Serotype-identifying ions in *Listeria monocytogenes* using matrix-associated laser desorption ionization-time of flight mass spectrometry

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**ABSTRACT**

*Listeria monocytogenes* is a foodborne pathogen that can cause a potentially life-threatening infection, and almost all cases of human listeriosis are caused by *L. monocytogenes* isolates in serotypes 1/2a, 1/2b, 1/2c, and 4b. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a rapid, accurate, and high-throughput tool for clinical diagnosis and microbiological research. In the current study, we examined the potential of MALDI-TOF MS for rapid identification of the foodborne pathogen *L. monocytogenes* and to identify high-risk serotypes. To achieve this, MALDI-TOF MS was applied to 50 *L. monocytogenes* strains. All strains were identified as *L. monocytogenes* species based on pattern matching against reference spectra for the species. Importantly, 83 specific mass ions were consistently and uniquely found in high-risk *L. monocytogenes* serotypes 1/2a, 1/2b, 1/2c, and 4b. These 83 mass ions were also unique to specific combinations of these serotypes, which enabled specific identification of these four serotypes using MALDI Biotyper analysis. Hence, this method shows potential for using MALDI-TOF MS for the rapid identification of *L. monocytogenes* species and to discriminate high-risk *L. monocytogenes* serotypes through specific serotype-specific biomarker ions.

**1. Introduction**

*Listeria monocytogenes*, a Gram-positive facultative intracellular pathogen, is the etiological agent of listeriosis, a potentially fatal foodborne infection (Freitag 2006). Because it is highly adaptable and survives well in the environment, *L. monocytogenes* is a frequent contaminant of food processing facilities and has been responsible for some of the largest and most expensive food recalls in US history (Mead et al., 1999; CDC 2001).

*Listeria* consists of 21 recognized species and six subspecies (Kaszon-Nückerl et al., 2020). Serological typing is a well-established typing method of *L. monocytogenes* isolates that is based on variation in the somatic cell wall (O) and flagellar (H) antigens (Seeliger and Höhne 1979). This method differentiates 13 serotypes, many of which are known to represent genetically diverse isolates. Only four of these serotypes (1/2a, 1/2b, 1/2c, and 4b) are implicated in almost all cases of listeriosis (McLauchlin et al., 1989). Among these serotypes, serotype 1/2a is isolated most frequently from foods and processing plants. Serotype 4b strains cause the majority of foodborne listeriosis outbreaks worldwide (Gilot et al., 1996; Kathariou 2002).

Because of the importance of *L. monocytogenes* to public health, several genetic typing methods such as pulsed-field gel electrophoresis (PFGE), PCR-based methods, RAPD (random amplification of polymorphic DNA), or ribotyping have been developed for subtyping *L. monocytogenes* (Tamburro et al., 2019). These methods are difficult to standardize (Ragon et al., 2008), but there is general agreement that *L. monocytogenes* isolates can be classified into three genetic lineages. Lineage I consists of serotypes 1/2b, 3b, 3d, 4d and 4e. Strains in this lineage tend to have tropism for human and animal host tissues and can infect many cell types. Lineage II consists of serotypes 1/2a, 1/2c, 3a and 3c; these strains tend to be more adapted to varying environmental conditions. Lineage III contains serotypes 4a and 4c and is usually not

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related to disease outbreaks, but strains are commonly isolated from various environmental and food specimens (Piffaretti et al., 1989; Bibb et al., 1990; Aarts et al., 1999). A subgroup of lineage III was separated into an independent lineage (lineage IV) (Orsi et al., 2008).

More than 98% of reported human listeriosis cases are caused by serotypes within lineages I and II (1/2a, 1/2c, 1/2b, and 4b) (Droumith et al., 2004). Thus, L. monocytogenes isolates vary in pathogenic potential and in genomic content, and there is some correlation with serotypes and lineages (Barbour et al., 2001; Roche et al., 2003; Kim et al., 2004; Werbrouck et al., 2008). A diagnostic method that would enable identification of isolates with potential to cause human or animal infection is needed (Vazquez-Boland et al., 2001; Ducey et al., 2007). Multiplex PCR-based methods were developed to facilitate prediction of L. monocytogenes isolates’ pathogenic potential using internalin genes (inlA, inlC, and inlJ) (Liu et al., 2007), and PrfA-regulated genes (plcA, actA, hlyA, tap, and prfA) (Notermans et al., 1991). Two novel genes (flaA [LMOSLCC2372.0508] and LMLG_0742) distinguish high-risk L. monocytogenes serotype 1/2a and 1/2c in lineage II using multiplex PCR (Nilo et al., 2015). However, these methods do not allow comprehensive separation of the high-risk serotypes 1/2a, 1/2b, 1/2c and 4b from the low-risk serotypes, which would enable studies on epidemiology of foodborne disease and the development of prophylactic strategies.

