

MOLECULAR TYPING OF *LISTERIA MONOCYTOGENES**

BARBARA JERŠEK

Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana,
Jamnikarjeva 101, 1000 Ljubljana, Slovenia

(Received: January 5, 2001; accepted: March 1, 2001)

Keywords: *Listeria monocytogenes*, molecular typing

Introduction

L. monocytogenes is a Gram-positive, nonsporeforming, facultatively anaerobic bacterium. It is widely spread in nature; in soil, plant, sewage, silage, slaughterhouse waste, dust and even water [1]. It has been found in at least 37 mammalian species, both domestic and wild, as well as in at least 17 species of birds and possibly some species of fish and shellfish. Humans (1–10 %) may be intestinal carriers of *L. monocytogenes*.

L. monocytogenes is present in a wide variety of foods. The natural exposure of vegetables, fruits, grains, food for animals, fish and poultry to the environmental sources of the organism makes impossible for such foods to be produced free from *L. monocytogenes*. *L. monocytogenes* is quite hardy and resists the deleterious effects of freezing, drying and heat remarkably well for a bacterium that does not form spores. Even normal refrigeration temperature (between 0 and 8 °C) does not kill it. The main growth-limiting parameters for *L. monocytogenes* are temperature (minimum –0,4 °C, maximum 45 °C) pH (minimum 4.39, maximum 9.4) and water activity (minimum 0.92). The bacterium does not change the taste or smell of contaminated food, which is the most frequent source of human infection with *L. monocytogenes*. For these reasons, it can be very difficult to suspect or trace the source of a listeria outbreak.

* Lecture presented at the International Training course for young scientists (Keszthely, Hungary, August 23–27, 2000) organized by the Hungarian Society for Microbiology and the UNESCO-Hebrew University of Jerusalem International School for Molecular Biology, Microbiology and Science for Peace

The disease

Listeriosis is the name of the general group of disorders caused by *L. monocytogenes* [2]. It has been known since 1911 that listeria can infect animals. According to the Food and Drug Administration (FDA), the first case of human infection was detected in 1929. While listeria has been monitored for decades, it is known only since the early 1980s that the bacteria may be spread in food as well as by contact with animals.

It is clinically defined when the organism is isolated from blood, cerebrospinal fluid, or an otherwise normally sterile site (e.g. placenta, fetus). The manifestations of listeriosis include septicemia, meningitis (or meningoencephalitis), encephalitis, intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion or stillbirth. The onset of disorders is usually preceded by influenza-like symptoms including persistent fever. It was reported that gastrointestinal symptoms such as nausea, vomiting, and diarrhoea may precede the more serious forms of listeriosis or may be the only symptoms expressed. The onset time to serious forms of listeriosis is unknown but may range from a few days to three months [2]. The infective dose of *L. monocytogenes* is unknown but is believed to vary with the strain and susceptibility of the victim. *L. monocytogenes* can survive and multiply in phagocytic host cells.

Incidence

According to data from CDC (Centres for Disease Control and Prevention, USA) there were 77 laboratory confirmed cases of listeriosis in 1997 (incidence 0.5 /100 000) and 15 of patients died, 106 cases in 1998 (incidence 0.5 /100 000) and 13 of them died (mortality rate 12%) (FoodNet). Although the incidence of listeriosis is low (2 to 15 people per 100,000 people per year), the high mortality rate (25%–30%) associated with this disease makes it a serious health problem [3].

Target populations

By humans listeriosis is an opportunistic infection, which most often affects people with severe underlying illness (cancer, AIDS), the elderly (over 65 years), pregnant women and infants. Patients without these risk factors can also become infected [2].

Associated foods

L. monocytogenes has been associated with foods such as raw milk, pasteurised fluid milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables,

fermented raw-meat sausages, raw and cooked poultry, raw meats (all types), and raw and smoked fish. Its ability to grow at temperatures as low as 3° C permits multiplication in refrigerated foods.

