Hindawi Publishing Corporation Scientifica Volume 2016, Article ID 5768526, 6 pages http://dx.doi.org/10.1155/2016/5768526



Research Article

Foodborne *Listeria monocytogenes*: A Real Challenge in Quality Control

Tünde Pusztahelyi, 1 Judit Szabó, 2 Zsuzsanna Dombrádi, 2 Szilvia Kovács, 1 and István Pócsi 3

¹Central Laboratory, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Böszörményi Út 138, 4032 Debrecen, Hungary

²Department of Medical Microbiology, Clinical Centre, University of Debrecen, Nagyerdei Körút 98, 4032 Debrecen, Hungary

Correspondence should be addressed to Tünde Pusztahelyi; pusztahelyi@yahoo.com

Received 30 December 2015; Accepted 14 April 2016

Academic Editor: Armen Trchounian

Copyright © 2016 Tünde Pusztahelyi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Listeria monocytogenes is a foodborne pathogen, and the detection and differentiation of this bacterium from the nonpathogenic Listeria species are of great importance to the food industry. Differentiation of Listeria species is very difficult, even with the sophisticated MALDI-TOF MS technique because of the close genetic relationship of the species and the usual gene transfer. The present paper emphasizes the difficulties of the differentiation through the standardized detection and confirmation according to ISO 11290-1:1996 and basic available L. monocytogenes detection methods and tests (such as API Listeria test, MALDI-TOF MS analysis, and hly gene PCR). With the increase of reports on the pathogenesis of atypical Listeria strains in humans, the significance of species level determination has become questionable, especially in food quality control, and the detection of pathogenic characteristics seems to be more relevant.

1. Introduction

Out of the six closely related Listeria species (L. monocytogenes, L. ivanovii, L. seeligeri, L. innocua, L. grayi, and L. welshimeri) and the newly isolated nonpathogenic L. marthii [1], L. rocourtiae [2], L. weihenstephanensis, and L. fleischmannii [3], L. monocytogenes is generally regarded as the only one that is capable of producing human listeriosis [4] with a high mortality rate (greater than 20%). Therefore, laboratory diagnosis has focused primarily on the detection of *L. monocytogenes* and its differentiation from other *Listeria* species [5]. In food samples, the contamination level is usually low (10 CFU/g), but higher than 100 CFU/g can be detected in some cases, for example, in minced meat or smoked fish [6]. Previously, some practical approaches to differentiation tests included culturing with and without preenrichment or with direct analysis from the food sample with biochemical confirmation tests and/or nucleic acid based techniques (reviewed by Churchill et al. [7]), and these methods presented some

success for the food industry. Others approached *Listeria* differentiation from a technical point of view by applying money-consuming methods for precise strain differentiation which is especially good for medical and/or research purposes (reviewed by Gasanov et al. [8]).

The standardized *L. monocytogenes* detection method in Europe requires a laborious testing scheme which is time-consuming, taking five to six days [9, 10]. Because of the high genome homology among *Listeria* species [11–13] the strains display similar morphological, biochemical, serological, and molecular characteristics [4, 14] that causes difficulty of the identification.

In *L. monocytogenes*, the central virulence gene cluster (pathogenicity island, e.g., LIPI-1) codes for the main activities that have importance in the intracellular life of the microbe inside the host. According to some researchers, nonpathogenic *L. innocua* species have lost their pathogenicity island [4, 11]. However, Johnson et al. [15] detected *hly* and *mpl* genes of the LIPI-1 pathogenicity island in atypical

³Department of Biotechnology and Microbiology, Faculty of Science and Technology, University of Debrecen, Egyetem Tér 1, 4032 Debrecen, Hungary

L. innocua and there are also reports on human infections caused by *L. innocua* [16], *L. seeligeri* [17], and *L. ivanovii* [18]. Here, a question is arising: what is more important in food hygiene? The species level determination of *L. monocytogenes* or the enumeration of possibly virulent *Listeria* species?