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is a comprehensive tool for rapid identification of bacterial species and for differentiation between different subtypes of pathogens (Bizzini et al., 2010; Ferroni et al., 2010; van Veen et al., 2007), and PrfA-regulated genes (Camara and Hays 2007; Wolters et al., 2011; Sandrin et al., 2013) for a particular L. monocytogenes strains was grown on BHI agar and broth. Two extraction methods were performed using the MALDI Biotyper protocol as described (Barbuddhe et al., 2008). For the direct colony method, individual bacterial colonies were transferred from BHI agar plate directly onto an MSP 96 target polished steel BC (Bruker Daltonik GmbH) and allowed to dry at room temperature. After drying, 1 μl matrix solution (saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) was added to the samples and allowed to crystallize with the samples.

For the standard extraction method, a loopful of bacteria was suspended in 300 μl distilled water. Then 900 μl absolute ethanol was added and mixed with the bacteria suspension by vortex. The sample was centrifuged at 16,000 × g for 2 min, and supernatant was decanted. The pellet was resuspended in 500 μl distilled water, centrifuged again with the same conditions, and supernatant was removed. Then 50 μl 70% formic acid was added, followed by vortex for 10 min and addition of 50 μl absolute acetonitrile. The sample was centrifuged for 2 min, and 1 μl of the supernatant was transferred onto an MSP 96 target polished steel BC (Bruker Daltonik GmbH). After the sample was dried, 1 μl of α-cyano-4-hydroxycinnamic acid matrix solution was added and allowed to crystallize with the sample.

2. Materials and methods

2.1. Bacterial strains

A collection of 50 L. monocytogenes strains were selected for analysis in this study (Table 1). The collection included 10 strains of serotype 1/2a, 5 strains of serotype 1/2b, 6 strains of serotype 1/2c, 1 strain of serotype 3a, 1 strain of serotype 3b, 2 strains of serotype 3c, 5 strains of serotype 4a, 12 strains of serotype 4b, 2 strains of serotype 4c, 2 strains of serotype 4d, 1 strain of serotype 4e, and 3 strains (F2-458, F2-208, and F2-270) of unidentified serotype. The strains were previously isolated from diverse animal sources, clinical human isolates, and food samples (Palumbo et al., 2003). Serotypes of the strains were previously performed using enzyme-linked immunosorbent assay (ELISA), slide agglutination serotyping, and a colony immunoblot procedure (Palumbo et al., 2003).

Bacteria were cultured on brain heart infusion (BHI) agar or broth (Becton Dickinson, Sparks, MD, USA) at 30 °C for 24 h.

2.2. Sample preparation for MALDI-TOF MS

L. monocytogenes strains were grown on BHI agar and broth. Two extraction methods were performed using the MALDI Biotyper protocol as described (Barbuddhe et al., 2008). For the direct colony method, individual bacterial colonies were transferred from BHI agar plate directly onto an MSP 96 target polished steel BC (Bruker Daltonik GmbH) and allowed to dry at room temperature. After drying, 1 μl matrix solution (saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) was added to the samples and allowed to crystallize with the samples.

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2.3. MALDI-TOF MS analysis

Mass spectrum analysis of the 50 L. monocytogenes strains was acquired using a Microflex LT mass spectrometer (Bruker Daltonik GmbH) under the control of flexControl software Version 3.0. The positive ions were extruded with default parameter settings (positive linear mode; laser frequency, 60 Hz; iso source 1 voltage, 20 kV; ion source 2 voltage, 16.7 kV; lens voltage, 7.0 kV; mass range, 2,000 to 20,000 Da). For each spectrum, 240 laser shots in 20 shot steps from different positions of the sample spot were accumulated and averaged (automatic mode, default settings). Before recording the sample measurements, each spectrum was calibrated with a bacterial test standard (Bruker Daltonik GmbH).

