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Persistence of *Listeria monocytogenes* strains in a frozen vegetables processing plant determined by serotyping and REP-PCR

Lorena Ballesteros[1], Yolanda Moreno[1], Gonzalo Cuesta[1], Alejandro Rodrigo[2], David Tomás[2], M. Hernández[1], Mª Antonia Ferrús[1]

[1] Departamento de Biotecnología, Universidad Politécnica de Valencia 46022 Valencia, Spain

[2]AINIA Centro Tecnológico, Valencia, Spain.

Summary. Seventy presumptive *Listeria monocytogenes* strains isolated from frozen vegetable products in a processing plant during a period of six months were characterized by serology, biochemical tests (API system), multiplex PCR, virulence characteristics and REP-PCR analysis. Most isolates belonged to 1/2a (62 strains) and 1/2b (3 strains) serotypes, the most common isolated from foods. Amplification of virulence gene *lmo*2821 was positive in 51 of the PCR-confirmed *L. monocytogenes* strains. Automated REP-PCR of *L. monocytogenes* isolates yielded 17 different patterns, formed by 70 to 80 different bands ranging from 150 to 7000 bp. A total of three genetic groups were defined at 82% homology degree. At this level, serovars 4b and 1/2b strains were discriminated from the rest of strains. Two REP-PCR patterns were frequently found for isolates sharing the same serotype and biochemical profile. These isolates were obtained from different vegetables during the sampling period, what clearly suggests the persistence of some *L. monocytogenes* strains in the processing plant. While the multiplex PCR assay applied in this work provided an accurate and rapid method for species identification, REP-PCR was confirmed as a rapid and reliable method for *L. monocytogenes* subtyping, providing useful information for epidemiological or risk assessment studies, as well as for tracking surveys in food processing plants.

Key words: *Listeria monocytogenes*, REP-PCR, multiplex PCR, virulence, serology.

Introduction

Listeria monocytogenes is the causal agent of one of the most important foodborne diseases worldwide. Pregnant women, neonates, newborns and inmunocompromized persons are especially susceptible to the infection, with a case-fatally rate until 75% [24]. Listeriosis is one of the most frequent causes of death due to food-borne illness, with rates ranging from 13 to 30% [4]. It is also responsible for the highest hospitalisation rates (around 90%) amongst known food-borne pathogens [10]. Infection can cause sporadic cases, as well as great outbreaks of illness [23], and it has been associated to ingestion of a great variety of fresh raw products as meat, milk or soft cheese [24], fish and mussels [6] and vegetables [17].

Listeria monocytogenes is widely distributed in the natural environment [6]. Being tolerant to adverse conditions such as extreme pH, high temperature or nutrient starvation [26], it can be found in soils, water, effluent and foods [16]. The extended distribution of *L. monocytogenes* and its ability to persist in food-processing environments cause the frequent contamination of foods, which represents the main source of human infection [20]. Freeze injury reversibility of this pathogen makes contaminated frozen vegetables a potential source of infection [7]. Incidence of the pathogen in commercial frozen vegetables has been shown to be high, from 1.2% [1] to 46% [19] depending on the study. Some authors have also suggested that some *L. monocytogenes* genetically similar strains may be specially adapted to colonizing the processing plant equipments, thus persisting in this environment for long periods of time [27, 19].

A great number of studies have demonstrated that this pathogen presents a great strain virulence and pathogenicity variation. Among the 14 known serotypes, only three (1/2a, 1/2b and 4b) produce the 95% of the infection cases [16]. However, heterogeneity in virulence levels has been reported even within serovars 1/2a and 1/2b [4]. Moreover,

studies on the virulence of *L. monocytogenes* have suggested that virulent strains can contain genes that are not present in non-.virulent isolates, which could be used for an evaluation by means of PCR of the potential virulence of this pathogen and for the specific detection of these more virulent strains in foods [15, 4]. *Lmo*2821 is a virulence region gene that encodes an internalin protein directly related to internalisation in the host cells. Some studies have shown that this gene is absent in non-virulent strains and it has been proposed as a species-specific virulence marker [15].