Here, ALOA medium, API *Listeria* biochemical test strip, MALDI-TOF MS analysis, and *hly* gene detection by PCR were compared to the standardized detection and confirmation according to ISO 11290-1:1996 on foodborne and reference strains.

2. Materials and Methods

2.1. Reference Strains. The following type strains were purchased from the National Collection of Agricultural and Industrial Microorganisms (NCAIM), Faculty of Food Sciences, Corvinus University of Budapest (Hungary): Listeria monocytogenes NCAIM B 01934 (ATCC 15313) type strain; Listeria monocytogenes NCAIM B 01966 (ATCC 35152) non-haemolytic nonvirulent variant [19]; L. innocua NCAIM B01933 (ATCC 33090) serotype 6a; L. welshimeri NCAIM B01872 (ATCC 35897) serotype 6b; L. seeligeri NCAIM B01873 (ATCC 35967); L. ivanovii NCAIM B02035 (ATCC 19119); L. grayi ssp. murray NCAIM B01372 (ATCC 25401); L. grayi ssp. grayi NCAIM B01871 (ATCC 19120).

2.2. Isolation of Foodborne Listeria. All applied enrichment media, selective media, and supplements were identical in composition to the described media of the ISO 11290-1:1996 standard [9] and supplied in dehydrated form. All media were tested with the *L. monocytogenes* NCAIM B 01934 reference strain.

Thirty-one commercially available frankfurter products originated from different producers were sampled and analyzed. From each of them 25 g was measured into 225 mL preenrichment medium (half Fraser broth, Merck, Hungary) in sterile bags and was homogenized in a Stomacher homogenizer (IUL Instruments, Spain) for 1 minute [9].

The homogenized samples were incubated in Schott flasks with loosening caps for 24 hours at 30°C. From each culture 0.1 mL was inoculated into 10 mL enrichment Fraser broth and incubated at 37°C for 48 hours. Black color development, which is characteristic of Listeria species, was detected in some of the culture broths. All cultures were streaked onto Oxford and PALCAM selective agar plates (Merck, Hungary) and incubated at 37°C for 24-48 hours. All colonies showed homogeneous morphology on these media. Subcultures of colonies were tested on sheep blood agar plates (OXOID, UK) $(24 \pm 2 \text{ hours})$ and with the Christie-Atkins-Munch-Petersen (CAMP) test for enhanced beta-haemolysis; on ALOA plates (VWR BDH Prolabo, USA) for β -glucosidase and phospholipase activities, catalase test and Gram staining were also performed. Gram-positive and catalase positive bacterial strains were stored in a Cryobank system (Mast Diagnostics, UK) at -75° C and were applied for further testing.

2.3. Biochemical Confirmation Tests. The biochemical confirmation reactions were performed with the API Listeria test (bioMérieux, France) according to the instructions of

the manufacturer using bacterial cultures cultivated freshly on sheep blood agar plates (OXOID, UK). Reading of the API test strips was evaluated with the mini-API instrument (bioMérieux, France).

2.4. MALDI-TOF MS. Bacterial isolates and Listeria reference strains were grown overnight at 37°C on sheep blood agar (OXOID, UK). Cells of a whole colony were placed in a spot on the steel target plate. Each sample was overlaid with 1μ L of matrix solution (saturated solution of α -cyano-4-hydroxycinnamic acid in 50% acetonitrile, 2.5% trifluoroacetic acid) and air-dried at room temperature. MALDI-TOF MS was performed on a Microflex LT instrument (Bruker Daltonik GmbH, Germany). For automated data analysis, raw spectra were processed using the MALDI Biotyper 3.0 software (Bruker Daltonik GmbH, Germany) with default settings. Generated peak lists were used for matches against reference libraries directly using the integrated pattern matching algorithm of the MALDI Biotyper 3.0 software [20].