2.4. Data analysis

After recording the averaged spectrum, flexAnalysis 3.3 (Bruker Daltonik GmbH) was used to analyze each spectrum and perform spectral preprocessing procedures, including smoothing, baseline subtraction, intensity normalization, and automated peak picking to create a list of the most significant peaks of a spectrum. Default MALDI Biotyper parameters were used for processing and automatic identification (smoothing algorithm Saviotzky-Golay and baseline subtraction algorithm TopHat). All spectra were compared to the reference spectra of L. monocytogenes strains Mb19348, RV412, ATCC 19115, and DSM 20600T from Bruker Daltonics database, resulting in identification scores at both the genus and species levels. Spectra peak matching patterns were determined by calculation of a logarithmic score by MALDI Biotyper 3.0 considering the proportion of matching peaks between the unknown spectrum and the reference spectra of the database as well as the consistency of the peak intensities between these spectra. The log score value ranged from 0 (no similarity) to 3.0 (absolute similarity). As suggested by the manufacturer, log score values over 2.000 are rated as a firm identification at species level (Hsu et al., 2014). Log score values between 1.700 to 1.999 are generally rated as identification of bacteria at genus level. Log score value below 1.700 indicated no reliable identification by MALDI Biotyper software.
2.5. Analysis of peak list for identification of specific biomarker ions

For identifying biomarkers to discriminate *L. monocytogenes* serotypes, peak masses obtained from each isolate were compared for presence/absence of peaks using MALDI Biotyper software; comparison was then conducted between all the isolates for each serotype using pattern matching algorithm of the software. First, to identify peaks unique to each strain, each peak in each strain was evaluated against Table 1. *Listeria monocytogenes* strains, origins, MALDI-TOF MS scores, and subgroups based on mass spectra profile dendrogram.

| Serotype | Strain | Origin         | Score | Subgroup |
|----------|--------|----------------|-------|----------|
| 1/2a     | RM2989 (35569A) | Bulk milk     | 2.166 | 2C       |
|          | RM3015 (51772)  | Cheese         | 2.144 | 2B       |
|          | RM3102 (12443)  | Monkey, clinical | 2.082 | 2C       |
|          | RM3152 (FSL-J2-020) | Cow      | 2.047 | 2C       |
|          | RM3160 (FSL-C1-056) | Human, sporadic | 2.350 | 2C       |
|          | RM3163 (FSL-J2-031) | Cow      | 2.195 | 2C       |
|          | RM3164 (FSL-J2-066) | Sheep     | 1.971 | 2C       |
|          | RM3175 (FSL-J1-101) | Human, sporadic | 2.258 | 2C       |
|          | RM3364 (F6854) | Turkey frank   | 2.007 | 2C       |
|          | RM3349 (EGD-e)  | Rabbit        | 2.127 | 2C       |
| 1/2b     | RM3368 (9900101) | Environmental | 2.220 | 2C       |
|          | RM2991 (17209)  | Sheep brain    | 2.300 | 2C       |
|          | RM3024 (G848)  | Unknown        | 2.119 | 2C       |
|          | RM3155 (FSL-J2-064) | Cow      | 2.323 | 2C       |
|          | RM3156 (FSL-J1-177) | Human, sporadic | 2.100 | 2C       |
| 1/2c     | RM3000 (C622N)  | Soil           | 2.240 | 2C       |
|          | RM3014 (G-3321) | Human          | 2.027 | 2C       |
|          | RM3017 (H9666) | Blood          | 2.218 | 2C       |
|          | RM3020 (H9067) | Cheese         | 1.968 | 2B       |
|          | RM3021 (H7973) | Blood          | 2.202 | 2C       |
|          | RM3367 (9900096) | Environmental | 1.790 | 2C       |
| 3a       | RM3026 (J0095) | Pie            | 2.067 | 2C       |
| 3b       | RM3121       | Chicken        | 2.058 | 2C       |
| 3c       | RM3027 (J0096) | Chicken        | 2.324 | 2C       |
|          | RM3159 (FSL-J1-049) | Human, sporadic | 2.126 | 2C       |
| 4a       | ATCC 19114 | Ruminant brain | 2.008 | 1C       |
|          | HCC23       | Catfish        | 2.220 | 2C       |
|          | HCC25       | Catfish        | 2.058 | 1B       |
|          | X1-002      | Food           | 2.120 | 1C       |
|          | RM3171 (FSL-X1-010) | Unknown | 1.970 | 2C       |
| 4b       | ATCC 19115 | Human          | 2.245 | 2C       |
|          | F2365       | Jalisco cheese | 2.043 | 2A       |
|          | RM2984 (013668A) | Cow brain     | 2.103 | 1C       |
|          | RM2996 (13565A) | Bulk milk     | 1.963 | 1A       |
|          | RM2997 | Bulk milk      | 2.183 | 1C       |
|          | RM3101 (ScottA) | Human, clinical | 2.098 | 2B       |
|          | RM3103 (12375) | Monkey, clinical | 2.137 | 1C       |
|          | RM3153 (FSL-J1-110) | Food, epidemic | 2.330 | 1C       |
|          | RM3173 (FSL-J1-158) | Goat      | 2.040 | 2A       |
|          | RM3176 (FSL-J1-108) | Human, epidemic | 2.314 | 2C       |
|          | RM3177 (FSL-J1-116) | Human, epidemic | 2.359 | 2B       |
|          | RM3390 (F-4565) | Human         | 2.090 | 2C       |
| 4c       | 874        | Cow brain      | 2.077 | 1C       |
|          | RM3030 (J0099) | Bull           | 2.369 | 2C       |
| 4d       | RM3025 (J0094) | Human         | 2.314 | 1C       |
|          | RM3108     | Chicken        | 2.347 | 1B       |
| 4e       | RM2218     | Oyster         | 2.235 | 2C       |
| Others   | F2-458     | Human          | 2.071 | 1C       |
|          | F2-208     | Human          | 2.219 | 1C       |
|          | F2-270     | Human          | 2.234 | 1C       |