Methods for detection of *L. monocytogenes* in food

There are several cultural methods available for detection of *L. monocytogenes* in food, e.g. standard method of FDA, AOAC, IDF and ISO [4]. Conventional microbiological detection methods are very time-consuming and can take up to 4 weeks until final results are obtained. A variety of detection methods for *L. monocytogenes* based on nucleic acid probes, monoclonal antibodies or polymerase chain reactions have been described. They tend to be simpler, labour-saving and capable of delivering results more quickly than conventional detection methods.

Typing of *L. monocytogenes*

Typing methods help to understand how *L. monocytogenes* enters the food chain and causes the illness. Phenotyping and genotyping methods used in epidemiological studies allow distinction of individual strains or groups of strains. They provide information on relationships between isolates, identify disease outbreaks, the source of infection and determine modes of transmission of *L. monocytogenes*. Typing methods can be separated into two major categories: conventional methods and molecular methods. World Health Organisation (WHO) organised international Multicentre *L. monocytogenes* Subtyping Study in 1996 to evaluate various typing methods [5].

Conventional typing methods

Serotyping and phage typing have traditionally been used for the differentiation and characterization of *L. monocytogenes* isolates [6, 7].

The antigenic scheme divides *L. monocytogenes* strains into 13 serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4ab, 4c, 4d, 4e, 7). Serotypes 1/2a, 1/2b and 4b cause most human listeriosis cases [1, 8]. Phage typing is more discriminative than serotyping and enables characterization of 52 to 78% of strains [9]. In spite of their usefulness, evidenced during epidemiological investigations, both typing methods remain of limited use due to the low discriminatory capacity of serotyping, and the large proportion of non-typeable strains by phage typing.

Molecular typing methods

Greatly improved results for typing *L. monocytogenes* strains have been attained following the development of different molecular typing methods such as multilocus enzyme analysis, restriction endonuclease analysis, pulsed-field gel electrophoresis with low-frequency cutting enzymes, ribotyping, plasmid analysis and different typing methods based on polymerase chain reaction.

Multilocus enzyme electrophoresis (MEE)

Characterization of isolates by MEE is based on differences in electrophoretic mobility of their metabolic enzymes. MEE was used for analysis of *L. monocytogenes* isolates of different origins (humans, animals, environment, and food products) in several epidemiological investigations [10–14]. Results of an international comparative study of MEE applied to *L. monocytogenes* isolates showed that reproducibility and discrimination power varied greatly within seven participating laboratories [15]. MEE was also not sufficiently discriminating (discrimination power (DI) 0.83–0.93) and should be supplemented with other typing method. MEE is a labour-intensive method that requires techniques and equipment available in relatively few laboratories. For these reasons, this method presently has limited application in epidemiological studies.

Restriction endonuclease analysis (REA)

REA requires digestion of chromosomal DNA with specific endonucleases and separation of fragments on agarose gel. REA has been used to characterize strains of *L. monocytogenes* recovered from different outbreaks of listeriosis [16, 17]. Wesley and Ashton [18] have shown that isolates from major outbreaks exhibit a unique restriction enzyme pattern. Gerner-Smidt et al. [19] evaluated REA typing of *L. monocytogenes*. The major limitation of REA was difficulty of comparing complex DNA patterns which consists of many bands that may be unresolved or overlapping. They concluded that differentiation criteria (the minimum number of band differences that indicate the differences between two strains) for each enzyme need to be clearly established. REA may be used as a definitive typing method if the typing procedure is standardized.

Pulsed-field gel electrophoresis (PFGE)

By macrorestriction analysis DNA is digested with one or more restriction endonucleases that cut infrequently, thus producing large fragments of DNA that are separated by PFGE. DNA patterns are more easily identified as those obtained by REA. DNA macrorestriction analysis using PFGE has shown to be a highly discriminatory

and reproducible typing method and has been successfully used for typing *L. monocytogenes* strains by several investigators [20, 21]. The method was particularly useful for subtyping serotype 4b strains, which were not subtyped satisfactorily by most other subtyping methods [22]. Typing of serotypes 1/2 and 3 was subsequently demonstrated by Buchrieser et al. [20]. PFGE was used to demonstrate the link between *L. monocytogenes* contaminated chocolate milk and sporadic listeriosis cases in Illinois [21]. Brosch et al. [23] evaluates the usefulness of PFGE for typing 80 strains of *L. monocytogenes* in four laboratories. Agreement of typing data among four laboratories ranged from 79 to 90%. PFGE was very discriminating with discrimination power (DI) ranged from 0.95 to 0.96. They have also confirmed the previous finding of Brosch et al. [22] that PFGE is particularly useful for typing serotype 4b isolates. The major disadvantages of PFGE are the long time required to complete the procedure (3 days), the requirement of large quantities of expensive restriction enzymes and the need for relatively expensive, specialized equipment for electrophoresis.

