Human health 20/17 0006 11 1538 4b Human health 6/6 0002 11 1538 4b H							
$ \left(\begin{array}{cccccccccccccccccccccccccccccccccccc$	ng ng	TS31	4b	Human health	24/12	0005	15
$ \left(\begin{array}{c} 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 $		TS12	4b	Human health	15/12	0008	
$ \left(\begin{array}{cccccccccccccccccccccccccccccccccccc$		TS69	4b	Human health	24/12	0005	15
$ \left(\begin{array}{c} 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 $	82						
$ \left(\begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\$							
Image: state of the state				Contract of the second states and			
Image: constraint of the second se							
Image: Constraint of the second se							10
Image: state of the state							21
Image: state of the state		to restance of					
Image: state of the state							5501.
1 TS35 4b Human health 6/6 0002 11 1 TS38 4b Human health 6/6 0002 19 1 TS35 4b Meat and products thereof 6/6 0002 19 1 TS45 4b Meat and products thereof 6/6 0002 19 1 TS75 4b Human health 6/8 0002 11 00EB258LM 4d unknown 401/50 0002 11 00EB258LM 4d unknown 401/50 0002 11 20082208 4b Human health 178/7 0002 20082208 20082208 4b Human health 178/7 0002 2008237 0002 20082310 4b Human health 6/237 0002 02 2008237 0002 002 20082376 4b Human health 95/136 0007 15/4 17/2b Human health 268/195 0001 45 TS48 1/2b Human health 269/196 0001		TS27	4b	Human health	22/167	0009	
Image: set of the set of		Clip80459	4b	unknown	104/175	0006	
Image: Selection of the se	92	1.000 B (1.000 B)					
Image: State of the s							
1 00EB258LM 4d unknown 401/50 0003 20082208 4b Human health 179/6 0002 20082310 4b Human health 178/7 0002 20082310 4b Human health 178/7 0002 20082310 4b Human health 6/237 0002 20082376 4b Human health 95/136 0007 1524 1/2b Human health 268/195 0001 1538 1/2b Human health 269/196 0001	L_65.6	100 Carl 6 2011					
set in teal 20082208 4b Human health 179/6 0002 20082310 4b Human health 178/7 0002 20082310 4b Human health 178/7 0002 20082310 4b Human health 6/237 0002 20082376 4b Food not specified 181/50 0002 02 20082376 4b Human health 95/136 0007 1524 1/2b Human health 269/195 0001		Contraction of the second					11
1 20092474 4b Hurnan health 178/7 0002 1 20082310 4b Hurnan health 6/237 0002 1 1 7572 4b Food not specified 181/50 0002 02 20082376 4b Hurnan health 95/136 0007 1524 1/2b Hurnan health 288/195 0001 1 T524 1/2b Hurnan health 269/196 0001	2						
Mail 20082310 4b Human health 6/237 0002 TS72 4b Food not specified 181/50 0002 02 20082376 4b Human health 95/136 0007 TS24 1/2b Human health 268/195 0001 TS48 1/2b Human health 269/196 0001							
314 TS72 4b Food not specified 181/50 0002 02 20082376 4b Human health 95/136 0007 001 ms TS24 1/2b Human health 268/195 0001 TS48 1/2b Human health 269/196 0001	748						
20082376 4b Human health 95/136 0007 msi TS24 1/2b Human health 268/195 0001 TS48 1/2b Human health 269/196 0001							02
TS48 1/2b Human health 269/196 0001		20082376	4b		95/136	0007	
	#1	11111111111					
L TS32 4b Food not specified 181/50 0002 02							
	20 Z	TS32	4b	Food not specified	181/50	0002	02

Fig. 2. Cluster analysis of 72 isolates based on combined PFGE using the categorical coefficient and UPGMA. Number, origin, serotype, MLVA typing results and combined PFGE results. The colors (including also white color) indicate distinct combined PFGE types.

same epidemiological group 16 displayed two different MLVA types, 05 and 09. The difference was related to a repeat in the locus JLR-4. Strain TS 67 and three other strains of group 03 displayed two different MLVA types. No amplification was observed for the strain TS 67 at locus LMCEB05.