* Score is the logarithmic score calculated by MALDI Biotyper 3.0 considering the proportion of matching peaks between the unknown spectrum and the reference spectra of the database as well as the consistency of the peak intensities between these spectra. Direct and Extract refer to the sample preparation procedure.

Subgroup refers to *L. monocytogenes* strain groupings based on the mass spectra profile dendrogram generated by MALDI Biotyper 3.0 shown in Figure 1.
Each peak in every other strain. A peak ‘x’ in strain A was considered unique if and only if no peak ‘y’ in strain B existed where (x - 2) ≤ y ≤ (x + 2). Next, peaks of each strain were pooled into groups based on serotype, and peaks were compared across serotypes. A peak ‘x’ in serotype A was considered unique if and only if no peak ‘y’ in serotype B existed where (x - 2) ≤ y ≤ (x + 2). Peaks common between strains and serotypes were identified using the same procedure and criteria. Peaks identified as common within four of the serotypes (1/2a, 1/2b, 1/2c, and 4b) were then compared against each other using the same criteria to identify peaks common between these high-risk serotypes.

2.6. Classification of L. monocytogenes strains

To further evaluate the proteomic relatedness of different serotypes, dendrogram analysis for the 50 strains was performed using the integrated tools of the MALDI Biotyper 3.0 software package based on a correlation matrix for calculating distance values. For graphical correlations, an average statistical algorithm was applied: distance measure was set as correlation, linkage was set as average, and score threshold value for a single isolate was set at a maximal value of 1,000 according to the manufacturer’s recommendation.

3. Results and discussion

3.1. Analysis of L. monocytogenes spectra

Based on comparison to the reference spectra of L. monocytogenes strains Mb19348, RV412, ATCC 19115, and DSM 20600T, all mass spectra obtained for the 50 L. monocytogenes strains matched with reference L. monocytogenes spectra. To determine the simplest procedure that has the potential for automation and results in MS spectra with high information content, two different sample preparation procedures were compared. Of the two sample preparation procedures that were compared, the standard extraction method yielded significantly more species level identifications from tested isolates (>2.0 score) than the direct colony method (Table 1). Sample identification scores ranged from 1.790 to 2.369 using the direct colony method, and they ranged from 2.043 to 2.567 using the standard extraction method. Thus, our results indicate that suspension of bacteria in water or solvent mixtures improved results compared to direct application of intact bacteria to the MALDI target, although this method required more time and reagents. Therefore, mass spectra generated from the standard extraction method were used in the current study for analysis and identification of unique peaks for serotypes.