2.5. Molecular Biological Identification. Genomic DNA was isolated from cultures of 1.5 mL of brain heart infusion broth (LAB M Limited, UK) or Tryptic Soy Broth (OXOID, UK) containing inoculated frankfurter products, using the Wizard Genomic DNA Purification Kit (Promega, USA) according to the instructions of the manufacturer. The integrity of the genomic DNA was tested by analyzing the samples on 0.8% agarose gel stained with ethidium bromide. Purity and concentration of the DNA were determined spectrophotometrically. Primers were designed with the Oligo Explorer software (version 1.2; Gene Link, http://www.genelink.com/), evaluated with the OligoAnalyzer (version 1.1.2., Teemu Kuulasma) software, and purchased from Integrated DNA Technologies (USA). Oligonucleotides used in the PCR reactions were the *hlyQF/hlyQR* primers of Rodríguez-Lázaro et al. [21] and the following designed primer pair:

hly F: 5'-CACTCAGCATTGATTTGCCA-3', hly R: 5'-CATACGCCACACTTGAGATA-3'.

PCR reactions for the *hlyF/hlyR* primers were run under the following conditions: the final volume of the 50 μ L PCR solution contained 2–4 μ L of genomic DNA, 5 μ L of 10x Taq buffer, 5 μ L 25 mM of MgCl₂, 2-2 μ L of each 10 μ M primer, 4 μ L of 10 mM dNTP mixture, and 2.5 U Taq polymerase (Fermentas, Germany). In the case of the *hlyQF/hlyQR* primers, PCR reaction conditions were determined by Rodríguez-Lázaro et al. [21]. The annealing temperature in the case of our *hlyF/hlyR* primers was 55°C. The amplification conditions were 3 min denaturation at 96°C, 35 cycles each consisting of 30 s at 94°C, 45 s at the corresponding annealing temperature, and 1.3 min at 72°C followed by a final extension for 10 min at 72°C. We applied *L. monocytogenes* NCAIM B 01934 genomic DNA template as a positive control in each PCR run.

Validation of the PCR protocol was carried out with artificially contaminated food samples. 25 g portions of commercial pasteurized frankfurter were suspended in 225 mL TSB (Tryptic Soy Broth; OXOID, UK) and homogenized

in a Stomacher homogenizer (IUL Instruments, Spain) for 1 minute. The suspensions were inoculated with decimal dilutions of exponential phase culture of the *L. monocytogenes* NCAIM B 01934 reference strain suspension from 1 to 10⁵ cells/mL concentrations, mixed well, and samples were taken immediately after inoculation. Following DNA isolation, *hlyF/hlyR* primers were applied in the PCR reactions. PCR products were sequenced in order to test their identity (data not shown). Reference sequences were gained from the National Center for Biotechnology Information Database (http://www.ncbi.nlm.nih.gov/) and aligned with the sequenced PCR products using the BioEdit Biological Sequence Alignment Editor Program [22].

3. Results and Discussion

From 31 frankfurter samples, 11 *Listeria* strains were isolated with the standardized method of *L. monocytogenes* detection. The isolates produced typical colonies on PALCAM agar (except Isolate 2 on Table 1) and/or Oxford selective agar media were Gram-positive and catalase positive.

L. monocytogenes should show haemolysis on sheep blood agar plates or with Staphylococcus aureus with the CAMP test and utilize rhamnose but not xylose [9, 10]. However, our foodborne strains showed the absence of haemolysis after 24 hours of incubation on sheep blood agar contrary to the reference L. monocytogenes NCAIM B 01934 and presented it weakly only after 48 hours of incubation. The ISO standard refers to the possibility of the isolation of *L. monocytogenes* strains with weak haemolysis or negative CAMP test [10, 23]. Foodborne L. monocytogenes strains with either gene alteration or deletion of the *hly* gene or its regulatory protein PrfA are frequently detected, which cause weak haemolytic or nonhaemolytic phenotype in the atypical, aberrant, or nonhaemolytic strains [24, 25]. This phenomenon is highly probable for bacteria isolated from processed food or natural sources. The absence of haemolysis at 24 hours of incubation makes species identification hardly possible because only rhamnose utilization can be applied as a confirmation test by the standard method. At least four Listeria species (L. monocytogenes, L. innocua, L. welshimeri, and L. grayi ssp. *murray*) are known to utilize rhamnose and not xylose [10]. Therefore, a Listeria strain can be determined only at the genus level.