3.2.2 MLVA assay applied on the test panel of strains

Based on MLVA results, the 72 isolates were divided into 21 types (Figure 1). MLVA types were clustered into two groups. All the isolates of serotypes 1/2a, 3a, 1/2c, 3c were classified in one group, while all the isolates of serotypes 4b, 1/2b, 3b, 4d were in another group (Figure 1). Nineteen of the 21 types contained isolates of the same serotype (Figure 1). Type "10" contained isolates of two serotypes 1/2a and 1/2c and the autoagglutinable strain. Type "7" contained isolates of two serotypes 4b and 1/2b and one isolate of the 1/2c serotype (Figure 1).

3.2.3 PFGE data

For PFGE, the two-enzyme combination divided the isolates into 48 distinct profiles (Figure 2). All the isolates of serotype 1/2a, 3a, 1/2c, 3c were classified in one group, while all the isolates of serotypes 4b, 1/2b, 3b, 4d were in another group. Combined PFGE types contained isolates of the same serotype, except the type "70/25", which contained isolates of serotypes 1/2c and 3c.

3.2.4 MLVA data compared with PFGE data

Six different MLVA types were encountered for nine distinct epidemiological groups. A single *ApaI/AscI* PFGE type was observed for each epidemiological group (Figure 1).

Five MLVA types ("19", "3", "4", "21", "16") contained one unique *ApaI/AscI* PFGE type. The other MLVA types contained at least two different PFGE types. The five *ApaI/AscI* PFGE types "19/15, "120/191", "70/25", "12/1", "146/73" were divided among two MLVA types ("9","5"), three MLVA types ("10","12","13"), two MLVA types ("10","14"), two MLVA types ("17","18") and two MLVA types ("11","15"), respectively (Figure 1).

4. Discussion

The objective of this work was to evaluate the feasibility of an MLVA scheme coupled with conventional agarose gel electrophoresis for subtyping *L. monocytogenes*. This type of scheme would be very useful for *L. monocytogenes* surveillance, because it can be implemented by any molecular laboratory and does not require an expensive capillary electrophoresis system.

Out of the 16 VNTRs published, only eight Lm-3, Lm-8, Lm-10, Lm-11, Lm-15, JLR4, LMV6-JLR and LMV9-JLR were selected here because (1) their repeat length was greater than or equal to 9 bp as demonstrated on a large panel of human and food strains (Sperry et al., 2008; Larson et al. 2010; Lindstedt et al., 2008; Murphy et al., 2007). For six out of eight loci (Lm-3, Lm-10, Lm-11, Lm-15, LMV6-JLR and LMV9-JLR), the size of the amplification products observed on the agarose gels differed between the runs. This result was observed

for different primer sets, both previously published and newly designed. This result was surprising, particularly regarding loci Lm-3 and Lm-10, for which Murphy et al. (2007) observed accurate detection on agarose gels. In this study, agarose gel electrophoresis does not appear to be sufficiently accurate for determining repeat number for these six loci. In contrast, agarose gel electrophoresis was suitable for loci Lm-8 and JLR-4. The sizing discrepancies need to be normalized to develop a standardized agarose gel protocol uding all the VNTRs selected here.

For Lm-8, the amplification protocol used here was as similar to that described by Sperry et al. (2008). The repeat number obtained here for the 34 "TS" strains on agarose gel was exactly the same as that obtained on the same panel on a CE Beckman Coulter CEQ 8000 genetic analyzer (Sperry et al., 2008). For Lm-8 and JLR-4, of 39 strains from a panel of 40, the repeat number on agarose gels was exactly the same as that obtained on the ABI 3130 genetic analyzer (Applied Biosystems) at SSI (Larsson et al., 2010). For only one strain, a low difference (maximum 0.56) was observed in the number of base pairs. We demonstrated here that the change in equipment used for the detection of JLR4 and Lm-8 did not affect the determination of repeat number. These data confirm the reliability of these two loci.