Similar to our results, a previous study reported that the standard extraction method yielded reliable bacterial strain identification compared to the direct colony method (Alatoom et al., 2011). Direct application of a colony may not consistently yield high scoring results because metabolites, pigments, and agar residue on the surface of the bacteria could interfere with the reflecting step (Du et al., 2002; Smole et al., 2002). However, in contrast to our results, suspension of Salmonella in water or solvent mixtures did not result in an improvement of strain identification compared to the direct application of bacteria to the MALDI target (Dieckmann et al., 2008). Therefore, although the standard extraction method clearly is preferred for identification of L. monocytogenes strains, the direct application method may be useful for other bacterial species.

In the current study, ten signal peaks (3194.79, 4326.11, 4878.47, 5302.59, 6718.63, 6863.864, 7405.272, 9040.504, 9754.854, and 11182.41 Da) were consistently identified in all our tested L. monocytogenes strains, and thus they are potentially useful as biomarkers for identification of L. monocytogenes at the species level. Other studies have also found that MALDI-TOF MS can consistently identify L. monocytogenes species (Barbuuddhe et al., 2008; Hsueh et al., 2014).

3.2. Assignment of biomarker peaks to high-risk L. monocytogenes serotypes

The list of mass spectra generated with MALDI Biotyper software was exported as a table in which every column represented the mass spectra of a strain in a certain mass range. A comparison of all mass ions occurring in the spectra was conducted. As a result of this comparison, 83 specific mass ions were consistently identified in high-risk L. monocytogenes serotype samples (1/2a, 1/2b, 1/2c, and 4b), and thus these mass ions have potential as biomarkers for rapid identification of L. monocytogenes high-risk serotypes.

MALDI-TOF MS has demonstrated potential for subtyping within bacterial species (Edwards-Jones et al., 2000; Stephan et al., 2011; Kuhn et al., 2012). This discriminating ability is attributable to measurement of constantly expressed highly abundant proteins including ribosomal proteins, DNA-binding proteins, and cold-shock proteins (Ryzhov and Fenselau 2001). In L. monocytogenes, MS peaks at mass to ion ratios (m/z) of 5590 and 11179 Da could differentiate between serotype 4a and 4c of L. monocytogenes (Barbuuddhe et al., 2008). In another study, five MS peaks at m/z 5594.85, 6184.39, 11871.31, 5601.21, and 11199.33 could be used to discriminate serotypes 1/2a (lineage II) from serotypes 1/2b and 4b (lineage I) for L. monocytogenes human isolates (Hsueh et al., 2014).

As shown in Table 2, the 83 mass ions were further differentiated into six serotype groupings 1/2a-1/2b, 1/2a-1/2c, 1/2a-4b, 1/2b-1/2c, 1/2b-4b, and 1/2c-4b (peak clusters 1–6, respectively). Unique peaks for individual serotypes were not identified. However, combinations of mass ions enable identification of individual high-risk L. monocytogenes serotypes using results from the MALDI Biotyper analysis. Serotype 1/2a strains have mass ions from peak clusters 1, 2, and 3; serotype 1/2b strains have mass ions from peak clusters 1, 4, and 5; serotype 1/2c strains have mass ions from peak clusters 2, 4, and 6; and serotype 4b strains have mass ions from peak clusters 3, 5, and 6.

Interestingly, peak clusters 2 and 5 had the highest number of peaks (17 and 21, respectively), which correlates with the relatedness of
L. monocytogenes genetic lineages. Peak cluster 5 is composed of isolates in genetic lineage I (serotypes 1/2b and 4b); because these two serotypes are closely related within the same genetic lineage, it would be expected that these two serotypes have a high number of shared mass ions. Likewise, peak cluster 2 is composed of isolates in genetic lineage II (serotypes 1/2a and 1/2c). The other peak clusters are each composed of serotypes from different genetic lineages, and they have lower numbers of shared mass ions.

Importantly, by using a combination of the mass ions unique to these groupings, it is possible to reliably determine these four L. monocytogenes serotypes using MALDI Biotyper analysis. Thus, these 83 mass ion peaks have potential for rapid serotyping of the high-risk L. monocytogenes serotypes 1/2a, 1/2b, 1/2c, and 4b. Further investigation of these biomarker peaks to identify the parent proteins is warranted, which could enable development of rapid antibody-based methods for differentiating L. monocytogenes high-risk serotypes.