ALOA medium, which was inserted into the standard method [10], differentiates phospholipase positive *Listeria* colonies from another phosphatidylinositol negative *Listeria* [26]. On ALOA plates, all foodborne *Listeria* isolates produced blue-green colonies with an opaque halo. However, other strains than *L. monocytogenes* (e.g., *L. seeligeri* and *L. ivanovii*) may also possess plcA gene coded phospholipase (PI-PLC) activity. Moreover, other bacteria species (e.g., *Bacillus circulans* [27]) also possess phospholipase and β -glucosidase activities that resulted in halo and blue-green color of the colonies, respectively. Therefore, application of ALOA medium independently from the application of preliminary selective enrichment or other selective media (which also needs, at least, two days of incubation) could lead to false positive results.

Results of the biochemical analysis with the API Listeria test were compared with the reference profiles given by the manufacturer (bioMérieux, France). All of our foodborne Listeria isolates showed negative arylamidase (DIM), Dxylose (DXYL), and D-tagatose (TAG) tests (API Listeria numeric profile: 6510) (Table 1) and were suspected to be L. monocytogenes. It is generally regarded that the API test fails in some (10-15%) percent [23, 24, 28, 29]. The main reason of the misidentifications is hidden in the doubtfulness of the color determination of the weak arylamidase (DIM) test. The negative DIM test gives the basis for the differentiation of L. monocytogenes from the closely related L. innocua [30]. A weak positive DIM reaction can be evaluated as a negative result and can increase the number of the false positive determinations. Furthermore, false negative identification of atypical strains is also frequent [29, 31, 32].

A quite new technique for species identification is the Matrix Assisted Laser Desorption Time of Flight (MALDI-TOF) analysis. The isolates are differentiated on the basis of spectral peaks that typically correspond to highly abundant conserved proteins (e.g., ribosomal, cold shock, and nucleic acid binding proteins) that are so-called "housekeeping" proteins [33]. The results of the pattern matching process of the fingerprints obtained in MALDI-TOF MS analysis are expressed in score values (0-3.000). More than half of the samples (n = 18) were determined by 2.000–2.299 score values that mean secure identification only at genus level while only seven strains were given a highly probable species identification with higher than 2.300 scores. The identification revealed L. monocytogenes-L. innocua misidentification of the reference strains (*L. monocytogenes* ATCC 35152 and *L.* innocua ATCC 33090) with scores of high probability species determination of *L. innocua* in case of the *L. monocytogenes* ATCC 35152 atypical strain and for L. seeligeri ATCC 35967 gave *L. monocytogenes* or *L. innocua* (Table 1).

Our PCR primers were designed for conserved regions of the hly gene of LIPI-1 in L. monocytogenes and amplified a 438 bp fragment, while primer pair hlyQF/hlyQR was constructed for RT-PCR and amplified a 64 bp fragment from the *L. monocytogenes hly* gene [21]. Considering that only one band appeared with agarose gel electrophoresis, the tested hlyQF/hlyQR primer pair [21] was much reliable than, for example, that of Border et al. [34] with more amplificants. Interestingly, the *hly* gene detection with *hlyQF/hlyQR* primer pair revealed atypical strain determination for L. seeligeri ATCC 35967 (Table 1). It is well known that nonhaemolytic L. seeligeri possess a distinct LIPI-1 structure [4, 14]. Moreover, this primer pair also gave a reaction with atypical L. monocytogenes NCAIM B 01966. The specificity of the primer pair constructed in this work was higher than *hlyQF-hlyQR* or the primers of Aznar and Alarcón [29], because they received positive PCR results with their hly gene probes for other Listeria species (L. ivanovii and L. seeligeri). Furthermore, better than that of Cooray et al. [35], which failed to detect the *hly* gene from *L. monocytogenes* ATCC 15313 strain. With our primer pair, six out of eleven of the foodborne bacterial isolates gave positive reaction (Table 1), and we did not get a reaction with atypical or nonpathogenic *Listeria* strains.

Table 1: Summary of the test results of the reference and foodborne $\it Listeria$ strains.

Bacteria tested	API test ^a	PCR with <i>hly</i> gene primers		MALDI-TOF MS
		In this work	Rodríguez-Lázaro et al. [21]	score values ^b
L. monocytogenes NCAIM B 01934 (ATCC® 15313™)	6510 L. monocytogenes	+	+	L. monocytogenes 2.430/2.312
L. monocytogenes NCAIM B 01966 (ATCC® 35152™)	6510	-	+	L. innocua 2.427/2.322/2.263/2.461
L. innocua NCAIM B01933 (ATCC® 33090™)	7510 L. innocua	-	-	L. innocua 2.151 L. monocytogenes 1.942
L. welshimeri NCAIM B01872 (ATCC® 35897™)	7311 L. welshimeri	-	-	L. welshimeri 2.211/2.109
L. seeligeri NCAIM B01873 (ATCC® 35967™)	3310 L. seeligeri/ivanovii	-	+	L. monocytogenes 2.241 L. innocua 2.179 L. seeligeri 2.050
L. ivanovii NCAIM B02035 (ATCC® 19119™)	3750 L. ivanovii	-	-	L. ivanovii 2.412/2.381
L. grayi ssp. grayi NCAIM B01871 (ATCC® 19120™)	7130 L. grayi	_	-	L. grayi 2.285/2.272
L. grayi ssp. murray NCAIM B01372 (ATCC® 25401™)	7130	-	-	L. grayi 2.266/2.283
Isolate 1	6510	-	+	L. monocytogenes 2.010/1.359
Solate 2 ^c	6510	-	+	L. monocytogenes 2.251/1.541
Solate 3	6510	+	+	L. monocytogenes 2.118/2.083
Solate 4	6510	+	+	L. monocytogenes 2.295/2.235
Solate 5	6510	-	+	L. monocytogenes 2.245/2.229
Solate 6	6510	+	+	L. monocytogenes 2.058/1.998
Solate 7	6510	+	+	L. monocytogenes 2.314/2.349
Solate 8	6510	+	+	L. monocytogenes 2.346/1.992
Solate 9	6510	-	+	L. monocytogenes 2.246/2.045
Isolate 10	6510	+	+	L. monocytogenes 2.120/2.395
Solate 11	6510	-	+	L. monocytogenes 2.380/2.258

^aNumeric profiles.

^bResults from repeated analyses.

^cNo growth on PALCAM agar.
+: detectable PCR product.
-: no PCR product.

4. Conclusions

Because of the close genetic relationship of the species and the usual presence of atypical strains in food, all tested species identification methods had drawbacks. The present work demonstrated that the standard tests [9, 10] are not always satisfactory for species determination and are not reliable for pathogenicity tests (e.g., haemolysis) of foodborne isolates.

MALDI-TOF MS is likely to become integral to microbiology laboratories for routine microorganism identification because of the short analysis time (<1 min per sample); thus one has the possibility to shorten the total identification time by at least 1-2 days. Here, some points should be addressed: (i) it needs a preliminary enrichment/purification of strains for the identification (needs at least 3 days) and (ii) the method needs validation because the settings of the software have great impact on the precision of the identification [36]. Additionally, identification of taxonomically closely related species has trouble, for example, differentiating *Listeria monocytogenes* from *Listeria innocua/Listeria ivanovii* [33]; therefore, it cannot be recommended for *Listeria* species differentiation in food quality control.

The PCR method is another possibility to shorten the identification time by detection of special genes without isolation steps. With our PCR primers, weak pathogenic or nonpathogenic *L. ivanovii*, *L. seeligeri*, *L. innocua*, and *L. welshimeri* strains and atypical nonhaemolytic *L. monocytogenes* were excluded.

It could be concluded that detection of potential pathogenicity is more important in food quality control than species determination as the specificity of the *Listeria monocytogenes* detection is 85.2% by the ISO standard method [10]. We propose that nucleic acid based detection of the LIPI-1 virulence gene cluster, which is a good marker of potential pathogenicity and does not need enrichment of the bacterium, should be used concomitantly with quick biochemical tests like phospholipase (PI-PLC) and β -glucosidase tests on selective ALOA medium to detect even atypical but pathogenic *Listeria* strains.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

The authors are indebted to the Debreceni Csoport Ltd, Hungary, the daughter company of Mecom Group (Slovakia) for their valuable support. The authors also would like to appreciate the technical assistance of the Department of Medical Microbiology, Faculty of Medicine, University of Debrecen.

References

[1] L. M. Graves, L. O. Helsel, A. G. Steigerwalt et al., "Listeria marthii sp. nov., isolated from the natural environment, Finger Lakes National Forest," International Journal of Systematic and Evolutionary Microbiology, vol. 60, no. 6, pp. 1280–1288, 2010.

[2] A. Leclercq, D. Clermont, C. Bizet et al., "Listeria rocourtiae sp. nov.," International Journal of Systematic and Evolutionary Microbiology, vol. 60, no. 9, pp. 2210–2214, 2010.

- [3] R. S. Hellberg, K. G. Martin, A. L. Keys, C. J. Haney, Y. Shen, and R. D. Smiley, "16S rRNA partial gene sequencing for the differentiation and molecular subtyping of *Listeria* species," *Food Microbiology*, vol. 36, no. 2, pp. 231–240, 2013.
- [4] J. A. Vázquez-Boland, G. Domínguez-Bernal, B. González-Zorn, J. Kreft, and W. Goebel, "Pathogenicity islands and virulence evolution in *Listeria*," *Microbes and Infection*, vol. 3, no. 7, pp. 571–584, 2001.
- [5] D. Liu, A. J. Ainsworth, F. W. Austin, and M. L. Lawrence, "Use of PCR primers derived from a putative transcriptional regulator gene for species-specific determination of Listeria monocytogenes," *International Journal of Food Microbiology*, vol. 91, no. 3, pp. 297–304, 2004.
- [6] B. Becker, S. Schuler, M. Lohneis, A. Sabrowski, G. D. W. Curtis, and W. H. Holzapfel, "Comparison of two chromogenic media for the detection of *Listeria monocytogenes* with the plating media recommended by EN/DIN 11290-1," *International Journal of Food Microbiology*, vol. 109, no. 1-2, pp. 127–131, 2006.
- [7] R. L. T. Churchill, H. Lee, and J. C. Hall, "Detection of *Listeria monocytogenes* and the toxin listeriolysin O in food," *Journal of Microbiological Methods*, vol. 64, no. 2, pp. 141–170, 2006.
- [8] U. Gasanov, D. Hughes, and P. M. Hansbro, "Methods for the isolation and identification of *Listeria* spp. and *Listeria* monocytogenes: a review," FEMS Microbiology Reviews, vol. 29, no. 5, pp. 851–875, 2005.
- [9] ISO 11290-1:1996, Microbiology of food and animal feeding stuffs—Horizontal method for the detection and enumeration of Listeria monocytogenes—Part 1: Detection method. (Brussels), 1996.
- [10] ISO 11290-1:1996/Amd 1:2004, Microbiology of food and animal feeding stuffs—Horizontal method for the detection and enumeration of Listeria monocytogenes—Part 1: Detection—Amendment 1: Modification of the isolation media and the haemolysis test, and inclusion of precision data. (Brussels), 2004.
- [11] T. Chakraborty, T. Hain, and E. Domann, "Genome organization and the evolution of the virulence gene locus in *Listeria* species," *International Journal of Medical Microbiology*, vol. 290, no. 2, pp. 167–174, 2000.
- [12] P. Glaser, L. Frangeul, C. Buchrieser et al., "Comparative genomics of *Listeria* species," *Science*, vol. 294, no. 5543, pp. 849–852, 2001.
- [13] M. W. Schmid, E. Y. W. Ng, R. Lampidis et al., "Evolutionary history of the genus *Listeria* and its virulence genes," *Systematic and Applied Microbiology*, vol. 28, no. 1, pp. 1–18, 2005.
- [14] E. Gouin, J. Mengaud, and P. Cossart, "The virulence gene cluster of Listeria monocytogenes is also present in Listeria ivanovii, an animal pathogen, and Listeria seeligeri, a nonpathogenic species," Infection and Immunity, vol. 62, no. 8, pp. 3550–3553, 1994.
- [15] J. Johnson, K. Jinneman, G. Stelma et al., "Natural atypical Listeria innocua strains with Listeria monocytogenes pathogenicity island 1 genes," Applied and Environmental Microbiology, vol. 70, no. 7, pp. 4256–4266, 2004.
- [16] M. Perrin, M. Bemer, and C. Delamare, "Fatal case of *Listeria innocua* bacteremia," *Journal of Clinical Microbiology*, vol. 41, no. 11, pp. 5308–5309, 2003.
- [17] J. Rocourt, H. Hof, and A. Schrettenbrunner, "Acute purulent meningitis due to *Listeria seeligeri* in an immunocompetent

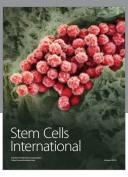
adult," Schweizerische Medizinische Wochenschrift, vol. 116, no. 8, pp. 248–251, 1986.

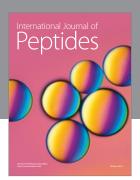
- [18] C. Guillet, O. Join-Lambert, A. Le Monnier et al., "Human listeriosis caused by *Listeria ivanovii*," *Emerging Infectious Diseases*, vol. 16, no. 1, pp. 136–138, 2010.
- [19] L. Pine, R. E. Weaver, G. M. Carlone et al., "Listeria monocy-togenes ATCC 35152 and NCTC 7973 contain a nonhemolytic, nonvirulent variant," Journal of Clinical Microbiology, vol. 25, no. 11, pp. 2247–2251, 1987.
- [20] S. B. Barbuddhe, T. Maier, G. Schwarz et al., "Rapid identification and typing of Listeria species by matrix-assisted laser desorption ionization-time of flight mass spectrometry," *Applied and Environmental Microbiology*, vol. 74, no. 17, pp. 5402–5407, 2008.
- [21] D. Rodríguez-Lázaro, M. Hernández, M. Scortti, T. Esteve, J. A. Vázquez-Boland, and M. Pla, "Quantitative detection of *Listeria monocytogenes* and *Listeria innocua* by real-time PCR: assessment of hly, iap, and lin02483 targets and AmpliFluor technology," *Applied and Environmental Microbiology*, vol. 70, no. 3, pp. 1366–1377, 2004.
- [22] T. A. Hall, "BioEdit: a user-friendly biological sequence alignment program for Windows 95/98/NT," *Nucleic Acids Symposium Series*, vol. 41, pp. 95–98, 1999.
- [23] R. Aznar and P. Elizaquível, "Reliability of Listeria monocytogenes identification by specific PCR assessed by phenotypic and genotypic techniques," Food Analytical Methods, vol. 1, no. 4, pp. 243–251, 2008.
- [24] J. McLauchlin, "The identification of *Listeria* species," *International Journal of Food Microbiology*, vol. 38, no. 1, pp. 77–81, 1997.
- [25] A. Roberts, Y. Chan, and M. Wiedmann, "Definition of genetically distinct attenuation mechanisms in naturally virulence attenuated *Listeria monocytogenes* by comparative cell culture and molecular characterization," *Applied and Environmental Microbiology*, vol. 71, no. 7, pp. 3900–3910, 2005.
- [26] G. Vlaemynck, V. Lafarge, and S. Scotter, "Improvement of the detection of *Listeria monocytogenes* by the application of ALOA, a diagnostic, chromogenic isolation medium," *Journal* of *Applied Microbiology*, vol. 88, no. 3, pp. 430–441, 2000.
- [27] A. Serraino, F. Giacometti, S. Piva, D. Florio, V. Pizzamiglio, and R. G. Zanoni, "Isolation of glucosidase and phospholipase positive *Bacillus circulans* on ALOA medium," *Letters in Applied Microbiology*, vol. 53, no. 2, pp. 244–246, 2011.