The great heterogeneity in *L. monocytogenes* virulence, associated to the high prevalence of the organism in foods have taken to numerous investigators to raise the necessity to develop discriminatory methods for epidemiological studies of *L. monocytogenes* strains present in the food-processing chain in order to evaluate risk for consumers [11,8]. Serotyping is not discriminative enough for epidemiological tracking, as most of the cases are caused by only three serotypes [18]. Moreover, it has been demonstrated that, in some occasions, wrong results are obtained as antigen sharing occurs frequently among *L. monocytogenes* serotypes and, as all phenotypic methods, its reproducibility is limited due to changes in environmental or growth conditions of the strains [16]. In this sense, molecular subtyping methods that differentiate between closely related strains could provide useful genetic, epidemiological and ecological information of *L. monocytogenes* isolates, allowing for an effective control in the food industry.

Repetitive Extragenic Palindromic based-PCR (REP-PCR) subtyping technique has been previously evaluated by other authors [12,5], showing to be more discriminative than ERIC-PCR, and having similar typing potential than RADP and PFGE methods. The DiversiLab System is an automated microfluidics-based detection method for REP-PCR microbial typing. It includes all the reagents kits, as well as software for data processing and analysis. The automated technique is less laborious

89 than PFGE and provides standardized results in less than 24 h [22]. Therefore, this
90 method could be a good alternative technique for subtyping *L. monocytogenes* [14].
91 The aim of this study was to investigate the occurrence of different serotypes of
92 *Listeria monocytogenes* in frozen vegetables; to compare the efficiency of biochemical
93 and molecular methods for accurate identification of *L. monocytogenes*, to characterize
94 the strains by phenotypic and molecular typing methods in order to predict the strain's
95 potential virulence for humans, and to evaluate the use of automated REP-PCR as a
96 molecular typing method for epidemiological or risk assessment studies.
97
98 **Materials and methods**
99 **Bacterial strains and culture conditions.** Five reference strains from the Colección
100 Española de Cultivos Tipo (CECT), Spain *Listeria monocytogenes* CECT 911 serotype
101 1/2c, *L. monocytogenes* CECT 4031 serotype 1a, *L. monocytogenes* CECT 4032
102 serotype 4b, *L. monocytogenes* CECT 933 serotype 3a, and *L. monocytogenes* CECT
103 936 serotype 1/2b, were included in the study. *Listeria ivanovii* CECT 913, *L. innocua*
104 CECT 910, *Vibrio vulnificus* CECT 529, *V. parahaemolyticus* CECT 511,
105 *Staphylococcus aureus* CECT 240, *S. epidermidis* CECT 231, *Micrococcus luteus*
106 CECT 245, *Citrobacter freundii* CECT 401, *Salmonella enterica* CECT 915,
107 *Pseudomonas aeruginosa* ATCC 10145 (American Type Culture Collection, U.S.A.),
108 *Enterobacter cloacae* CECT 194, *E. faecalis* DSMZ 20478 (Deutsche Sammlung von
109 Mikroorganismen und Zellen, Germany), *Campylobacter jejuni* NCTC 11828 (National
110 Collection of Type Cultures, United Kingdom), *C. coli* NCTC 11366, *Helicobacter*
111 *pylori* NCTC 11637, *Escherichia coli* CECT 349 and *Arcobacter butzleri* NCTC 12481
112 were also used to evaluate the specificity of the PCR assays. All of them were
113 rehydrated and cultured according to their Culture Collections instructions.
114 A total of 70 presumptive *Listeria monocytogenes* isolates were obtained from
115 different frozen vegetables samples in a processing plant during a period of six months.