Ribotyping

Ribotyping reduces the complexity of genomic DNA restriction profiles by rendering visible only those restriction fragments that contain nucleotide sequences homologous to the rRNA or rDNA probe. It has been applied for typing *L. monocytogenes* strains and has great value when *EcoRI* was used as the restriction endonuclease [17, 24]. Ribotyping has failed to discriminate particularly among *L. monocytogenes* serotype 4b isolates that caused most human listeriosis cases. Swaminthan et al. [24] and Kerouanton et al. [25] suggested combination of ribotyping with another typing methods such as PFGE for molecular epidemiology of *L. monocytogenes* strains of serotype 4b.

Plasmid analysis

Plasmid profile analysis has not been widely used for typing *L. monocytogenes* isolates because of the relatively rare occurrence of plasmids and their similarity in size [26].

Molecular typing methods based on polymerase chain reaction (PCR)

Based on the principle of polymerase chain reaction (PCR) a number of molecular typing methods have been developed and used for differentiation of *L. monocytogenes* strains. Among those methods randomly amplified polymorphic DNA, PCR-ribotyping, repetitive element sequence-based PCR and amplified fragment length

polymorphism have been applied successfully for the typing of *L. monocytogenes* strains.

Randomly amplified polymorphic DNA (RAPD)

RAPD is a method in which a single arbitrarily selected primer amplifies DNA fragments of different sizes. DNA patterns are suitable for typing. This technique is advantageous since no DNA sequence information is needed. The method was first applied to type *L. monocytogenes* by Mazurier et al. [27]. They analysed 53 isolates from 6 listeriosis outbreaks. All isolates classified by RAPD were in complete agreement with phage typing. They suggested RAPD as an attractive alternative to phage typing. Farber and Addison [28] applied RAPD to 64 *Listeria* isolates. Method allowed differentiation among *Listeria* species and also serotypes within species of *L. monocytogenes*. Many other researchers have also found RAPD to be highly discriminating for typing *L. monocytogenes* strains isolated from patients, food and food processing environment [29–32]. Despite the simplicity and high discriminating ability of RAPD, much work is still needed to develop standardized RAPD protocol. The major disadvantage of the method is an inconstant reproducibility of patterns [33].

Repetitive element sequence-based PCR (rep-PCR)

Repetitive DNA sequences are the basis for molecular typing methods that distinguish bacteria by hybridization or PCR amplification. Different repetitive DNA sequences utilized by DNA-based typing methods include rRNA and tRNA genes, insertion sequences and transposons and smaller highly repetitive extragenic sequences [34]. Versalovic et al. [34] introduced DNA fingerprinting technique for prokaryotic organisms, rep-PCR, which is based on presence of conserved extragenic repetitive DNA sequences in bacteria. Rep-PCR is based on the fact that outwardly facing oligonucleotide primers complementary to repetitive sequences will enable the amplification of differently sized DNA fragments consisting of sequences lying between these elements. Because the distances between these repetitive elements vary, differently sized PCR products will be produced and can be separated by agarose gel electrophoresis to yield specific DNA patterns. DNA patterns are strain specific. The rep-PCR method has been applied successfully for typing several bacterial species and strains that possess repetitive elements. Key advantages of rep-PCR typing include its speed and reproducibility. Either purified genomic DNA or crude cell lysate can be added directly to PCR reactions.

We have used rep-PCR for characterization species in the genus *Listeria* and strains within the species *L. monocytogenes* [35, 36].