- [28] J. Bille, B. Catimel, E. Bannerman et al., "API Listeria, a new and promising one-day system to identify Listeria isolates," *Applied* and Environmental Microbiology, vol. 58, no. 6, pp. 1857–1860, 1992
- [29] R. Aznar and B. Alarcón, "On the specificity of PCR detection of Listeria monocytogenes in food: a comparison of published primers," *Systematic and Applied Microbiology*, vol. 25, no. 1, pp. 109–119, 2002.
- [30] R. R. Beumer, M. C. Te Giffel, M. T. C. Kok, and F. M. Rombouts, "Confirmation and identification of *Listeria* spp.," *Letters in Applied Microbiology*, vol. 22, no. 6, pp. 448–452, 1996.
- [31] M. Vaneechoutte, P. Boerlin, H.-V. Tichy, E. Bannerman, B. Jäger, and J. Bille, "Comparison of PCR-based DNA finger-printing techniques for the identification of *Listeria* species and their use for atypical *Listeria* isolates," *International Journal of Systematic Bacteriology*, vol. 48, no. 1, pp. 127–139, 1998.
- [32] P. A. Gouws and I. Liedemann, "Evaluation of diagnostic PCR for the detection of *Listeria monocytogenes* in food products," *Food Technology and Biotechnology*, vol. 43, no. 2, pp. 201–205, 2005.

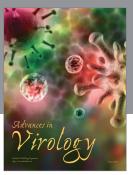
[33] J. Kok, S. C.-A. Chen, D. E. Dwyer, and J. R. Iredell, "Current status of matrix-assisted laser desorption ionisation-time of flight mass spectrometry in the clinical microbiology laboratory," *Pathology*, vol. 45, no. 1, pp. 4–17, 2013.

- [34] P. M. Border, J. J. Howard, G. S. Plastow, and K. W. Siggens, "Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction," *Letters in Applied Microbiology*, vol. 11, no. 3, pp. 158–162, 1990.
- [35] K. J. Cooray, T. Nishibori, H. Xiong, T. Matsuyama, M. Fujita, and M. Mitsuyama, "Detection of multiple virulence-associated genes of *Listeria monocytogenes* by PCR in artificially contaminated milk samples," *Applied and Environmental Microbiology*, vol. 60, no. 8, pp. 3023–3026, 1994.
- [36] S. Jadhav, D. Sevior, M. Bhave, and E. A. Palombo, "Detection of Listeria monocytogenes from selective enrichment broth using MALDI-TOF mass spectrometry," Journal of Proteomics, vol. 97, pp. 100–106, 2014.

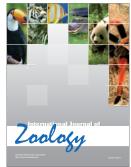


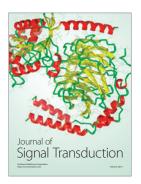






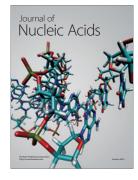






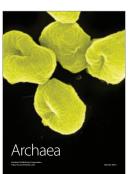


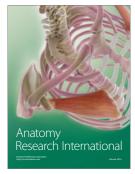
Submit your manuscripts at http://www.hindawi.com











Enzyme

Reséarch