Isolation was performed according to standard protocol ISO 11290-1:1996 [2]. All *Listeria* presumptive colonies were purified and subcultured onto blood agar plates (Columbia Blood Agar base supplemented with 5% horse blood) to check the haemolytic activity. Oxidase, catalase and Gram-stained tests were also applied. Presumptive *L. monocytogenes* strains were stored in glycerol broth (10% [vol/vol] glycerol in 1% [wt/vol] nutrient broth num. 2 [NB, Oxoid CM67]) with glass beads at -80ºC until required. Pure cultures were recovered on Tryptone Soy agar (TSA, Merck, Darmstadt, Germany) and used for biochemical and molecular identification.

Biochemical identification. API *Listeria* identification system (Biomerieux, Mercy L'Etoile, France) was used according to manufacturers´ instructions.

Serotyping. Serotyping was determined by heat-inactivating bacteria agglutination by using the commercial system *Listeria* antisera set (Denken Seiken.Co, Ltd., Tokio, Japan) according to manufacturers' instructions. For the assay, *L. monocytogenes* strains were subcultured three times on Brain Heart Infusion Agar (Merck, Darmstadt., Germany) plates and suspended with 0,2 w/v% sodium chloride.

DNA extraction. Cells from an overnight culture of field isolates and reference strains were harvested by centrifugation at 14000 x g for 30 s, washed twice with sterile phosphate buffer (PBS, 130 mmol l^{-1} sodium chloride, 10 mmol l^{-1} sodium phosphate, [pH 7.2]), suspended in Tris-EDTA (TE) buffer and processed with Realpure kit (Durviz, Paterna, Spain).

DNA was concentrated with ethanol, followed by centrifugation at 14000 x g for 5 min. Pellets were suspended in 200µl of DNA hydratation solution and incubated overnight at room temperature to solubilise DNA. Concentrated DNA was stored at -20ºC.

Multiplex PCR identification. Identification was confirmed by multiplex PCR by using specific primers described by Bansal et al. [3]. These primers amplify a genus specific 938 bp fragment of 16S rDNA of *Listeria* sp. and a species specific 750 bp

fragment of *hly*A gene of *L. monocytogenes*. Primers were confirmed to be genus and species specific, respectively, by a BLAST (National Centre for Biotechnology Information, [http://www.ncbi.nlm.nih.gov/blast/]) search and by amplification of the DNA of all *Listeria* and non-*Listeria* reference strains included in this paper.

Amplification was carried out according to Zamora et al. [28]. Briefly, amplification mixtures were prepared in a final volume of 30µl containing 2.5 µl of 10X reaction buffer, 2.5mM of $MgCl_2$, 0.2mM each of dNTPs, 20 pmol each of the four primers, 2U of *Taq* polymerase (Bioron GmbH, Sludwigshafen, Denmark) and 3µl of DNA template. The amplification was conducted with an initial DNA denaturing step at 95°C for 1m followed by 40 cicles of 94°C for 30 s, 51°C for 20 s, 74°C for 30 s, and a final elongation step of 74°C for 8 min. to ensure full extension of the product. All PCR reactions were performed with an automatic thermal cycler (TC-312 Techne, Barloworld Scientific Ltd., Staffordshire, UK). PCR products were analyzed by electrophoresis at 100 V for 1 h through 1% (wt/vol) SeaKem LE agarose (FMC Bioproducts, Denmark) in TAE buffer and visualized under UV illumination after staining with ethidium bromide. The expected molecular weight of the amplicons was confirmed by comparison to the GeneRuler 100-bp DNA Ladder Plus (MBI Fermentas, Burlington, Canada). DNA templates from *L. monocytogenes* CECT 4032 and *L. monocytogenes* CECT 936 were used as positive controls. Nuclease-free water replacing DNA was also included as negative control in all the assays.

