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Molecular Characterization of *Listeria monocytogenes* Based on the PFGE and RAPD in Korea

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ABSTRACT

This study was performed to characterize 35 *L. monocytogenes* isolates from animals, foods, environmental samples collected between 1997 and 2007 with no apparent epidemiological relations, and five reference isolates using serotypic, genotypic and molecular typing methods to understand the pattern of strain distribution in Korea. For this study, we used serotyping and detected 6 different virulence-associated genes (*inlA*, *inlB*, *plcA*, *plcB*, *hlyA*, and *actA*) and 16s rRNA using multiplex-PCR. We also compared RAPD and PFGE to determine genetic characterization of *L. monocytogenes* strains to define the genetic diversity. Serotype patterns of the 30 *L. monocytogenes* strains was as follows; 9 isolates (30.0 %) belonged to serotype, 7 isolates (23.3%) belonged to serotype 4b, 4 isolates (13.3%) belonged to serotype 1/2b, 3 isolates (10.0%) belonged to serotype 1/2c, 2 (6.7%) isolates belonged to 4c, 2 (6.7%) isolates belonged to NT (Non Type), one isolate (3.2%) belonged to 3a and 3b, and 4a, respectively. Although, a limited number of isolates were analyzed in this study, molecular typing with RAPD and PFGE indicated that PFGE is more discriminatory for the subtyping *L. monocytogenes* than RAPD. Some *L. monocytogenes* isolates by RAPD and PFGE types are associated with specific sources. And, combining data obtained by these methods will increases the likelihood of strain discrimination.

Keywords: *Listeria monocytogene*; PFGE; RAPD

1. Introduction

Listeriosis is a foodborne disease caused by *L. monocytogenes*, and a severe disease characterized by abortion, meningitis and septicemia with lethality around 20% - 30% of cases. *L. monocytogenes* is a ubiquitous and psychotropic micro-organism which can contaminate food at all steps of the food chain [1].

L. monocytogenes is a Gram-positive, non-spore forming, intracellular, facultative anaerobic rod, growing at refrigeration temperature that causes invasive, often fatal, disease in susceptible hosts [2].

Epidemiological investigations have revealed that *L. monocytogenes* is ubiquitously distributed throughout the environment and in foods [3]. Foodstuffs associated with listeriosis epidemics have included among other foods both milk and dairy products [4].

Phenotypic methods often yield a low power of discrimination in strains (e.g., serotyping), suffer from biological variability and may not be applicable to all strains [5]. The classical method of bacteriological identification for Listeria spp., including *L. monocytogenes*, is laborious and time consuming.

Recently, the use of multiple key virulence factors such as *hlyA* [6], internalin B (*inlB*) [7], *actA*, *plcA* [8], *plcB* [9], to detect *L. monocytogenes* have been described. Several molecular typing have been used to differentiate Listeria species that include RAPD (Random Amplification of Polymorphic DNA), AP-PCR (Arbitrarily Primed PCR), ERIC-PCR (Enterobacterial Repetitive Intergenic Consensus PCR), REP-PCR (Repetitive sequence-based PCR) and virulence gene sequencing have been used to characterize strains of *L. monocytogenes* [10-13]. PFGE (Pulsed Field Gel Electrophorosis) method is standardized, reliable and reproducible, and as such is useful when conducting comparative genetic analysis and for molecular subtyping of *L. monocytogenes* isolates [13].

The sources of infection in Korea are unknown to a

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great extent, and no food associated and environment cases have been reported. So, the purpose of the present study was to characterize epidemiologically unrelated environmental, animal and food *L. monocytogenes* isolates using serotypic, genotypic and molecular typing methods to understand the pattern of strain distribution in Korea.

2. Materials and Methods

2.1. Isolation of *L. monocytogenes*

A total of 35 *L. monocytogenes* isolates were used for experiment. 16 *L. monocytogenes* isolates were obtained from Dr. Byeong Yeal Jung (National Veterinary Research & Quarantine Service, Anyang). In particular, *L. monocytogenes* obtained from different animal species (porcine and bovine), environment (poultry slaughtering plant and dairy plant waste water) and meat (imported beef and pork) and from different foods (milk and cheese) from 1997 to 2007, were used. And five additional strains were purchased from the ATCC. Data are summarized in **Table 1**.

