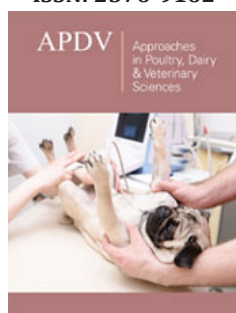


Molecular Detection Methods for *listeria spp.* Occurring in Food Products

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Abstract

Listeria monocytogenes is of growing scientific interest due to the spread in the natural and productive environment of food as well as the increasing number of world-registered cases of listeriosis. *L. monocytogenes* as a food-borne pathogen representative of the genus *Listeria* is associated with 20%-30% of cases of listeriosis. Monitoring and tracking the source of *L. monocytogenes* infection involves the use of molecular biology methods. The use of genetic methods proved to be better than phenotype-based methods. The number of *Listeria* species has increased significantly in recent years, which is related to the development of the latest molecular diagnostics and sequencing methods.

Keywords: *Listeria monocytogenes*; Food; Typing; REA PFGE; MLST

Introduction

Listeria spp. is a group of microorganisms related to bacteria belonging to the species *Bacillus*, *Clostridium*, *Enterococcus*, *Streptococcus* and *Staphylococcus*. These microorganisms are gram-positive, intracellular rod of 0.4 to 1.5µm, optionally anaerobic, which do not form spores. They are characterized by an increase in a wide temperature range of 0-45 °C and pH 6-9 and show the ability to move at temperatures from 10 to 25 °C [1-5]. *Listeria spp.* are widespread in the environment. They are isolated from various environments, including water, sewage, soil, human and animal faeces, but mainly from food products. The most frequently contaminated cheeses and dairy products from unpasteurized milk, raw maturing sausages, smoked fish and industrially produced food, chilled and ready to eat, without prior heat treatment are [6-9,5]. Food intended for sale should not contain more than 100cfu/g during the shelf-life. If these values are exceeded, such food constitutes a threat to the health and life of humans and animals [10].

The species of the genus *Listeria* is the subject of research of many scientists around the world, which results in a continuous increase in the number of species of this genus. By 2015, the genus *Listeria* included 17 species: *L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. grayi*, *L. marthii*, *L. aquatica*, *L. booriae*, *L. cornellensis*, *L. fleischmannii*, *L. floridensis*, *L. grandensis*, *L. newyorkensis*, *L. riparia*, *L. rocourtiae*, *L. seeligeri*, *L. weihenstephanensis*, *L. welshimeri*. In 2015-2019, another 3 species were added to the genus: *L. thailandensis*, *L. costaricensis*, *L. goaensis* [11-16]. *L. thailandensis* was isolated when testing fried chicken samples in Thailand in 2018. Phenotypically, it was very similar to the genus *Listeria*, but it could not be attributed to the previously known species due to the production of acid from d-tagatose and inositol [17]. *L. costaricensis* was isolated from the drainage system of a food processing plant in the province of Alajuela in northern Costa Rica. The colonies of this species are opaque, yellow colored, which is an unusual phenotypic feature for species from the genus *Listeria*. It shows mobility at 37 °C, it is also negative in terms of catalase, hemolysis and nitrite reduction [18-20]. *L. goaensis* is the newest type classified to the genus *Listeria*. It was isolated from the mangrove swamp sediment of the Mandovi River swamp in Goa Province in India. The 16S rRNA gene sequences showed 93.7-99.7% identity of nucleotides with other *Listeria spp.*, which allowed it to be classified as a new type [21].

L. monocytogenes possesses pathogenic features and is the cause of a disease called listeriosis. Impaired immunity, cancer and diabetes contribute to the development of the disease, that include elderly, newborn and pregnant women, allergy sufferers, diabetics, transplants are also susceptible. People at high risk so-called YOPI (Young, Old, Pregnant, Immunocompromised) [22-24]. In addition to *L. monocytogenes*, *L. ivanovii* is another potential pathogenic microorganism within the genus. It is, responsible mainly for infection of animals, wild and domesticated mammals and birds [25]. It is the main etiological factor of listeriosis in sheep and has rarely been associated with human disease [26]. *L. seeligeri* is very rarely found in the course of listeriosis in animals and is associated mainly in the course of mastitis [27]. Listeriosis can occur both in the form of sporadic disease and an genetic outbreak. Therefore, fast and effective methods for identifying these types of rods are needed. They are most effective methods of differentiating representatives of the genus *Listeria*.

Molecular biology methods have found wide application in the diagnosis of listeriosis and the species identification of the pathogens isolated. Currently it is PCR (Polymerase Chain Reaction) that is the main diagnostic method. Using species-specific PCR, bacteria can be identified to species level. It is also possible to use genetic probes that detect genes controlling p60 protein synthesis in the material tested, as well as fragments of the nucleotide sequences of the gene encoding listeriolysin hlyA and the gene encoding the regulatory protein for this prfA toxin. Multiplex PCR techniques that detect fragments of many genes are also used. Typing with the use of molecular methods allows for faster and more efficient detection and identification of epidemic outbreaks caused by infection with *L. monocytogenes* [28]. Available genetic methods of subtyping allow for learning about the genotype, pathogenicity and occurrence of *L. monocytogenes* [29]. The phenotyping methods of *Listeria* rods include macro-restriction analysis of genomic DNA using alternating electric field electrophoresis (REA-PFGE). This method is used to study strains derived from epidemic outbreaks caused by *L. monocytogenes* as well as individual cases of listeriosis. It is characterized by high discriminatory power and repeatability of the results [30,31]. In relation to the epidemic, this method may show affiliation to the same clonal group of pathogens isolated from patients and their food [32].

For a quick comparison of the relationship of strains other genotyping methods are used, based on the polymerase chain reaction (PCR) technique. The most important methods for strains of *L. monocytogenes* are:

A. PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism)-a fragment of one or several housekeeping genes is subjected to amplification and then to restriction analysis using a specific endonuclease or combination of nucleases (HhoI, SacI or HinfI). The products undergo electrophoretic separation and the obtained profiles allow *L. monocytogenes*.

B. AFLP (Amplified Fragments Length Polymorphism) -DNA tested is subjected to restriction analysis using two enzymes: a cutting restriction enzyme and an enzyme that recognizes a few restriction sites in the genome under study. The result of electrophoresis separation is visible as numerous band patterns that correspond to 40 to 200 DNA fragments.

C. Methods from the RAPD group (Random Amplification of Polymorphic DNA), among which RAPD-PCR, AP-PCR, REP-PCR and ERIC-PCR are used most commonly. It is a method used to analyze similarities between strains of microorganisms tested. It involves random amplification of polymorphic DNA fragments using one oligonucleotide (10-20bp) primer. The amplification products are separated on an agarose gel by electrophoresis.

D. MLST (Multilocus Sequence Typing)-this method is based on comparing highly stable allele sequences of selected "housekeeping" genes-coding proteins involved in basic metabolism, conditioning cell survival.

E. MLVA (Multi- Locus Variable-Number Tandem- Repeats Analysis)-this method is based on the analysis of tandem repeats found in DNA. It is mainly used to determine the phylogenetic origin of the strains tested [33].

Molecular biology methods are currently the most effective tool in detecting, identifying and differentiating *Listeria spp.* REP-PCR and ERIC-PCR techniques play the most important role in the commercial identification of *L. monocytogenes* strains. This is related to the speed and ease of performing the test, as well as the high rate of discriminatory power. To describe the species identification different methods are used including techniques of sequencing entire genomes of isolated pathogens. Methods such as MLST or MLVA allow for not only species identification but also to perform a relationship analysis and determine phylogenetic relationships and has been highly efficient and more profitable.

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