However, locus Lm-8 revealed low levels of diversity (2 alleles) on the tested panel of human and food strains. This result corroborates those obtained by Sperry et al. (2008) who report only two alleles from a panel of 193 isolates. Locus JLR-4 showed the highest number of alleles. Locus Lm-26 also showed low diversity (3 alleles), as previously demonstrated by Sperry et al. (2008). This locus overlaps with locus LM-TR2, included in the scheme of Murphy et al. (2007). It had the lowest diversity index in comparison to the five other VNTR loci.

The five VNTR loci found here, LM 02, 05, 06, 12, 14, were identified from the sequenced genomes of three reference strains. They have never been described before. Our results demonstrate that these loci show reliable amplification.

With 71 of 72 strains, our MLVA scheme of eight loci (Lm 02, 05, 06, 12, 14, Lm-8, Lm-26 and JLR-4) confirmed the division of *L. monocytogenes* strains into two distinct genetic lineages. One strain of the 1/2c serotype showed an MLVA type common to strains of serotype 1/2b and 4b. This strain belonged to molecular serogroup IIc and has a combined PFGE profile specific to 1/2c and IIc strains. Other molecular methods are needed to further investigate the genetic profile of this strain.

Five VNTR loci, LM 02, 05, 06, 12, 14, exhibited low diversity on the total test strain panel. These data indicate that the MLVA scheme developed here was less discriminating than *ApaI/AscI* PFGE. However, the eight VNTR loci selected in this study have proved useful and can be included in a larger MLVA scheme coupled with CE, including VNTR loci with shorter repeat motifs and with higher polymorphism. The more polymorphic loci were excluded from this study, either because they are too short to be visible on agarose gels or because sizing discrepancies were observed on agarose gels. It is absolutely necessary to normalize these sizing discrepancies for accurate and standardized detection on agarose gels. Moreover, in the future, it is necessary to compare all the data obtained in different laboratories and to harmonize VNTR loci and allele naming for a standardized *L. monocytogenes* MLVA scheme.

312

5. Acknowledgements

This work was conducted as part of the activities of the European Union Reference Laboratory for *Listeria monocytogenes* and was supported by a grant from the Directorate-General for Heath and Consumers (DG Sanco) of the European Commission.