Twenty-five mL of buffer including the swabs were inoculated in 225 mL of Fraser Broth (Becton, Dickinson and company sparks, USA), and blended. All samples were incubated at 30˚C for 48 h. A portion (10 μL) of the enrichment broth was streaked on Palcam agar plate (Merk, Germany). After 24 - 48 h incubation at 37˚C the plates were examined for typical *L. monocytogenes* colonies, which were streaked for purity on horse blood agar plates. Hemolytic colonies on horse blood agar were confirmed as *L. monocytogenes* by API Listeria kit (Biomerieux, Korea).

2.2. Serotyping

Serotyping was performed using commercial Listeria antisera according to the instructions given by the manufacturer (DENKA SEIKEN CO., LTD., Tokyo, Japan). This method differentiates *L. monocytogenes* into 13 different serotypes based on the association of somatic (O) and flagellar (H) antigens with a series of polyvalent and monovalent antisera.

2.3. Preparation of Genomic DNA

Genomic DNA was extracted using the AccuPrep® Genomic DNA Extraction Kit (Bioneer, Korea) according to the manufacturer's instruction.

2.4. Identification by the Multiplex-PCR of Virulence-Associated Genes

All of the primers used for specific PCR amplifications of the entire coding sequences of virulence-associated genes are reported in **Table 2**. PCR was performed in a PCR 9600 thermal cycler (Perkin-Elmer Corporation). A 50-μL aliquot contained buffer (10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$ [pH 8.3]), the dNTP mixture (TaKaRa, Japan) 0.25 mM each, 10 pmols of primer, 25 ng of DNA, and 0.8 U of Taq DNA polymerase (TaKaRa, Japan). The cycling conditions were the template DNA was denatured at 94˚C for 3 min followed by 35 cycles of amplification (each cycle consisted of denaturation at 94˚C for 1 min, annealing at 60˚C for 2 min and elongation at 72˚C for 1 min). 5 microlitre of the amplified products was separated by electrophoresis in 1.5% agarose gel containing ethidium bromide, and visualized under UV.

2.5. RAPD Fingerprinting

For RAPD-PCR, the HLWL 74 (5'-ACG TAT CTG C-3') was used. The PCR mixture consisted of buffer (10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$ [pH 8.3]), the dNTP mixture (TaKaRa, Japan) 0.25 mM each, 10 pmols of primer, 25 ng of DNA, and 0.8 U of Taq DNA polymerase (TaKaRa, Japan) in a total volume of 25 μL. Each sample was subjected to an initial denaturation step of 95˚C for 4 min, followed by 45 amplification cycles of 1 min of 95˚C, 2 min at 35˚C, and 2 min at 72˚C 1 min and followed by final extension of 72˚C for 10 min. All PCR amplifications were carried out in a TGRADIENT (Biometra, Germany) [14].

2.6. PFGE

PFGE was performed according to the PulseNet standardized protocol, with ApaI as restriction endonuclease (Roche, Germany). Bacteria were grown on BHI agar plates at 37˚C for 16 - 18 h. Cell were removed from the plate to plastic tubes (Falcon 2057, 14 mL - 17 × 100 mm) containing 3 mL of TE buffer using a sterile cotton swab and the cell density adjusted. The electrophoretic parameters used were as follows; initial switch time, 4.0 s; final switch time, 40.0 s; sum time. 22 h; angle, 120˚; gradient, 6.0 V/cm; temperature, 14˚C; ramping factor, linear. After electrophoresis, the gels were stained for 15 - 20 min in 250 mL of deionized water containing 25 μL of ethidium bromide (10 mg/mL) and destained by three washes of 20 - 30 min each using 500 mL of deionized water [15].

2.7. Discrimination Index

The discriminatory power of the subtyping methods employed in this study was determined by calculating the discrimination index (DI) using the formula of Hunter and Gaston [16].

3. Results

The serotype distribution of the 30 *L. monocytogenes*

Table 1. List of the strains used in this study.

Strain	Isolate number	Source	Isolated from	Serotype
Listeria monbocytogenes	1	animal	porcine	2c
	3	animal	porcine	2a
	4	animal	bovine	2b
	10	animal	bovine	2c
	13	animal	porcine	2b
	19	animal	bovine	3b
	31	animal	bovine	2c
	32	animal	bovine	2b
	37	animal	bovine	3a
	43	meat	imported beef	NT
	44	meat	imported beef	2a
	45	meat	imported beef	4b
	46	meat	imported beef	NT
	47	environment	poultry slaughtering plant	2a
	48	environment	poultry slaughtering plant	2b
	49	environment	poultry slaughtering plant	2a
	50	environment	poultry slaughtering plant	2a
	60	milk	raw milk	2a
	61	milk	raw milk	2a
	62	milk	raw milk	4b
	63	milk	raw milk	2a
	67	environment	dairy plant waste water	4c
	68	environment	dairy plant waste water	4a
	69	environment	dairy plant waste water	4c
	70	environment	dairy plant waste water	2a
	80	meat	pork	4b
	81	meat	pork	4b
	82	meat	pork	4b
	83	meat	pork	4b
	84	meat	pork	7
1) ATCC 13932	s1		spinal fluid	4b
ATCC 51773	s2		cheese	2a
ATCC 51780	s3		cheese	2b
ATCC 51779	s4		cheese	2c
ATCC 15313	s5		quinea pig	NT

