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PCR Techniques as An Alternative for Rapid Detection of *Listeria Monocytogenes* in Cheese

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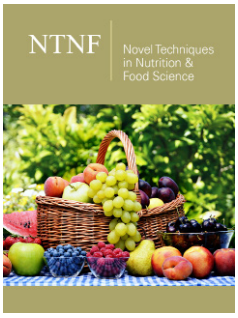

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Abstract

Rapid methods in milk industry ensure quality and safety in finished products. In Mexico, the only techniques to be used in detection of *Listeria monocytogenes* in fresh cheese and dairy products are mainly based on traditional culture methods, here we carry out a narrative minireview of PCR techniques are used to detect *Listeria monocytogenes*, which can be rapid alternative techniques in Mexican milk industry.

Keywords: Detection methods; Listeria; PCR

Introduction

Milk industry has a global market projected to reach more than 1.2 billion USD by 2028 [1]. In Mexico, more than 13 billion liters of milk were produced with a value greater than 112 billion Mexican pesos in 2022, highlighting the states of Coahuila, Chihuahua, Durango, Guanajuato, Jalisco and Veracruz as principal milk producers [2]. Non-fermented or aged dairy products, such as fluid milk and fresh cheeses (e.g. Panela, Ranchero and Oaxaca), provide good conditions for the growth of microorganisms (low acidity, high moisture, low salt content), including *Listeria monocytogenes* [3]. *L. monocytogenes* causes a disease known as listeriosis; a disease transmitted to humans by food consumption often associated with the consumption of fresh cheeses [4]. In Mexico, *L. monocytogenes* has been detected in raw milk (at farms), and manufactured cheeses made either with pasteurized milk (commercial) or with raw milk (artisanal), including Fresh, Ranchero, Chihuahua, Adobera and Panela cheeses [3]. Several reports of detection of *Listeria monocytogenes* in cheese using PCR techniques have been carried out and many reviews in molecular methods to detect this pathogen in dairy products. This paper aimed to provide a narrative minireview of PCR techniques to detect *L. monocytogenes* in cheese, we will focus on giving an overview of the potential of PCR molecular methods to be implemented in protocols of detection of this pathogen in fresh cheeses in Mexico.

Listeria monocytogenes detection methods for fresh cheese in Mexico

Rapid and accurate detection of *L. monocytogenes* in raw materials (raw milk), processing areas and finished product, is important for Mexican diary producers to ensure the quality and safety of fresh cheeses and dairy products [5]. In Mexico, the official standard NOM-243-SSA1-2010 establishes zero tolerance for *L. monocytogenes* (absence in 25g or mL) in milk and dairy products, such as fresh cheeses. The standard also establishes the test method for detection of *Listeria* in foods, mainly based on traditional culture methods, which involve enrichment steps (2 days), plating in selective culture media (2 days of incubation) and finally confirmatory and identification tests (1-5 days), which are based on morphological and biochemical characteristics of this pathogenic bacteria [6]. Although the enrichment culture

method is a standard in the food industry in Mexico, due to lower costs, results obtention require around 5 to 10 days, causing it to be not efficient enough for the dairy industry to make quick decisions.

Molecular detection methods

Implementation of molecular techniques based by in the Polymerase Chain Reaction (PCR) is an alternative to rutinary analysis in cheeses and general food, which can reduce the overall detection time of *L. monocytogenes* (e.g. enrichment about 18h, and detection 4-6h), even when is present in small quantities in cheese, obtaining results (detection, characterization and confirmation of the microbial species) in approximately 1-2 days [7,8], compared to 5-10 days of traditional culture methods [6], allowing Mexican dairy producers to improve production processes and routine practices and the ability for short-term decision making.

In general, molecular methods for the detection of *L. monocytogenes*, such as PCR and biosensors, are mainly based on the detection of nucleic acids such as DNA or RNA, as well as the detection of surface antigens, to identify at the species level, as well as serovar [9-12]. Among the PCR-based molecular methods that have been used for the detection of *Listeria monocytogenes* in cheeses and other foods, there are endpoint PCR, real-time PCR; Droplet digital PCR that detects different pathogenicity genes of the microorganism (PrFA, PRS, Hyla, etc.) Some examples of these methods are briefly described in Table 1; [13-20]. These molecular methods based in PCR allow a rapid detection and they do not present limitations to be used in samples of foods with high fat content such as cheeses [21], but specialized equipment is required (e.g. thermocycler), which increases the initial cost for the implementation of these methods, however the precision, specificity, sensitivity and response time in the analysis not only of *Listeria* spp. but other pathogenic or food spoilage microorganisms, are the alternative trend for microorganisms detection in the short term.

Table 1: Detection of *Listeria monocytogenes* with molecular methods based by PCR.

Molecular Technique	Equipment	Comentaries
End-point PCR	Thermocycler	Lower sensitivity Less specificity Identifies viable and non-viable cells. Electrophoresis is needed to visualize the product at the end of PCR Not automated
Nested-point PCR	Thermocycler	Major sensitivity Major specificity Needs two pair of primers Electrophoresis is needed to visualize the product at the end of PCR Not automated
Real-time PCR (qPCR)	Real-time PCR thermocycler	Higher sensibility Accessibility (commercial kits available) Highly specific Measures PCR amplification during each cycle Requires reference samples or standard curves Uses fluorescent reagents (fluorochromes) Identifies viable and non-viable cells
Multiplex real-time PCR (qPCR)	Real-time PCR thermocycler	Higher sensibility Sensibility may increase false positives due to cross contamination Can detect living populations Measures PCR amplification during each cycle Uses different oligonucleotide pairs, each specific to virulence genes
Droplet digital PCR (ddPCR)	Digital PCR thermocycler	Exact quantification Higher sensibility Requires additional equipment Do not require standards nor reference samples

Recombinase polymerase amplification (RPA)	Water bath Real-time PCR thermocycler	High sensibility when sample was pre-enriched Minimal sample preparation Reaction can operate at temperatures between 37 and 42 °C DNA amplification requires 10-20 min Enzyme can be denatured at >60°C.
Loop-mediated isothermal amplification (LAMP)	Water bath (only for colorimet Real-time PCR thermocycler (only for fluorescence LAMP)ric and turbidimetry LAMP)	High sensibility End product measured by turbidimetry, colorimetry or fluorometry Uses at least 4 to 6 oligonucleotide pairs Amplification requires 30 min

Related to price of the analysis, traditional culture methods, as established by the official Mexican standard NOM-243-SSA1-2010 and depending on the analysis laboratory, have an average price per sample of $800.00 Mexican pesos [22], and using molecular methods based on PCR, already established in a specialized analysis laboratory, this price can be reduced by up to 60% [23]. Therefore, they can be an attractive alternative due to lower costs and time involved for cheese and food producers in general, who send their samples to be analyzed in specialized external laboratories. Nevertheless, there are few laboratories in Mexico that currently perform this type of analysis for the detection of *L. monocytogenes*, and other pathogenic microorganisms, by molecular methods, due to initial investment in the equipment and trained personnel required in this area. But the implementation of these molecular techniques is common at research settings such as University graduate projects, allowing technology transfer to large analysis laboratories and eventually becoming more available to support food producers.

Some molecular methods using real-time PCR are currently valid commercial alternatives for the detection of *L. monocytogenes* in foods within the legislation of countries such as the United States [24] and Canada [25]: BAX® Automated System Assurance, GDS® Listeria spp. Tq Genetic Detection System and iQ-Check *Listeria monocytogenes* PCR Detection Kit. In particular, BAX® Automated System method is used to detect *L. monocytogenes* in food, and it was validated by AOAC International (official method AOAC 2003.12), however molecular methods based by PCR are not part of the official methods to detect *L. monocytogenes* in food, including dairy products in Mexico [6]. Therefore, cheese and other dairy producers in Mexico still cannot resort to non-traditional methods for routine quality and safety control analysis that they must comply with by standard.

Conclusion

Detection of *L. monocytogenes* by molecular methods arises as a need for companies producing fresh cheeses, since they need to know the safety of their cheeses produced, as well as validate the cleaning and disinfection processes within the plant and be able to make decisions about corrective actions, such as the frequency of cleaning and disinfection of the plant, replacement of equipment that does not have a sanitary design, the use of food additives to inhibit *L. monocytogenes* in the finished cheese, among others. The establishment of protocols for the rapid detection of *L. monocytogenes* in fresh cheeses would allow producing companies in Mexico to obtain data faster for making decisions more effectively, at convenient costs and times, directly impacting the improvement of safety and a lower risk of infection by *L. monocytogenes* for consumers of fresh cheeses in Mexico.

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