Amplified fragment length polymorphism (AFLP)

AFLP is a novel PCR-based typing technique developed by Vos et al. [37]. It is based on selective amplification of genomic restriction fragments using PCR. It basically consists of three steps: (1) digestion of genomic DNA with two restriction enzymes and ligation of restriction half-site specific adaptors to all restriction fragments, (2) selective amplification of these fragments with two primers that have corresponding adaptor- and restriction-site-sequences as their target sites; (3) electrophoretic separation of the PCR products on polyacrylamide gel. AFLP can be used to generate DNA patterns from DNA of any origin and from any complexity without prior sequence knowledge. It is highly reproducible and has high discrimination power [38]. Critical points of procedure include preparation of genomic DNA of high purity to ensure complete digestion by restriction enzymes and the correct choice of restriction enzymes. The best combination of endonucleases should be determined empirically for each organism.

We have investigated 27 clinical and 6 animal isolates of *L. monocytogenes* with AFLP [39]. Reactions on these DNAs were performed after digestion of DNAs with *EcoRI* and *TaqI* using 7 combinations of primers with 1 to 3 selective nucleotides. All obtained patterns were very complex and strains were clustered irrespective of their serotype. Differences were observed also between strains from the same patient. Discrimination index DI was 1 and that indicated that each isolate would have its own AFLP type. Therefore human and animal isolates were not typed on the basis of AFLP results. As a high-resolution typing method AFLP requires a correct choice of restriction enzymes, corresponding adapters and primers.

AFLP was used for typing *L. monocytogenes* strains isolated from different foods and cheese producing environments [40]. *EcoRI* and *MseI* were used for DNAs digestion. They showed that AFLP in combination with automated laser fluorescent analysis (ALFA) is a useful alternative that can be used for rapid and reliable typing of *L. monocytogenes* strains. Results obtained by AFLP matched the results of other phenotypic or genotypic typing methods.

Characterisation of *L. monocytogenes* strains by rep-PCR

The aim of our research work was characterization of *L. monocytogenes* strains on the basis of repetitive elements that are conserved in DNA [35, 36].

Thirty-four *L. monocytogenes* strains and 14 strains representing other *Listeria* species were used in the first part of our work.

Sixty-four strains of *L. monocytogenes* isolated from patients, animals and foods were examined in the second part of our work. Among them 52 strains were of unrelated origin.

Primer sets based on repetitive elements, being the 124 bp to 127 bp enterobacterial repetitive intergenic consensus (ERIC) sequence and the 35 bp to 40 bp repetitive extragenic palindromic (REP) sequence were used to generate fingerprints through REP- and ERIC-PCR amplifications.

The results of the first part of our work showed that:

REP and ERIC elements are present in the genus *Listeria*;

ERIC-PCR generated unique DNA pattern for each species in the genus *Listeria*;

REP-PCR DNA patterns are discriminative for *Listeria* spp. with the exception of two species, *L. murrayi* and *L. grayi*

L. monocytogenes serotypes 1/2a, 1/2b, 1/2c, 3b and 4b could be clearly distinguished from each other by REP and ERIC-PCR;

within serotype 1/2a REP-PCR had a comparable discriminative potential (DI 0.92) as RAPD with 3 to 4 primers (DI 0.91).

The results of the second part of our work showed that:

L. monocytogenes strains could be divided into four major clusters matching the origin of isolation; rep-PCR patterns of human and animal isolates were different from those of food isolates;

within each major cluster rep-PCR allowed differentiation of tested serotypes 1/2a, 1/2b, 1/2c, 3b and 4b;

the discrimination power (DI) calculated for 52 epidemiologically unrelated strains of *L. monocytogenes* were 0.98 for rep-PCR and 0.72 for serotyping.

Our result suggest that rep-PCR can provide an alternative method for *L. monocytogenes* typing. The potential of rep-PCR as an efficient molecular typing method for *L. monocytogenes* isolates should be further evaluated by examination of isolates associated with food-borne epidemics.

Results of our studies add further evidence to the idea that rep-PCR may be broadly applicable for typing bacterial strains that possess repetitive elements such as REP and ERIC sequences.

Conclusions

The ideal typing method should:

- be able to type the vast majority of strains;

- have good discrimination power to recognize the reasonable number of types;
- show good reproducibility over a long period of time in different laboratories;
- should not be too complicated or expensive.

In 1996 World Health Organisation (WHO) organized Multicentre *L. monocytogenes* Subtyping Study. On the basis of results from Phase I, where different typing methods were compared, serotyping, phage typing, REA, PFGE and RAPD were selected for standardization in Phase II [5]. This effort should provide a selection of standardised typing methods for *L. monocytogenes* (Table 1). Current strategy for typing *L. monocytogenes* is a combination of both conventional and molecular typing methods.

Table I

Characteristics of typing methods used in WHO Multicentre L. monocytogenes Subtyping Study
[15, 19, 23, 24, 33, 41, 42]

Method	Discrimination power (DI)	Intra-laboratory reproducibility (%)	Inter-laboratory reproducibility (%)
Serotyping	0.68	82–100	83
Phage typing	ND	NA	79
MEE	0.83–0.93	27–91	ND
REA	0.93–0.98	88–97	98
PFGE	0.95–0.96	ND	84
Ribotyping	0.83–0.88	80–100	ND
RAPD	0.75–0.95	0–100	0–100

ND, not done; NA, not available

References

1. Farber, J.M., Peterkin, P.I.: *Listeria monocytogenes*, a food-borne pathogen. *Microbiol Rev* **55**, 476 (1991).
2. McLauchlin, J.: The pathogenicity of *Listeria monocytogenes*: a public health perspective. *Rev Med Microbiol* **8**, 1 (1997).
3. Alterkruse, S.F., Cohen, M.L., Swerdlow, D.L.: Emerging foodborne diseases. *Emerg Infect Dis* **3**, 285 (1997).

4. Waak,E., Tham,W., Danielsson,T.M.L.: Comparison of the ISO and IDF methods for detection of *Listeria monocytogenes* in blue veined cheese. *Int Dairy J* **9**, 149 (1999).
5. Bille,J., Rocourt,J.: WHO international multicenter *Listeria monocytogenes* subtyping study – rationale and set-up of the study. *Int J Food Microbiol* **32**, 251 (1996).
6. Audurier,A., Martin,C.: Phage Typing of *Listeria monocytogenes*. *Int J Food Microbiol* **8**, 251 (1989).
7. Seeliger,H.P.R., Hoehne,K.: Serotyping of *Listeria monocytogenes* and related species. In: *Methods in Microbiology*, Bergan,T., Norris,J. (Ed.) Academic Press, New York, London, 1979, pp. 31–48.
8. Manzano,M., Cocolin,L., Cantoni,C., Comi,G.: A rapid method for the identification and partial serotyping of *Listeria monocytogenes* in food by PCR and restriction enzyme analysis. *Int J Food Microbiol* **3**, 207 (1998).
9. Low,J.C., Donachie,W.: A review of *Listeria monocytogenes* and listeriosis. *Vet J* **153**, 9 (1997).
10. Bibb,W.F., Schwartz,B., Gellin,B.G., Plikaytis,B.D., Weaver,R.E.: Analysis of *Listeria monocytogenes* by multilocus enzyme electrophoresis and application of the method to epidemiologic investigations. *Int J Food Microbiol* **8**, 233 (1989).
11. Bibb,W.F., Gellin,B.G., Weaver,R., Schwartz,B., Plikaytis,B.D., Reeves,M.W., Pinner,R.W., Broome,C.V.: Analysis of clinical and foodborne isolates of *Listeria monocytogenes* in the United States by multilocus enzyme electrophoresis and application of the method to epidemiologic investigations. *Appl Environ Microbiol* **56**, 2133 (1990).
12. Boerlin,P., Piffaretti,P.: Typing of human, animal, food and environmental isolates of *Listeria monocytogenes* by multilocus enzyme electrophoresis. *Appl Environ Microbiol* **57**, 1624 (1991).
13. Noerrung,B., Skovgaard,N.: Application of multilocus enzyme electrophoresis in studies of the epidemiology of *Listeria monocytogenes* in Denmark. *Appl Environ Microbiol* **59**, 2817 (1993).
14. Harvey,J., Gilmour,A.: Application of multilocus enzyme electrophoresis and restriction fragment length polymorphism analysis to the typing of *Listeria monocytogenes* strains isolated from raw milk, nondairy foods and clinical and veterinary sources. *Appl Environ Microbiol* **60**, 1547 (1994).
15. Caugant,D.A., Ashton,F.E., Bibb,W.F., Boerlin,P., Donachie,W., Low,C., Gilmour,A., Harvey,J., Noerrung,B.: Multilocus enzyme electrophoresis for characterization of *Listeria monocytogenes* isolates: results of an international comparative study. *Int J Food Microbiol* **32**, 301 (1996).
16. Nocera,D., Bannerman,E., Rocourt,J., Jaton-Ogay,K., Bille,J.: Characterisation by DNA restriction endonuclease analysis of *Listeria monocytogenes* strains related to the Swiss epidemic of Listeriosis. *J Clin Microbiol* **28**, 2259 (1990).
17. Baloga,A.O., Harlander,S.K.: Comparison of methods for discrimination between strains of *Listeria monocytogenes* from epidemiological surveys. *Appl Environ Microbiol* **57**, 2324 (1991).
18. Wesley,I.V., Ashton,F.: Restriction enzyme analysis of *Listeria monocytogenes* strains associated with foodborne epidemics. *Appl Environ Microbiol* **57**, 969 (1991).
19. Gerner-Smidt,P., Boerlin,P., Ischer,F., Schmidt,J.: High-frequency endonuclease (REA) typing: results from the WHO collaborative study group on subtyping of *Listeria monocytogenes*. *Int J Food Microbiol* **32**, 313 (1996).
20. Buchrieser,C., Brosch,R., Catimel,B., Rocourt,J.: Pulsed-field gel electrophoresis applied for comparing *Listeria monocytogenes* strains involved in outbreaks. *Can J Microbiol* **39**, 395 (1993).
21. Proctor,M.E., Brosch,R., Mellen,J.W., Garrett,L.A., Kaspar,C.W., Luchansky,J.B.: Use of pulsed-field gel electrophoresis to link sporadic cases of invasive listeriosis with recalled chocolate milk. *Appl Environ Microbiol* **61**, 3177 (1995).

22. Brosch,R., Buchriesre,C., Rocourt,J.: Subtyping of *Listeria monocytogenes* serovar 4b by use of low-frequency-cleavage restriction endonucleases and pulsed-field gel electrophoresis. *Res Microbiol* **142**, 667 (1991).
23. Brosch,R., Brett,M., Catimel,B., Luchansky,J.B., Ojeniyi,B., Rocourt,J.: Genomic fingerprinting of 80 strains from WHO multicenter international typing study of *Listeria monocytogenes* via pulsed-field gel electrophoresis (PFGE). *Int J Food Microbiol* **32**, 343 (1996).
24. Swaminthan,B., Hunter,S.B., Desmarchelier,P.M., Gerner-Smidt,P., Graves,L.M., Harlander,S., Hubner,R., Jacquet,C., Pedersen,B. Reineccius,K., Ridley,A., Saunders,N.A., Webster,J.A.: WHO-sponsored international collaborative study to evaluate methods for subtyping *Listeria monocytogenes*: restriction fragment length polymorphism (RFLP) analysis using ribotyping and southern hybridization with two probes derived from *L. monocytogenes* chromosome. *Int J Food Microbiol* **32**, 263 (1996).
25. Kerouanton,A., Brisabois,E., Denoyer,F., Dilasser,J., Grout,G., Salvat,B., Picard,A.: Comparison of five typing methods for the epidemiological study of *Listeria monocytogenes*. *Int J Food Microbiol* **43**, 61 (1998).
26. McLauchlin,J., Hampton,M.D., Shah,S., Threlfall,E.J. Wieneke,A.A., Curtis,G.D.W.: Subtyping of *Listeria monocytogenes* on the basis of plasmid profiles and arsenic and cadmium susceptibility. *J Appl Microbiol* **83**, 381 (1997).
27. Mazurier,S.I., Audurier,A., Marquet Van der Meer,N., Notermans,S., Wernars,K.A: Comparative study of randomly amplified polymorphic DNA analysis and conventional phage typing for epidemiological studies of *Listeria monocytogenes* isolates. *Res Microbiol* **143**, 507 (1992).
28. Farber,J.M., Addison,C.J.: RAPD typing for distinguishing species and strains in the genus *Listeria*. *J Appl Bacteriol* **77**, 242 (1994).
29. Kerr,K.G., Kite,P., Hertige,J., Hawke,P.M. Typing of epidemiologically associated environmental and clinical strains of *Listeria monocytogenes* by random amplification of polymorphic DNA. *J Food Protect* **58**, 609 (1995).
30. Boerlin,P., Bannerman,E., Ischer,F., Rocourt,J., Bille,J.: Typing of *Listeria monocytogenes*: a comparison of polymorphic DNA with 5 other methods. *Res Microbiol* **146**, 35 (1995).
31. O'Donoghue,K., Bowker,K., Mc Lauchlin,J., Reeves,D.S., Bennett,P.M., MacGowan,A.P.: Typing of *L. monocytogenes* by random amplified polymorphic DNA (RAPD) analysis. *Int J Food Microbiol* **27**, 245 (1995).
32. Lawrence,L.M., Gilmour,A.: Characterisation of *Listeria monocytogenes* isolated from poultry products and from poultry-processing environment by random amplification of polymorphic DNA and multilocus enzyme electrophoresis. *Appl Environ Microbiol* **61**, 2139 (1995).
33. Wernars,K., Boerlin,P., Audurier,A., Russell,E.G., Curtis,G.D., Herman,L., van der Mee-Marquet,N.: The WHO multicenter study on *Listeria monocytogenes* subtyping: random amplification of polymorphic DNA (RAPD). *Int J Food Microbiol* **32**, 325 (1996).
34. Versalovic,J., Koeuth,T., Lupski,J.R.: Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* **19**, 6823 (1991).
35. Jeršek,B., Tcherneva,E., Rijpens,N., Herman,L.: Repetitive element sequence based PCR for species and strain discrimination in the genus *Listeria*. *Lett Appl Microbiol* **23**, 55 (1996).
36. Jeršek,B., Gilot,P., Gubina,M., Klun,N., Mehle,J., Tcherneva,E., Rijpens,N., Herman,L.: Typing of *Listeria monocytogenes* strains by rep-PCR. *J Clin Microbiol* **37**, 103 (1999).

37. Vos,P., Hogers,R., Bleeker,M., Reijans,M., van de Lee,T., Hornes,M., Frijters,A., Pot,J., Peleman,J., Kuiper,M., Zabeau,M.: AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res* **23**, 4407 (1995).
38. Blears,M.J., De Grandis,S.A., Lee, H., Trevors,J.T.: Amplified fragment length polymorphism (AFLP): a review of the procedure and its applications. *J Ind Microbiol Biotechnol* **21**, 99 (1998).
39. Jeršek,B.: Typing of *Listeria monocytogenes* with polymerase chain reaction: Dissertation thesis, Ljubljana, University of Ljubljana, Biotechnical Faculty, 1999, pp. 1–88.
40. Aarts,H.J.M., Hakemulder,L.E., Van Hoef,A.M.A.: Genomic typing of *Listeria monocytogenes* strains by automated laser fluorescence analysis of amplified fragment length polymorphism fingerprint patterns. *Int J Food Microbiol* **49**, 95 (1999).
41. McLauchlin,J., Audurier,A., Frommelt,A., Gerner-Smidt,P., Jacquet,C., Loessner,M.J., van der Meer-Marquet,N., Rocourt,J., Shah,S., Wilhelms,D.: WHO study on subtyping *Listeria monocytogenes*: results of phage-typing. *Int J Food Microbiol* **32**, 289 (1996).
42. Schoenberg,A., Bannerman,E., Courtieu,A.L., Kiss,R., McLauchlin,J., Shah,S., Wilhelms,D.: Serotyping of 80 strains from WHO multicentre international typing study of *Listeria monocytogenes*. *Int J Food Microbiol* **32**, 279 (1996).