NT, non-typeable. 1) ATCC, American Type Culture Collection.

Table 2. Primer pairs used for amplification of virulence genes and 16s rRNA in *Listeria* isolates.

Primers	Sequences (5'→3')	Product size (bp)
inlA	F CCT AGC AGG TCT AAC CGC AC	255
	R TCG CTA ATT TGG TTA TGC CC	
inlB	F AAA GCA CGA TTT CAT GGG AG	146
	R ACA TAG CCT TGT TTG GTC GG	
actA	F GAC GAA AAT CCC GAA GTG AA	268
	R CTA GCG AAG GTG CTG TTT CC	
hlyA	F GCA TCT GCA TTC AAT AAA GA	174
	R TGT CAC TGC ATC TCC GTG GT	
plcA	F CGA GCA AAA CAG CAA CGA TA	129
	R CCG CGG ACA TCT TTT AAT GT	
plcB	F GGG AAA TTT GAC ACA GCG TT	261
	R ATT TTC GGG TAG TCC GCT TT	
16S rRNA	F CAG CAG CCG CGG TAA TAC	938
	R CTC CAT AAA GGT GAC CCT	

strains was as follows; 9 isolates (30.0 %) belonged to serotype 1/2a, 7 isolates (23.3%) belonged to serotype 4b, 4 isolates (13.3%) belonged to serotype 1/2b, 3 isolates (10.0%) belonged to serotype 1/2c, 2 (6.7%) isolates belonged to 4c, 2 (6.7%) isolates belonged to NT (Non typeable), one isolate (3.2%) belonged to 3a and 3b, and 4a, respectively (**Table 1**).

PCR products of the 6 different virulence-associated genes and 16s rRNA (**Table 2**) were obtained DNA from all Listeria strains considered in this study, except for strain No.37, 62, 80, 82, 83, 84, and s3 which was negative for the *hlyA* gene. All of the amplification products were of the expected size, except for the *actA* gene PCR products from 10 strains of serotypes 1/2b, 4b and 7 which had molecular weights lower than expected. Twenty-one isolates (70%) showed the expected *actA* product size of 268 bp, while nine isolates (30%) showed a higher product size of 385 bp. Most of the reference strains (s1, 2, 4, and s5) showed 385 bp, while s3 had 268 bp product. In this study, the isolates each had 16S rRNA and six virulence-associated genes (*hlyA*, *plcA*, *plcB*, *inlA*, *inlB*, *and actA*), suggesting that they are potentially pathogenic (**Table 3**).

RAPD (HLWL 74) types of all isolated strains are presented in **Figutr 1** and **Table 4**. It demonstrates examples of all RAPD types indicated in our studies. Among the 35 strains of *L. monocytogenes* examined (30 isolates and 5 reference/type strains) it was possible to identify 19 different banding types at a relative genetic similarity of 80%. RAPD (HLWL74) of genomic DNA from *L. monocytogenes* isolates generated multiple DNA fragments in sizes ranging between 100 and 2000 bp. Among them, five types (D, O, P, Q, and S) had two more isolates and D and S types had same cluster having more than two different strains. RAPD (HLWL 74) type S was the dominant type (10 strains) and was isolated all animal (porcine and bovine) isolates, some imported beef (No.43), and cheese isolates (s3 and s4). The most predominant type was S1. Interestingly, an identical RAPD profile (S1) was observed in all porcine samples. Three types found in bovine belonged to E1, K1, and S1. Especially, profile S1 was detected in isolates from the bovine (66.7%, 4/6). Four types had shown in imported beef and poultry slaughtering plant, respectively. Bovine and raw milk had three types. S1 cluster represented 66.7% (n = 4) in bovine and D cluster accounted for 50% (n = 2) in raw milk. Dairy plant waste water had Q1 (75%, n = 3) and R1 (25%, n = 1) and pork isolates was identified in O1 (40%, n = 2) and P1 (60%, n = 3). Five reference strains belonged to four types (K1, M1, N1 and S1), and profile S1 represented of two reference strains (s3 and s4).