***Lmo* gene PCR identification.** Amplification of a 611 bp fragment of *lmo*2821 virulence gene was performed with primers Lmo2821 (5'-TGTAACCCCGCTTACACAGTT-3') and Lmo2821 (5'-TTACGGCTGGATTGTCTGTG-3') according to Liu et al. [15]. Each reaction consisted of 0,5 U *Taq* Polymerase, 50 µM dNTPs, 25 pmol each of the two primers and 10 ng of DNA template in a final volume of 25 µl. Cycling parameters consisted of an initial denaturalization step of 94°C for 2 m, 25 cycles (94°C-20 s,60°C-20 s and 72°C-

45 s) and a final step of 72°C for 2 m. DNA of the *Lmo* gene positive *L. monocytogenes* strain CECT 4032 was added as a PCR positive control.

REP-PCR. DNA for REP-PCR analysis was extracted with Ultra Clean Microbial DNA Isolation kit (MoBio, Laboratories, Inc). Amplification was carried out by using Diversilab Listeria kit (Bacterial Barcodes Inc.) and AmpliTaq DNA Polymerase (Applied Biosystems), following manufacturers' instructions. Amplifications were performed with a Mastercycler personal (Eppendorf) Cycler and analysed with an Agilent Technologies 2100 Bioanalyzer B.02.05.SI360. Cycling conditions consisted of an initial denaturing step of 94º C for 2 min; 35 cycles of 94º C for 30 seg, 50º C for 30 seg and 70º C for 90seg and a final elongation step of 70º C for 3 min.

Results

Biochemical identification. Sixty six out of the 70 presumptive *L. monocytogenes* strains were identified by API Listeria system as *L. monocytogenes*, presenting three different numerical profiles: 6110 (47 strains, 71.2 %), 6510 (11 strains, 16.6 %) and 6550 (8 strains, 12.1 %). Table 1. Only one isolate identified as *L. monocytogenes* was negative for haemolytic activity. Four strains were identified as *L. inocua* (numerical API profile 7510) (data not shown).

Table 1. *Listeria monocytogenes* strains isolated in this studio

Serotyping. Serotypes of reference strains were determined and, in all cases, results were in agreement with Culture Collection information. All the biochemically confirmed *L. monocytogenes* isolates were also analysed by serology. Only one *L. monocytogenes* isolate belonged to serotype 4b. The rest of isolates belonged to 1/2a (62 strains, 93.9 %) and 1/2b (3 strains, 4.5 %) (Table 1).

Multiplex PCR identification. All isolated strains were *Listeria* spp.-PCR positive. The 66 strains biochemically identified as *L. monocytogenes* yielded two amplification bands, one of them of 938 bp corresponding to genus identification and the second one of 750 bp corresponding to species identification (Fig. 1). None of the five *L. innocua* strains yielded the *L. monocytogenes* species-specific band.

Fig. 1. Multiplex PCR analysis of *Listeria monocytogenes* isolates with primers showing the genus specific 938 bp fragment of 16S of *Listeria* sp. and a species specific 750 bp fragment of *hly*A gene of *L. monocytogenes*.

Virulence gene identification. Amplification of *lmo*2821 region showed a 611 bp band in 51 of the *L. monocytogenes* isolates. The assay was negative for 15 strains, all of them presenting the same API profile (6110). All of the *lmo*2821-positive strains belonged to serotype 1/2a except one strain which belonged to serotype 1/2b. All *L. monocytogenes* which presented API profiles 6510 and 6550 were positive for *lmo*2821-PCR detection assay (Table1).

REP-PCR. REP-PCR *L. monocytogenes* DNA analysis yielded 17 different patterns formed by 70-80 different bands ranging from 150 to 7000 bp. A band of approximately 1200 bp was present only in *L. monocytogenes* strains. A total of three genetic groups grouped at 82% similarity level were defined (Fig 2). Group 1 included profiles 1, 2, 3, 4, 5, 6 and 7, all of them grouped at 94% similarity level, with a total of 32 isolates. All of them belonged to 1/2a serotype except isolate L86, which belonged to 1/2b serotype and presented a unique profile. Group 2 (93.5% similarity level) included profiles 8, 9, 10, 11, 12, 13 and 14, containing a total of 31 isolates belonged to 1/2a serotype and three reference strains with serotypes 1/a, 1/2c and 3a respectively. REP-PCR profiles 3 and 10 were the most frequently found. They were shared by 22 and 20 isolates,

220 respectively, which also presented the same serotype (1/2a in both cases) and
221 biochemical profile (6110). These isolates were obtained from different kinds of
222 vegetables during all the sampling period. Profile 13, corresponding to 3a serotype
223 shared a low number of bands with the rest of profiles of this group.