3.3. Biotyping of L. monocytogenes by mass spectrometric analysis

By using a maximal distance level of 1,000 as the cutoff for differentiating the mass signal patterns, a cluster analysis with all strains was performed using the MALDI Biotyper 3.0 software based on a correlation matrix. The resulting phylogenetic dendrogram classified L. monocytogenes into two groups (Figure 1). Group 1 contained 14 strains, all of which were in serotypes 4a, 4b, 4c, and 4d. Group 1 had most of the serotype 4a isolates (3 out of 5), some of the 4b isolates (5 out of 12), half of the 4c isolates (1 out of 2), and all the 4d isolates (2 out of 2). Group 2 contained 36 isolates, including all the isolates in serotypes 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, and 4e. Each of the two groups could be further subdivided into three subgroups (designated 1A, 1B, 1C, 2A, 2B, and 2C) (Figure 1). Four of the subgroups (1A, 1B, 2A, and 2B) had only 1–4 isolates each, with the majority of isolates being in subgroups 1C and 2C. Subgroup 2C was the largest (30 isolates); it contained all the 1/2b, 3a, 3b, and 3c isolates and almost all the 1/2a and 1/2c isolates. Subgroup 1C had eleven isolates, and it had the highest number of serotype 4b isolates (four). However, the twelve serotype 4b isolates were scattered between five subgroups: 1A, 1C, 2A, 2B, and 2C.

A previous study demonstrated the ability of the MALDI Biotyper database to identify L. monocytogenes isolates, and it separated them into two clusters according to five peaks, cluster 1 (serotype 1/2a) and cluster 2 (serotypes 1/2b and 4b) (Hsueh et al., 2014). In the current study, our phylogenetic groups did not correlate with the major L. monocytogenes genetic lineages or listerial serotypes. However, all isolates in high-risk serotypes 1/2a, 1/2b, and 1/2c were in MALDI Biotyper group 2, and almost all were in subgroup 2C. Serotype 4b was the most diverse, with isolates in five of the subgroups. Interestingly, serotype 4b is known to be genetically diverse; although most serotype 4b isolates are in genetic lineage I, some serotype 4b isolates are in genetic lineage III (Liu et al., 2006). Hence, use of mass signal patterns from a MALDI Biotyper could be an alternative approach to discriminate and phylogenetically analyze L. monocytogenes strains, particularly strains in high-risk serotypes 1/2a, 1/2b, 1/2c, and 4b.

Figure 1. Mass spectra profile dendrogram generated by MALDI Biotyper 3.0 to determine the relationship between 50 L monocytogenes strains investigated. The dendrogram was generated with the following settings: distance measure was set at correlation, linkage at average, and score threshold value for a single strain at 1,000. Strains clustering with distance levels lower than 800 could be classified up to new classified group level.
4. Conclusion

We have shown a robust extraction method to analyze L. monocytogenes by MALDI-TOF MS. Ten predominant signal peaks have potential as biomarkers for identification of L. monocytogenes species. Cluster analysis of mass spectral patterns generated from L. monocytogenes strains by MALDI-TOF MS shows potential for phylogenetic analysis and differentiation at the subspecies level. Finally, 83 spectral peaks were identified with potential to rapidly and reproducibly classify L. monocytogenes isolates belonging to serotypes 1/2a, 1/2b, 1/2c, and 4b. This method can facilitate rapid identification of these high-risk serotypes to distinguish them from the L. monocytogenes isolates that pose low risk of infection, which can support epidemiological investigations of L. monocytogenes foodborne disease and L. monocytogenes research.

These findings are significant because the method enables differentiation at the species and serotype levels for L. monocytogenes, and it is a novel approach compared with standard methods (Doumith et al., 2004; Palumbo et al., 2003). The strengths of this method are that it can be conducted with a MALDI Biotyper, it is rapid, and reproducible; however, its current major limitation is that further validation is needed with a larger panel of L. monocytogenes strains.

Declarations

Author contribution statement

Hossam Abdelhamed: Analyzed and interpreted the data; wrote the paper. Seong Won Nho: Conceived and designed the experiments; performed the experiments; wrote the paper. Si Won Kim: Performed the experiments; wrote the paper. Seong Bin Park, Joseph S. Reddy and Tae Sung Jung: Contributed reagents, materials, analysis tools, or data; wrote the paper. Mark Lawrence: Conceived and designed the experiments, analyzed and interpreted the data, and wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest’s statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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