PFGE (ApaI) types of all isolated strains are presented in **Figure 2** and **Table 5**. It demonstrates examples of all PFGE types indicated in our studies. Among the 35 strains of examined (30 isolates and 5 reference/type strains), dendrogram analyses of the PFGE profiles showed that the 35 *L. monocytogenes* isolates from 21 different PFGE profiles with discriminatory indexes 0.937 at 80% relative genetic similarity. The most predominant type was R. Interestingly, an identical PFGE profile (R3) was observed in all porcine samples. Also profile R3 was detected in isolates from the bovine (33.3%, n = 2). Dairy plant waste water and pork were clustered in (B1, F, and G1) and (I1 and L1), respectively. Five reference strains belonged to four types (E1, H1, and R2), and profile S represented of two reference strains (s1 and s4).

Among the 30 *L. monocytogenes* and 5 reference strains examined, results calculated for RAPD (HLWL74) and PFGE (ApaI) typing at 70%, 80%, 90%, and 95% relative genetic similarity were shown in **Table 6**.

4. Discussion

Studies have implicated contaminated foods such as cheese, milk and beef in the transmission of *L. monocytogenes* to human [3,5,17]. Similarly, animals can be infected through the consumption of contaminated feeds and environment also can be contaminated with *L. monocytogenes*. It is also important to investigate relation of contaminated foods and source of *L. monocytogenes* (e.g. animals, raw materials, and environment source). Thus, we used serotyping and PCR (6 different virulence-associated genes and 16s rRNA). We also compared RAPD and PFGE analyses to determine ge-

Table 3. Genotypic characterization of virulence genes in *L. monocytogenes* isolates.

Isolate number	PCR (bp)						
	16S rRNA	*hlyA*	*plcA*	*plcB*	*inlA*	*inlB*	*actA*
1	938	174	129	260	255	150	385
3	938	174	129	260	255	150	385
4	938	174	129	260	255	150	385
10	938	174	129	260	255	150	385
13	938	174	129	260	255	150	385
19	938	174	129	260	255	150	385
31	938	174	129	260	255	150	385
32	938	174	129	260	255	150	385
37	938	174	129	260	255	150	385
43	938	174	129	260	255	150	385
44	938	174	129	260	255	150	268
45	938	174	129	260	255	150	268
46	938	174	129	260	255	150	385
47	938	174	129	260	255	150	268
48	938	174	129	260	255	150	385
49	938	174	129	260	255	150	268
50	938	174	129	260	255	150	385
60	938	174	129	260	255	150	385
61	938	174	129	260	255	150	268
62	938	174	129	260	255	150	385
63	938	174	129	260	255	150	385
67	938	174	129	260	255	150	385
68	938	174	129	260	255	150	385
69	938	174	129	260	255	150	385
70	938	174	129	260	255	150	268
80	938	174	129	260	255	150	385
81	938	174	129	260	255	150	268
82	938	174	129	260	255	150	385
83	938	174	129	260	255	150	268
84	938	174	129	260	255	150	268
s1	938	174	129	260	255	150	385
s2	938	174	129	260	255	150	385
s3	938	174	129	260	255	150	268
s4	938	174	129	260	255	150	385
s5	938	174	129	260	255	150	385

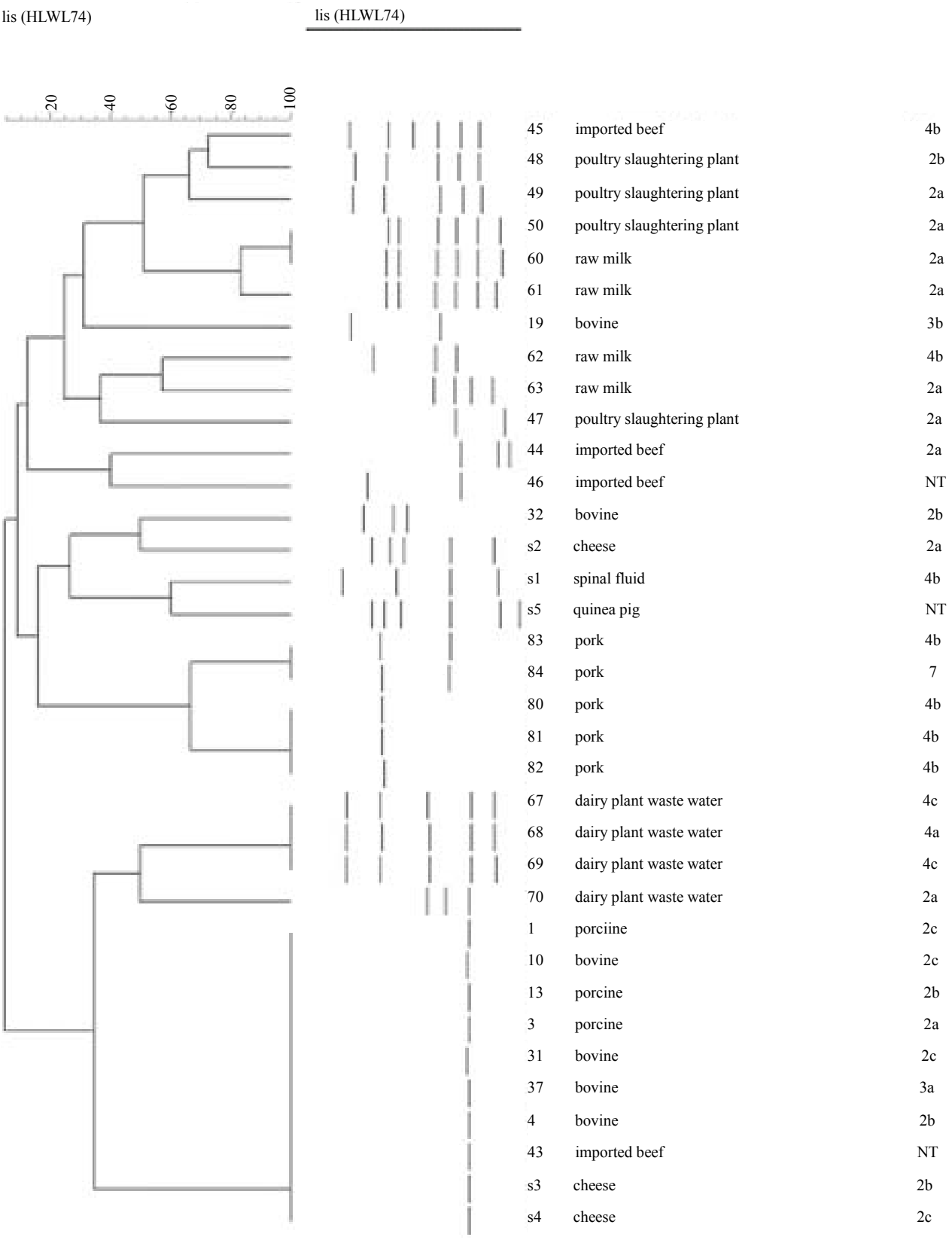

Figure 1. Dendrogram for 30 *L. monocytogenes* isolates and *L. monocytogenes* ATCC analyzed by RAPD (HLWL74). Percentages of similarity are shown above the dendrogram. Perpendicular line was stand for 80% relative genetic similarity. The origins of strains are shown in Table 1; 2a:1/2a, 2b:1/2b, 2c:1/2c.

Table 4. Distribution of RAPD types determined with HLWL74 primer in *L. monocytogenes* isolates from different sources.

Source	No. of isolates with following RAPD type																			
	A1	B1	C1	D1	D2	E1	F1	G1	H1	I1	J1	K1	L1	M1	N1	O1	P1	Q1	R1	S1
porcine																				3
bovine						1						1								4
imported beef	1									1	1									1
poultry slaughtering plant		1	1	1					1											
raw milk				1	1		1	1												
dairy plant waste water																		3	1	
pork																2	3			
ref. strains													1	1	1					2
Total	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	2	3	3	1	10

Similiarity: 80%, DI = 0.896.

netic characterization of *L. monocytogenes* strains isolated from animals, raw materials, and environment to define the genetic diversity. Most of the pathogenic *L. monocytogenes* serotype is limited to 1/2a, 1/2b, and 4b. Especially, at least 95% of *L. monocytogenes* strains isolated from human listeriosis cases are of those three serotypes [18]. Out of 30 isolates, serovar 1/2a was predominant with 9 strains followed by serovars 4b and 1/2b with 7 and 4 strains, respectively. **Figure 1** shows that serovars 1/2a, 1/2b, and 4b were predominant with 20 strains (66.7%) of 30 strains, in agreement with a recent report in Korea [19], showing that 90% of *L. monocytogenes* isolates were serotype 1 and only 4.1% were type 4. It was reported that these serotypes were widely distributed in food, animal, and environment of Korea. Chung HC *et al.* indicated that some serotype 1/2 isolates which showed that the H-antigenic factors could not be fully determined by traditional antiserum serotyping [20]. Because most human infections are reported to be associated with 1/2a, 1/2b, and 4b, our results were suggested that three major serotypes may be particularly important. Therefore, most of the organism isolated in this study that could lead to human infections and were then possibly pathogenic for human. 1/2c was shown in only animal. This finding may reflect a greater capacity of 1/2c strains to survive and multiply in the skin of animals. Also, it is interesting to note that serovar 4b, which causes 2/3 of the human infections, was not detected in animal. It may be assumed that serovar 4b does not play an important role in animal contamination. Out of 47 strains of L. *monocytogenes*, 16 belonged to serovar 1/2a, 30 to serovar 1/2c and only one to serovar 4b [21]. This study was similar to our results that most of the serovar was 1/2a, 1/2b, and 4b. Chung HC *et al.* reported that 54.4% (31/57) of human isolates belonged to serotype 4b, and 31.6% (18/57) belonged to serotypes 1/2a and 1/2b. These three serotypes were predominant among the *L. monocytogenes* isolated from human listeriosis cases and other types of isolates (food, environment, and animal) [19]. Many countries have seen a shift in the *L. monocytogenes* serotypes causing human infections from predominantly serotype 4b to 1/2a and 1/2b [22].

In general, PCR products of the virulence genes did not show polymorphism except for the *actA* gene [23]. Nine (30%) of these isolates showed the expected 268 bp *actA* gene products, whereas twenty one (70%) produced the 385 bp product. Among them, all isolates (100%) of animals (porcine and bovine) showed 385 bp products. Polymorphism of the *actA* gene for *L. monocytogenes* demonstrated by Wiedmann M *et al.* and can divided into two groups based on the *actA* gene sequence [24]. Many reports has been identified polymorphism of *L. monocytogenes* genes such as for *hlyA* [25], *iap* (murein hydrolase) [26] and *inlA* and *inlB* (internalin) [27]. However, in our study, we did not identify any polymorphism in the PCR products of these genes and other genes excepting *actA*. It might be that the primers used here have the target sequence located outside the areas of polymorphism, or the target regions may have been too small to detect any variations [23]. Jaradat ZW *et al.* also insisted that sequence analysis of these genes can possibly reveal a better picture of the relatedness of the isolates. Jacquet C *et al.* found that low molecular mass of ActA was due to a deletion in *actA*. As this deletion is detected in human strains, (*i.e.*, pathogenic strains), it is probably not in the amino-terminal region, which is essential for F-actin assembly and movement [28]. In vitro motility assays showed that this deletion decreases the motility of the bacteria [29]. In mouse model, a mutant with a mutation in the proline-rich region was less virulent than the

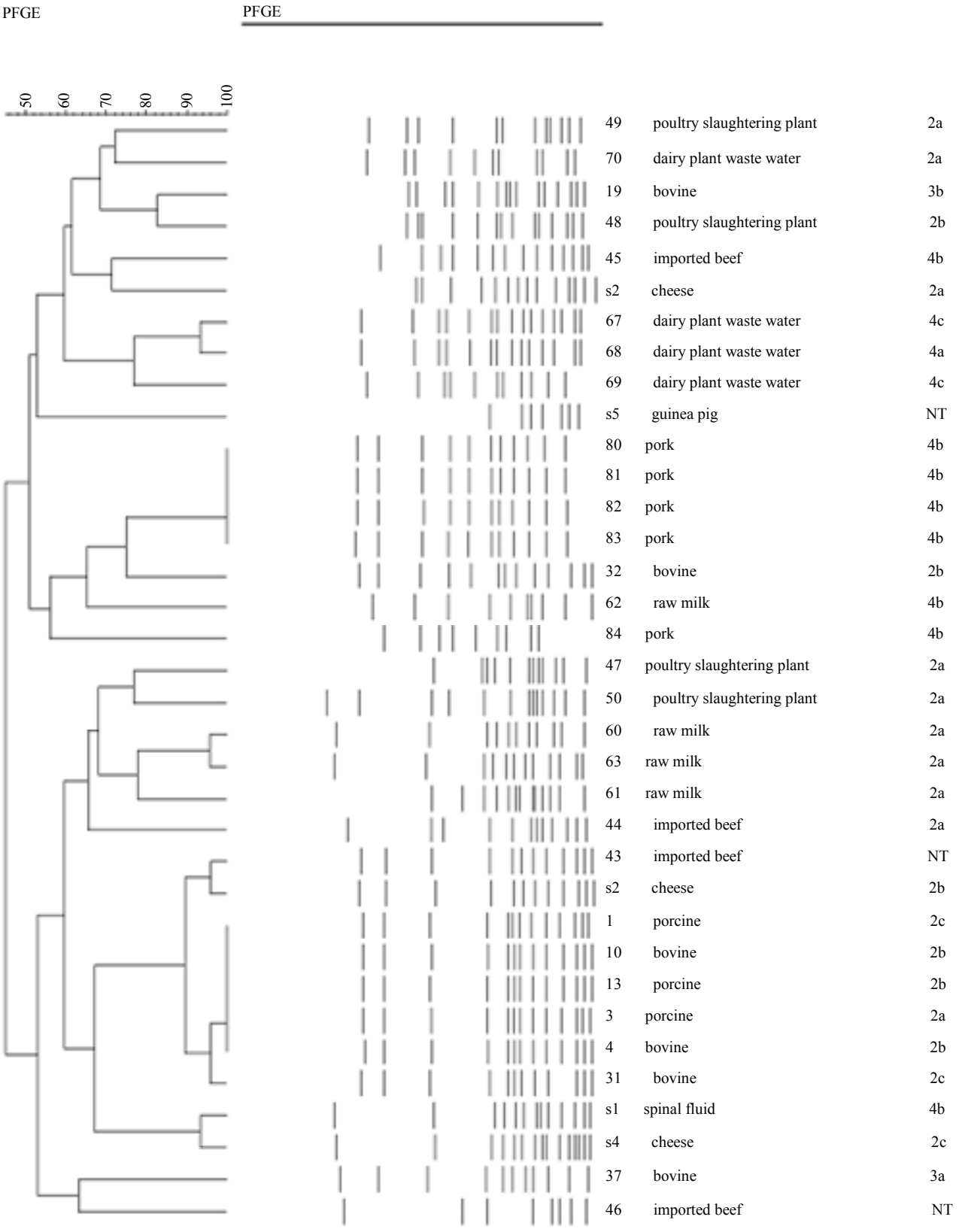

Figure 2. Dendrogram for 30 *L. monocytogenes* isolates and *L. monocytogenes* ATCC analyzed by PFGE (ApaI). Percentages of similarity are shown above the dendrogram. Perpendicular line was stand for 80% relative genetic similarity. The origins of strains are shown in Table 1.; 2a:1/2a, 2b:1/2b, 2c:1/2c.

Table 5. Distribution of PFGE types determined with *ApaI* in *L. monocytogenes* isolates from different sources.

Source	No. of isolates with following PFGE type																												
	A1	B1	C1	C2	D1	E1	F1	F2	G1	H1	I1	J1	K1	L1	M1	N1	O1	O2	P1	Q1	R1	R2	R3	R4	S1	S2	T1	U1	
porcine																							3						
bovine			1									1											2	1			1		
imported beef					1															1	1							1	
poultry slaughtering plant	1			1											1	1													
raw milk													1				1	1	1										
dairy plant waste water		1					1	1	1																				
pork											4			1															
ref. strains						1				1												1			1	1			
Total	1	1	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1	

Similiarity : 80%, DI = 0.937.

Table 6. Comparison of discriminative ability among RAPD (HLWL74) and PFGE (ApaI) methods used to subtyping of *L. monocytogenes*.

Methods	Relative genetic similarity (%)			
	70	80	90	95
RAPD (HLWL74)	0.893	0.896	0.900	0.900
PFGE (ApaI)	0.918	0.937	0.938	0.962

wild type [30]. Further studies are required to understand virulence among isolates compared to different molecular size of *actA* gene.

RAPD typing has been shown to be of value in resolving some specific epidemiological questions [31]. Some authors have been comparing different typing methods PFGE and RAPD showing the highest discriminatory efficiency on a set of *L. monocytogenes* [32,33].

In this study, we have identified 19 different banding types at a relative genetic similarity of 80%. 19 patterns were found after RAPD typing *L. monocytogenes* using primer HLWL74, of which Q type accounted for 75.0% (3/4) of the isolates from dairy plant waste water. Of the animal isolates, 7 (77.8%) belonged to RAPD type S. Pork samples were found to type O (2/5, 40%) and P type (3/5, 60%). Recently, Luca C. *et al.* published a study on the characterization of *L. monocytogenes* isolated from various sources (food, environment, and animals), and investigate their capability to define the strain origin [11]. Mazurier SI *et al.* found that identical patterns were found with the primer HLWL74 of *L. monocytogenes* and suggested that RAPD offers an attractive alternative to phage typing [34]. Niederhauser C *et al.* used a 19-mer primer to subtype 57 *L. monocytogenes* isolates and reported that the method allowed the tracing of *L. monocytogenes* contamination in several food outlets to be traced back to a food processing plant. They found RAPD to be highly discriminating for subtyping [35].

PFGE has been considered as a standard subtyping method for *L. monocytogenes* [36]. And PFGE is a method with high discriminatory power and it has shown to be very accurate and reproducible for fine structure comparison and molecular typing of *L. monocytogenes* [2, 37]. Types of PFGE did not show any significant match to bacterial serotypes. It is generally considered laborious and time-consuming and thus also too expensive for preliminary screening of hundreds of isolates as required in extensive hygiene surveys for tracing contamination sources [38]. Our data indicate that the PFGE molecular subtypes of the 35 *L. monocytogenes* strains including ATCC reference strains are presented in a dendrogram that utilized the PFGE data following ApaI digestion. Results indicate that PFGE (ApaI) digestions provided high levels of discrimination between isolates from animal, meat, milk, and environment samples with discriminatory indexes 0.937 at 80% relative genetic similarity. The two enzymes have also been recommended and used in subtype differentiating and epidemiologic studies for *L. monocytogenes* [39]. Our result showed that the 35 *L. monocytogenes* isolates from various different PFGE profiles (21 pulsotypes) with discriminatory indexes 0.937 at 80% relative genetic similarity. We have identical pattern from same source (F type: dairy plant waste water, I type: pork, O type: raw milk, R type: animal isolates). We noted that animal isolates (R type) unrelated imported beef (No. 43) shared identical patterns, suggesting that they are related. This result was consistent with RAPD. In many cases, the genetic patterns of strains showed no association with any of the

properties that correlated with their origin [40]. It was also demonstrated that similar subtypes of strains can be found in different product types and in different processing environments and that, therefore, the recovery of identical patterns form various food and patient strains does not prove that a particular food is the vehicle of infection [41]. It was reported a similar finding with an identical PFGE pattern that was shared by different serotypes, even from different flagella antigen groups [42]. It was indicated that the poor correlation between serotyping and molecular subtyping may be due to horizontal gene transfers or point mutations in genomic DNA resulting in phenotypic shifts that affect serotyping [43]. In the results of the present study, specific PFGE types could not be connected with serotype, especially most animal isolates could differentiated with Q type showing serotypes 1/2a, 1/2b, and 1/2c which is in agreement with the results of other studies having examined the relation of *L. monocytogenes* between subtypes and genotypes. For example, a study was carried in pork slaughtering and cutting plants inspected 287 isolates that produced 17 ApaI PFGE types and four serotypes (serotypes 1/2a, 3a, 1/2c, and 3c) [44]. This study showed that there was overrepresentation of one *L. monocytogenes* PFGE type that accounted for 90% of the isolates (1/2a). A report that investigated the prevalence of *L. monocytogenes* in frankfurter packages from seven plants showed that 90% had the same ribotype profile and serotype [45]. Other investigators have also shown that PFGE and serotyping showed that *L. monocytogenes* is heterogeneous serologically and genetically as observed herein [13]. Correlations between molecular subtyping and serotyping of *L. monocytogenes* have been reported previously [43]. There is currently no thorough knowledge of the molecular basis for the relationship between serotypes and molecular subtyping of *L. monocytogenes* [13]. But, genomic DNA isolates of serotype 4b digested by ApaI enzyme in our study showed relatively distinguishable patterns not including No.45 sample and reference strain s1. It was indicated that identical PFGE patterns belonged to the same serotype [20,41]. The use of both RAPD and PFGE for typing *L. monocytogenes* has previously been reported to identify similar group and isolates from different sources [11,44]. However, Destro MT *et al.* insisted that using more than one method may increase the discriminatory ability [46].

Our results showed that DI values varied at different relative genetic similarity (70% - 95%). The most discriminate DI value was 0.900 at 95% relative genetic similarity. It was proposed that the higher the DI, the more discriminatory is the procedure [47]. But, it was insisted that DI values must be regarded with caution for small samples and typing schemes should not be validated with limited sample sizes [16]. So, we choose 80% relative genetic similarity for differentiating *L. monocytogenes* isolated various sources. Most of the serotype showed not consistent with RAPD type but 4b serotype had same RAPD type (except s1 and No.45). And these results were same as PFGE.

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