224

225 **Fig. 2.** Dendrogram derived from REP-PCR analysis of 66 isolated and 5 *L.*
226 *monocytogenes* reference strains showing the three genetic groups, the serotypes and
227 number of isolates strains.

228

229 An isolate (L74) and the reference strain *L. monocytogenes* CECT 4032, identified both
230 as 4b serotypes, and two isolates and the reference strain *L. monocytogenes* CECT 936
231 belonged to 1/2b serotypes represented the genetic Group 3. Strains of both serotypes
232 joined at a 93.6 % homology degree.

233 Profiles for other *Listeria* species were clearly distinguishable from *L. monocytogenes*
234 patterns (data not shown).

235 **Discussion**

236 In this study we have identified and characterized presumptive *L. monocytogenes* strains
237 isolated from different frozen vegetables products in a processing plant by different
238 phenotypic and genotypic methods. In order to confirm biochemical identification, all
239 the isolates were tested by specific multiplex PCR. According to previous reports [9]
240 multiplex PCR and API methods had 100% agreement for *L. monocytogenes* species
241 identification. Most of isolates presented the 1/2a serotype (n=62) while three of them
242 were found to be 1/2b serotype. This result is in agreement with other previous works
243 [24,13] which have shown that 1/2a and 1/2b are the most frequent serotypes isolated
244 from foods. Serotypes usually involved in cases of listeriosis are 4b, 1/2a and 1/2b [16,
245 9], which were also the serotypes mainly detected in our study. According with other

246 authors [24] serotype 4b, which is usually associated with raw meet products, was found
247 in very slow percentage (only in one strain). Notably, we found that serotyping was
248 correlated with biochemical profiles: while 75.8% of 1/2a strains presented 6110 API
249 profile, all 1/2b and 4b isolates were characterized as 6550 or 6510 API profiles.
250 All *L. monocytogenes* strains are potentially pathogenic, but virulence can vary,
251 changing their ability to produce infection. Many authors have studied the target
252 proteins and genes present in virulent and avirulent strains to establish differences for
253 determination of *L. monocytogenes* virulence and pathogenicity [14]. The putative
254 internalin gene *lmo*2821 seems to be present in all virulent *L. monocytogenes* strains.
255 Therefore specific amplification of this target has been proposed as a rapid and specific
256 method to determine the potential of infection of an isolate and then to establish a
257 protocol to prevent the outbreak by foods [15]. Among all the isolates identified as *L.*
258 *monocytogenes* in this work, about 77% were *lmo*2821-PCR positive, showing its
259 virulence potential. In contrast with some authors, who reported that persistent strains
260 found in fish processing plants seem to be less virulent than clinical strains in cellular
261 and animal models [11], the persistent strains found in our work maintain their
262 virulence potential. This is an important fact that must be considered when evaluating
263 risk to consumers.
264 Only 22.7 % were negative for *lmo*2821 amplification, showing as potentially non-
265 pathogenic strains. All these strains, curiously, presented the API profile 6110, whose
266 main characteristic is to be rhamnose-negative, and belonged to 1/2a serotype. Liu et al.
267 [16] studied the virulence of an unusual rhamnose-negative Lineage III (4a and 4c
268 serotypes) strains in mice and conclude that strains from this group negative for
269 *lmo*2821 appeared to be virulent via intraperitoneal inoculation but not via oral route.
270 On the other hand, it has been demonstrated that *lmo*2821 internalin is implicated in
271 crossing the host intestinal barrier [21], and then these atypical *L. monocytogenes*
272 strains can not be considered infective via oral route. To our knowledge, there is not any

study about the virulence of atypical rhamnose-negative serotype 1/2a strains and then further studies will be necessary to evaluate "*in vivo*" the virulence of these strains.

Although biochemical tests and serotyping are the usual methods to characterize *L. monocytogenes* isolates, these techniques have less discriminatory power and reproducibility than molecular methods, specially for epidemiological studies or for tracking persistent strains in processing plants environments. REP-PCR (repetitive extragenic palindromic repetitive element–based PCR) has been used for typing *L. monocytogenes* from different sources [12,5]. Our results showed the high reproducibility and discrimination power of this rapid method. All the isolates were typeable by REP-PCR, yielding 17 different profiles. Dendrogram of REP-PCR analysis showed three different clusters or genetic groups. Cluster I and II included all the serovar 1/2a strains and the 911, 4031 and 933 NCTC reference strains, all of them belonging to the proposed Lineage II of *L. monocytogenes* [25] and joined at ≥90% of similarity. Cluster III contained the strains belonging to 4b and 1/2b serovars, joined both at 93.6% of homology and corresponding to Lineage I of *L. monocytogenes*. While in Clusters I and II isolates presenting different API profiles (6550, 6110 and 6510) could be found, in Cluster III all isolates shared the 6510 profile. Although in our work serovars 4b and 1/2b (Lineage I) were discriminated from serotypes from Lineage II, more strains should be included in future works in order to better assess these results.

Two REP-PCR patterns were frequently found for isolates sharing the same serotype and biochemical profile. Thus, these isolates were considered subclonal variants from one original strain. They were obtained from different kinds of vegetables during all the sampling period, what clearly suggests that the processing plant was the most likely source of this strain. The abundance of a strain in a particular vegetable, its special resistance to cleaning and disinfection procedures or its ability to produce biofilms have been proposed as the most probable causes for the persistence of a particular strain of *L. monocytogenes* in the processing plant environment [1] The persistence of *L.*

300 *monocytogenes* is a cause of concern, as it can cause contamination of vegetables
301 processed in the plants, leading to a possible increased risk for consumers.
302 We also observed that rhamnose-negative strains (API profile 6110, n=47 strains) were
303 grouped only in four different REP-PCR types, while rhamnose-positive strains (n=19)
304 yielded 12 different REP types, showing a higher genetic heterogeneity.
305 Finally, we found that most of *L. monocytogenes* isolates from frozen vegetables were
306 potentially virulent. Therefore, besides the biochemical and serological typing,
307 subtyping methods are necessary to control the source of contamination and possible
308 transmission routes through the processing plants and finally to the consumer. While the
309 multiplex PCR assay applied in this work provided an accurate and rapid method for
310 species identification, REP-PCR was confirmed as a rapid and reliable method for *L.*
311 *monocytogenes* subtyping, providing useful information for epidemiological or risk
312 assessment studies, as well as for tracking surveys in food processing plants.
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Table 1. *Listeria monocytogenes* strains isolated in this studio

Strains-serotype	API profile	Haemolytic activity	lmo gen	REP type
CECT 4031[a]-1/a	6550	-	+	8
CECT 911[a]-1/2c	6510	+	+	9
L4- 1/2a	6110	+	-	3
L1- 1/2a	6110	+	-	
L6- 1/2a	6110	+	-	
L34- 1/2a	6110	+	+	
L14- 1/2a	6110	+	-	
L7- 1/2a	6110	+	-	
L8- 1/2a	6110	+	-	
L84- 1/2a	6110	+	+	
L10- 1/2a	6110	+	-	
L88- 1/2a	6110	+	+	
L3- 1/2a	6110	-	-	
L25- 1/2a	6110	+	+	
L24- 1/2a	6110	+	+	
L22- 1/2a	6110	+	-	
L23- 1/2a	6110	+	+	
L18- 1/2a	6110	+	-	
L19- 1/2a	6110	+	-	
L98- 1/2a	6110	+	+	
L58- 1/2a	6110	+	+	
L67- 1/2a	6110	+	+	
L38- 1/2a	6110	+	+	
L21- 1/2a	6510	+	+	
L51- 1/2a	6110	+	+	10
L46- 1/2a	6110	+	+	
L55- 1/2a	6110	+	+	
L9- 1/2a	6110	+	-	
L76- 1/2a	6110	+	+	
L53- 1/2a	6110	+	+	
L66- 1/2a	6110	+	+	
L50- 1/2a	6110	+	+	
L37- 1/2a	6110	+	+	
L60- 1/2a	6110	+	+	
L2- 1/2a	6110	+	-	
L36- 1/2a	6110	+	+	
L35- 1/2a	6110	+	+	
L62- 1/2a	6110	+	+	
L11- 1/2a	6110	+	-	
L70- 1/2a	6110	+	+	
L40- 1/2a	6110	+	+	
L63- 1/2a	6110	+	+	
L44- 1/2a	6110	+	+	
L41- 1/2a	6110	+	+	
L12- 1/2a	6110	+	-	5
L54- 1/2a	6110	+	+	
L71- 1/2a	6110	+	+	
L48- 1/2a	6110	+	+	
L42- 1/2a	6110	+	+	4
L64- 1/2a	6110	+	+	
L75- 1/2a	6510	+	+	1
L79- 1/2a	6550	+	+	11
L32- 1/2a	6510	+	+	2
L85- 1/2a	6510	+	+	6
L86- 1/2b	6510	+	+	7
L57- 1/2a	6550	+	+	12
CECT933- 3a	6510	-	+	13
L82- 1/2a	6550	+	+	14
L83- 1/2a	6550	+	+	
L97- 1/2a	6510	+	+	
L33- 1/2a	6510	+	+	
L17- 1/2a	6510	+	+	
L27- 1/2a	6550	+	+	
L59- 1/2a	6550	+	+	
L56- 1/2a	6550	+	+	
L73- 1/2a	6550	+	+	
CECT 4032[a]- 4b	6510	+	+	16
L74- 4b	6510	+	+	15
CECT 936 [a]- 1/2b	6510	+	+	17
L39- 1/2b	6510	+	+	
L78- 1/2b	6510	+	+	

[a] Reference strains

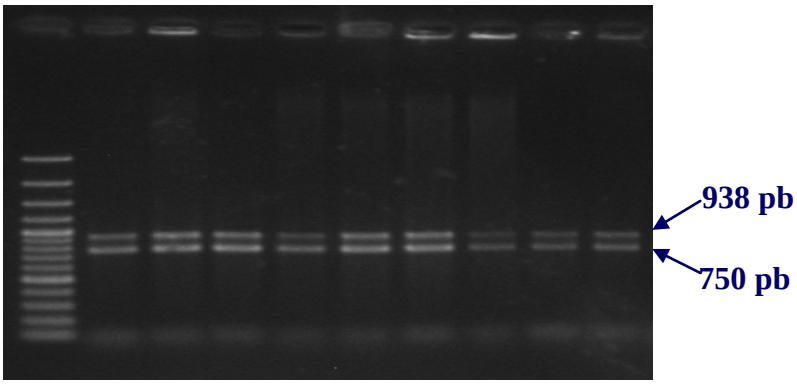

Fig. 1. Multiplex PCR analysis of *Listeria monocytogenes* isolates with primers showing the genus specific 938 bp fragment of 16S of *Listeria* sp. and a species specific 750 bp fragment of *hly*A gene of *L. monocytogenes.*

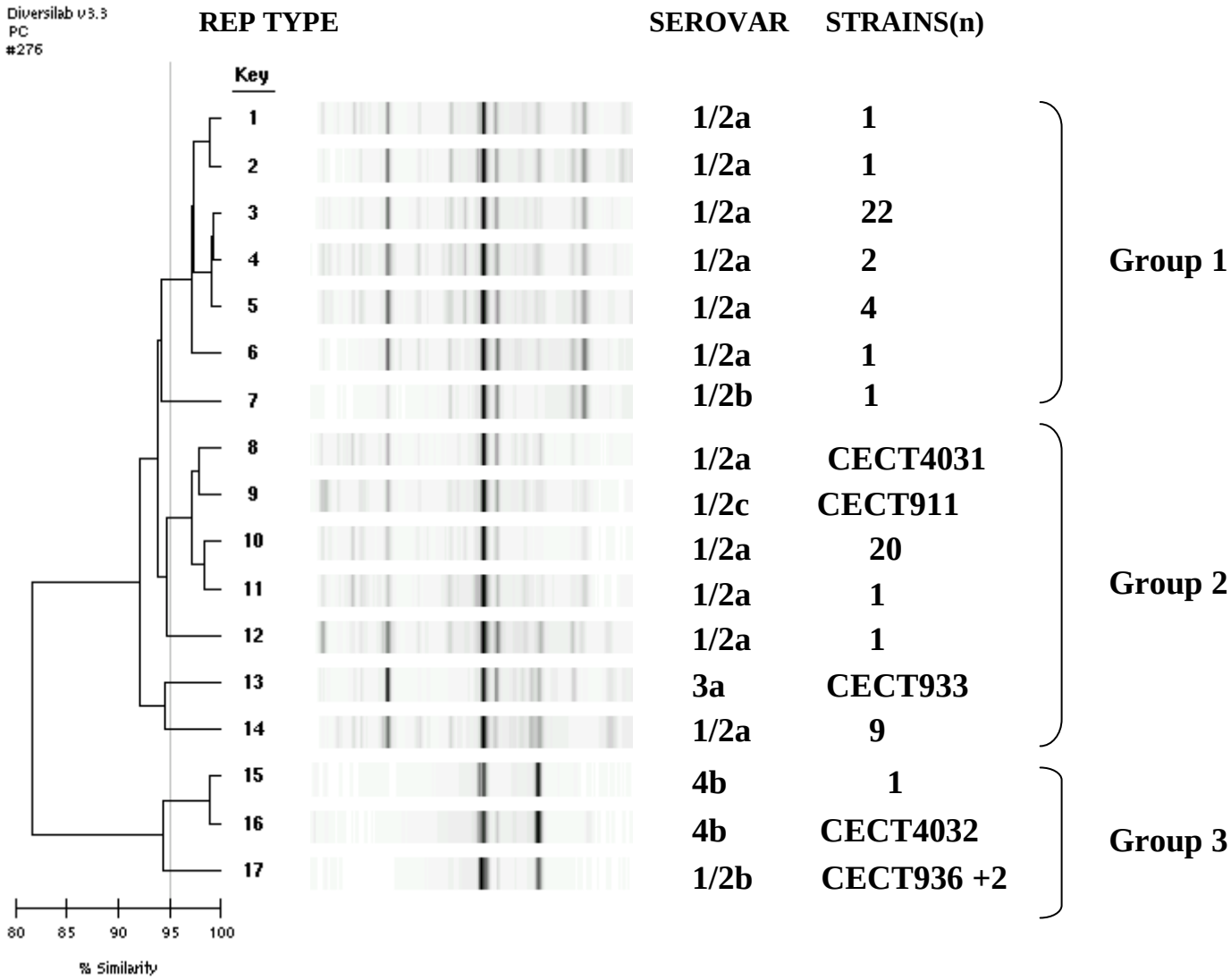

Fig. 2. Dendrogram derived from REP-PCR analysis of 66 isolated and 5 *L. monocytogenes* reference strains showing the three genetic groups, the serotypes and number of isolates strains.