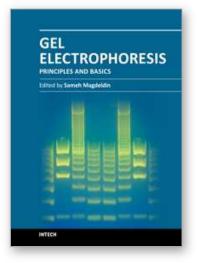
6. References

- Barrett, T.J., Gerner-Smidt, P. & Swaminathan, B. (2006). Interpretation of pulsed-field gel electrophoresis patterns in foodborne disease investigations and surveillance. *Foodborne Pathog Dis.* 3, 20-31
- Bille, J. & Rocourt, J. (1996). WHO international multicenter *Listeria monocytogenes* subtyping study- rationale and set-up of the study. *Int J Food Microbiol* 32(3), 251-62
- Graves, L.M. & Swaminathan, B. (2001). PulseNet standardized protocol for subtyping Listeria monocytogenes by macrorestriction and pulsed-field gel electrophoresis. Int J Food Microbiol 65(1-2), 55-62
- Hyytia-Trees, E. (2010). Genetic diversity of *Listeria monocytogenes* measured by multiplelocus VNTR analysis. *ISOPOL XVII* may 5-8th, 2010, Porto, Portugal
- Kerouanton, A.; Marault, M.; Petit, L.; Grout. J.; Dao, TTD. & Brisabois, A. (2010). Evaluation of a multiplex PCR assay as an alternative method for *listeria monocytogenes* serotyping. J Microbiol Methods 80(2), 134-7
- Larsson, JT.; Roussel, S. & Moller Nielsen, E. (2010). Better and faster typing, MLVA shall we play together?. *ISOPOL XVII* may5-8th, 2010, Porto, Portugal
- Lindstedt, BA.; Tham, W.; Danielsson-Tham, ML.; Varvund, T.; Helmersson, S. &Kapperud, G. (2008). Multiple-Locus Variable-Number Tandem-Repeats Analysis of *Listeria monocytogenes* Using Multicolour Capillary Electrophoresis And Comparison With Pulsed-Field Gel Electrophoresis Typing. J Microbiol Methods 72(2), 141-8
- Martin, P.; Jacquet, C.; Goulet, V.; Vaillant, V. & de Valk, H. (2006). Pulsed-Field Gel Electrophoresis of *Listeria monocytogenes* Strains: The PulseNet Europe Feasibility Study. *Foodborne Pathog Dis* 3(3), 303-8
- Miya, S.; Kimura, B.; Sato, M.; Takahashi. H.; Suda, T.; Takakura, C.; Fujii, T. &Wiedmann,
 M. (2008). Development of a Multilocus Variable-Number of Tandem Repeat
 Typing Method for *Listeria monocytogenes* serotype 4b strains. *Int J Food Microbiol* 124(3),239-249
- Murphy, M.; Corcoran, D.; Buckley, JF.; Mahony, M.; Whyte, P. & Fanning, S. (2007). Development and application of Multiple-Locus Variable Number of Tandem Repeat Analysis (MLVA) to subtype a collection of *Listeria monocytogenes*. Int J Food Microbiol 115(2), 187-94
- Schönberg, A.; Bannerman, E. & Courtieu, AL. (1996). Serotyping of 80 strains from the WHO mulitcenter international typing study of *Listeria monocytogenes*. Int J Food Microbiol; 32, 279-287
- Vergnaud, G. & Pourcel, C. (2006). Multiple-Locus VNTR (variable-number tandem-repeat) Analysis. Molecular Identification, Systematic, and Population Structure of Prokaryotes. e. stackebrandt (ed.). springer-verlag berlin heidelberg 2006. chap4, 83-104

Volpe-Sperry, KE.; Kathariou, S.; Edwards, J.S. &Wolf LA. (2008). Multiple-Locus Variable-Number Tandem-Repeat Analysis As A Tool For Subtyping *Listeria Monocytogenes* Strains. J Clin Microbiol 46(4),1435-1450



IntechOpen



Gel Electrophoresis - Principles and Basics Edited by Dr. Sameh Magdeldin

ISBN 978-953-51-0458-2 Hard cover, 346 pages Publisher InTech Published online 04, April, 2012 Published in print edition April, 2012

Most will agree that gel electrophoresis is one of the basic pillars of molecular biology. This coined terminology covers a myriad of gel-based separation approaches that rely mainly on fractionating biomolecules under electrophoretic current based mainly on the molecular weight. In this book, the authors try to present simplified fundamentals of gel-based separation together with exemplarily applications of this versatile technique. We try to keep the contents of the book crisp and comprehensive, and hope that it will receive overwhelming interest and deliver benefits and valuable information to the readers.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Sophie Roussel, Marie-Leone Vignaud, Jonass T Larsson, Benjamin Felix, Aurore Rossignol, Eva Moller Nielsen and Anne Brisabois (2012). The Use of Pulsed Field Gel Electrophoresis in Listeria monocytogenes Sub-Typing - Comparison with MLVA Method Coupled with Gel Electrophoresis, Gel Electrophoresis -Principles and Basics, Dr. Sameh Magdeldin (Ed.), ISBN: 978-953-51-0458-2, InTech, Available from: http://www.intechopen.com/books/gel-electrophoresis-principles-and-basics/feasibility-of-the-mlva-methodcoupled-with-agarose-gel-electrophoresis-for-subtyping-listeria-monoc

INTECH

open science | open minds

InTech Europe

University Campus STeP Ri Slavka Krautzeka 83/A 51000 Rijeka, Croatia Phone: +385 (51) 770 447 Fax: +385 (51) 686 166 www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai No.65, Yan An Road (West), Shanghai, 200040, China 中国上海市延安西路65号上海国际贵都大饭店办公楼405单元 Phone: +86-21-62489820 Fax: +86-21-62489821 © 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the <u>Creative Commons Attribution 3.0</u> <u>License</u>, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen