Evaluation of a rapid molecular method for detection of Listeria monocytogenes directly from broth culture

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Abstract

The present study was carried out to know the lowest detection limit of *Listeria monocytogenes* by PCR. The quantification of organisms was done by CFU counting from ten fold serial dilution and PCR amplification of *inlA* gene fragment was performed from each dilution. The PCR could detect as low as 20 organisms indicating the lowest PCR detection limit. Thus, lowest number of *L. monocytogenes* detectable by PCR is a low-cost and rapid procedure that can be appropriated for the detection in real time of low *L. monocytogenes* levels in naturally contaminated food and is suitable to implement in the food industry.

Keywords: Quantification, CFU counting, Serial dilution, PCR, Low-cost, Food industry**.**

Introduction

Listeria monocytogenes is considered an emerging food-borne pathogen. This kind of bacterium, easily isolated from soil, silage, and other environmental sources (Bad Bug Book, 2006), resists the effects of freezing, drying and heat remarkably well. Agent of listeriosis, a serious infection caused by eating food contaminated with these bacteria, this pathogen has been recognized as an important public health problem in most industrialised countries (USDA/FSIS, 2000). In human listeriosis during the early stages of infection it often displays non-specific flu-like symptoms (e.g. chills, fatigue, headache, and muscular and joint pain) and gastroenteritis. This facultative intracellular pathogen has a unique ability to cross three barriers during infection: the intestinal barrier, the blood-brain barrier, and/or the placental barrier (Lecuit *et al.,* 2004; Vazquez-Boland *et al.,* 2001). However, without appropriate antibiotic treatment, it can develop into septicaemia, meningitis, encephalitis, abortion and, in some cases, death and mortality rates on average approaching 30 %.

Listeric infection in animals is usually acquired by consumption of spoiled silage, in which these bacteria multiply, resulting in herd outbreaks. Encephalitis associated with *Listeria monocytogenes* infection is an important disease of ruminants worldwide (Bakulov and Kotliarov, 1966). Although *Listeria* infection also causes clinical syndromes of abortion or neonatal septicemia, encephalitis localized to the brain stem or spinal cord is the most common illness of adult animals. Listeric mastitis is the most stubborn and difficult to treat and results in culling of the infected animals from a herd (Stewart, 1998).

Materials and methods

The study was carried out at Department of Veterinary Microbiology, Veterinary College, Anand. Reference strain of *Listeria monocytogenes* 4b (MTCC 1143), BHI broth, Potassium tellurite agar, Glasswares, Chemicals and Plasticwares for PCR.

a) Ten fold serial dilution and plating of culture

The ten fold serial dilution was done as per method described by Barocci *et al.*, (2008). The serial dilution was prepared in ten tubes, each tube containing 9.0 ml of Brain Heart Infusion (BHI) broth. Then, 1.0 ml of broth culture was added in first tube, mixed thoroughly, from this tube 1.0 ml of suspension was transferred to second tube containing 9.0 ml of Brain Heart Infusion (BHI) broth and mixed thoroughly. The same process was repeated up to tenth tube.

Plating of serially diluted culture was done by spread plate method by taking an inoculum of 0.1 ml of each diluent was spread over the entire surface of Potassium Tellurite Agar plate. Plating of three plates were done for each dilution and it was incubated at 37^0C for 48 hrs. The count for dilution was determined by taking the average count of the three plates and the multiply by ×10 × dilution factor.

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b) PCR detection of serially diluted culture

1.0 ml of serially diluted culture was taken from each dilution and DNA was isolated by boiling method. The PCR amplification of *inlA* gene fragment was done by *inlA* (F) (5'-CCTAGCAGGTCTAACCGCAC-3') and *inlA* (R) (5'-TCGCTAATTTGGT-

TATGCCC-3') primers (Vines and Swaminathan, (1998). The reaction volume of 25 ml with 3 ml DNA template of each sample performed in a thermocycler (Eppendorf, Germany), with the following program: initial denaturation at 94°C for 3 min, then 35 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 2 min, elongation at 72°C for 1 min and a final extension at 72 °C for 10 min. The PCR products were loaded on 2.0% agarose gel stained with bromo-phenol blue loading dye and visualized with ethidium bromide on a UV light and documented by gel documentation system. A 100 bp DNA ladder was used as a marker.

Results and Discussion

The concentrations of organisms in ten fold serially diluted BHI broth were approximately 2×10^8, 2×10^7, 2×10^6, 2×10^5, 2×10^4, 2×10^3, 2×10^2, 2×10^1 and 2 CFU/ml in different tube corresponding to dilution undiluted, 10^{-1}, 10^{-2},10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8}. The PCR amplification showed the amplification up to 2×10^1 CFU/ml using primer pair of *inlA* gene fragment with band size of approximately 255 bp. The result indicates the lowest detection of *L. monocytogenes* organism was 2×10^1 CFU/ml broth by PCR.

The finding of the present study is partial accordance with Almeida and Almeida (2000) reported the sensitivity of PCR detection of an overnight culture of *L. monocytogenes* Scott A was serial diluted and amplification of *inlA* gene fragment of 760 bp and PCR assay could detect as few as 10 CFU of *L. monocytogenes* in a broth culture.

Barocci *et al.*, (2008) studied the detection limit by PCR using *hlyA* gene, amplified 267 bp fragment,

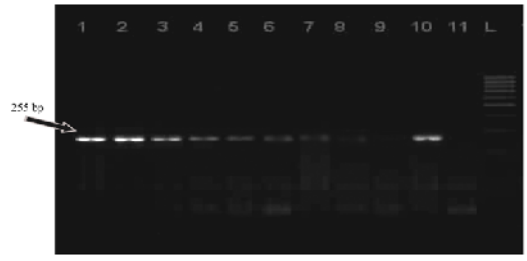

Plate 11 : Agarose gel electrophoresis pattern of *L. monocytogenes inlA* gene
255bp of ten fold serially diluted reference strain of *L. monocytogenes*
L : DNA molecular weight ladder 100 bp
1-9 : Serially diluted sample showing PCR amplification
10 : Positive control
11 : Negative control

of the samples analyzed at 24 h incubation is approximately 3×10^1 CFU/ml, while after the whole 48 h incubation, it is possible to detect 3×10^0 CFU/ml. Longhi *et al*., (2003) reported the lowest levels of *L. monocytogenes* in mozzarella and crescenza cheese homogenates 0.04–0.4 and 4 CFU per gram, respectively whereas in ricotta cheese the detection limit was higher 40 CFU per gram.

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A rapid method for the detection of Listeria monocytogenes in foods combining culture enrichment and real-time PCR was compared to the ISO 11290-1 standard method. The culture enrichment component of the rapid method is based on the ISO standard and includes 24 h incubation in half-Fraser broth, 4 h incubation in Fraser broth followed by DNA extraction and real-time PCR detection of the ssrA gene of L. monocytogenes.Â Listeria monocytogenes is a food-borne pathogen widely distrib-uted in nature. Those most at risk of infection are pregnant women, neonates, newborns, immunocompromised persons and the elderly (Schuchat et al.,1991). Rapid detection and risk assessment of pathogenic organisms, which can endanger health, are necessary to ensure public interests, such as food safety. Therefore, direct and exact quan-tification of pathogenic organisms is becoming more and more relevant. This was emphasized, for example, for foodborne pathogens by Hoorfar (2011) [1]. However, micro-biological methods based on enrichments do not produce quantitative results necessary for an appropriate risk analysis, and they are also both time- and cost-intensive.Â These techniques are mostly derived from molecular biology, such as real-time quantitative polymerase chain reaction (qPCR) detection.Â Quantitative detection of the Listeria monocytogenes prfA locus. Fig. Listeria monocytogenes, a foodborne pathogen that can cause listeriosis through the consumption of food contaminated with this pathogen. The ability of L. monocytogenes to survive in extreme conditions and cause food contaminations have become a major concern.Â Hence, these plating media are not able to provide a rapid detection of L. monocytogenes from foods. This has led to the development of chromogenic media which can improve the isolation of L. monocytogenes as they are able to differentiate L. monocytogenes and/or pathogenic Listeria sp. from other non-pathogenic Listeria sp.Â TABLE 1. Summary of each culture reference method for the isolation and detection of L. monocytogenes in foods and the detection limit of each method. The detection of Listeria spp . traditionally involve culture methods based on selective enrichment and plating followed by the characterization of Listeria spp . based on colony morphology, sugar fermentation and haemolytic properties. This method is the gold standard; but it can take up to five days to obtain a result. Recently, alternative methods have been proposed for the detection of Listeria spp . in food.Â The mini-VIDAS Â® LMO2 was used for detection and confirmation of Listeria monocytogenes and the mini-VIDAS Â® LDUO was used for the simultaneous determination of Listeria sp and Listeria monocytogenes . The immunological techniques described above consist of screening methods that require confirmation of positive results by the conventional method. Rapid screening methods for Listeria monocytogenes. Please note that these methods do not screen for Listeria spp. and therefore may not be suitable for situations in which the identification of Listeria spp. is desired. AOAC Official Method 993.09. Listeria in select foods Colorimetric deoxyribonucleic acid hybridization method (GENE-TRAK Listeria Assay).Â For the approved rapid methods, use the selective isolation agar recommended by the manufacturer but auxiliary use of chromogenic L. monocytogenes-L. ivanovii differential agars is also recommended. Note: The chromogen used in both the R&F LMCPM and RAPIDâ€™ L. mono agars is indicative of phosphoatidylinositol-specific phospholipase C (PI-PLC) activity.