

PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF
NUTRITION

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Detection of *Listeria monocytogenes* Bacteria in Four Types of Milk Using PCR

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Abstract: In this study, detection of *prf A* gene of *Listeria monocytogenes* bacteria in four types of milk (cow, buffalo, camel and sheep) electrophoresis was showed products of gene amplification clear bands at 274 bp. In all samples inoculated with bacteria after 0 h and 24 h. The numbers of bacteria ranging from 44×10^5 - 55×10^5 cfu/ml directly after inoculation and 29×10^2 - 65×10^6 cfu/ml after 24 h at inoculation.

Key words: *Listeria monocytogenes*, milk types, PCR

INTRODUCTION

Listeria monocytogenes is a motile, gram positive, coccobacillus, non-spore forming, aerobic to facultative anaerobic and an intracellular bacterium within leukocytes (Rocourt, 1988). *L. monocytogenes* has been causative agent of major food-borne epidemics in which dairy products including cheese, raw milk and pasteurized milk have been incriminated as the contaminated foods (Vardar-Unlu *et al.*, 1998).

The classical detection method for *L. monocytogenes* in milk and milk products involves enrichment for 48 h and subsequent colony formation on selective agar medium for 48 h, followed by a set of biochemical and morphological confirmation tests which typically take days to complete (IDF, 1990). This process takes a minimum of 5 days to confirm a sample free of *Listeria* and about 10 days to characterize to the species level (Niederhauser *et al.*, 1992).

If a 24-h detection method is preferred, total analysis time for DNA extraction and PCR detection methods in high-throughput volumes must be within approximately 4-5 h after a 19-20 h enrichment process. Fluorescence technology has aided in significantly decreasing post-PCR analysis time by replacing gel electrophoresis steps for PCR product detection (Cano *et al.*, 1995).

The aim of this study was use of PCR in the detection of *Listeria monocytogenes* in types of milk (cow, buffalo, camel and sheep) samples.

MATERIALS AND METHODS

Bacterial strains and growth conditions: The standard strain of *Listeria monocytogenes* ATCC 9525 was obtained from (ATCC; Manassas, Va., USA) and used for artificial contamination of pasteurized milk samples. Standard strain was grown on Brain-Heart Agar (BHA) (Hi-media, India) and buffered peptone water at 37°C.

Milk samples and preparation: Milk samples were obtained from the Agricultural Research Station in Agriculture college at Basrah University, 25 ml of pasteurized milk (cows, buffalo, camel and sheep) for the inoculated bacteria *Listeria monocytogenes* ATCC

9525 by 1 ml contains 1×10^5 cfu/ml DNA was extracted from 10 mL of milk Samples, after 0 h and 1 mL after 24 h of incubation at 37°C.

Amplification of DNA extracted from milk: DNA in all the eluate was extracted by standard methods. 2.5 volumes of ethanol and 1/10 volume of 0.3 M sodium acetate pH 5.2 were added to the sample. After 30 min at -20°C, the sample was centrifuged at 15000 g for 15 min, washed with 50 μ l of 70% ethanol, centrifuging for 5 min as above and resuspended with 20 μ l of nuclease-free water (Sambrook *et al.*, 1989). 10 μ l of the extracted DNA were used for PCR assay.

As a positive control, 5 μ l of *L. monocytogenes* ATCC 9525 DNA, obtained by lysis at 100°C, were included. PCR and PCR product detection by gel electrophoresis.

Multiplex PCR assay: The *LM lip1* primers (Forward 5-GAT ACA GAA ACA TCG GTT GGC-3 Reverse 5- GTG TAA TCT TGA TGC CAT CAG-3) 274 bp are specific for the *prf A* gene of *L. monocytogenes* serovar. The reaction mixture consisted of 2 μ l of extracted DNA, 2.5 μ l of 10 x PCR buffer, 1.5 μ l MgCl₂ (50 mM), 0.5 μ l dNTP (10 mM), 1.25 μ l of each primer, 0.4 μ l of Taq DNA polymerase (5U/ μ l) and deionized water to a final volume of 25 μ l. The reaction mixture was amplified in a thermocycler (Thermo, U.S.A.) with the following PCR conditions: denaturation at 94°C for 5 min, 33 cycles with denaturation at 94°C for 45 sec, annealing at 56°C for 30 sec and extension at 72°C for 1 min and final extension at 72°C for 5 min. The PCR product was separated by electrophoresis in 1.5% agarose gel at 100 V for 40 min in Tris-acetate buffer, visualized by ethidium bromide staining, illuminated by UV transilluminator. A 100-1500 bp DNA ladder was used as a size reference for PCR assay (Jami *et al.*, 2010).

RESULTS

The detection method for *L. monocytogenes* was applied in 10 and 1 ml of types milk (cows, buffalo, sheep, camel and goats), The samples was artificially contaminated with *L. monocytogenes* (Table 1).

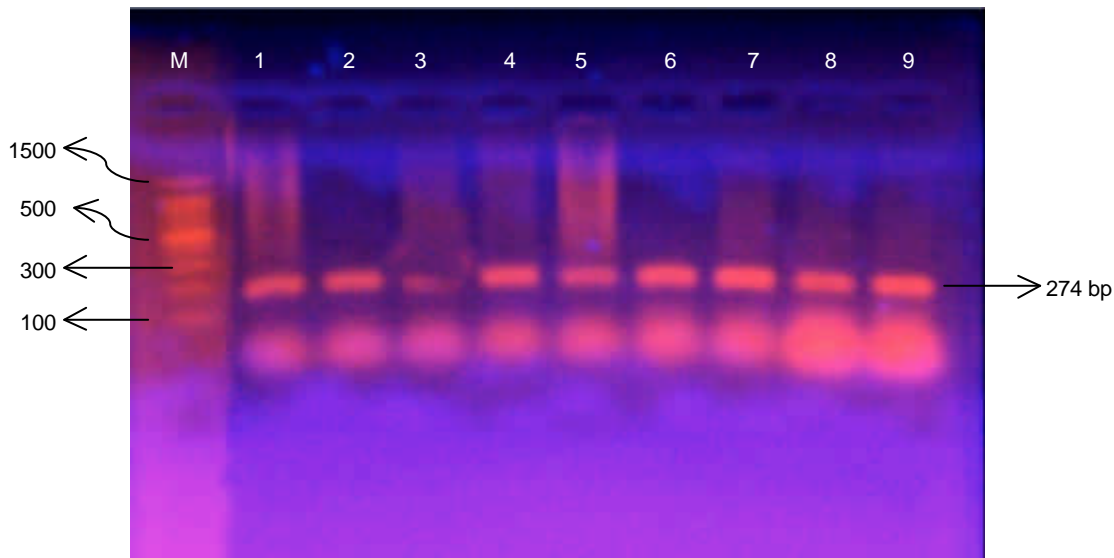


Fig. 1: Agarose gel electrophoresis of the amplification *LM lip1* gene obtained from artificial milk samples and *L. monocytogenes*. Lanes: M, Marker (1), (2) (3) and (4) The milk (cow, buffalo, camel and sheep) samples after 0 h of incubation. (5), (6), (7) and (8) The milk (cow, buffalo, camel and sheep) samples after 24 h of incubation, Lane (9) The *LM lip1* gene extract from *L. monocytogenes* ATCC 9525

Table 1: The numbers *L. monocytogenes* ATCC 9525 in types milk samples

Times (h)	Numbers <i>L. monocytogenes</i> ATCC 9525 (cfu/ml)			
	Cow's milk	Buffalo milk	Sheep's milk	Camel's milk
0	52x10 ⁴	49x10 ⁴	55x10 ⁴	44x10 ⁴
24	65x10 ⁶	43x10 ⁶	104x10 ⁶	92x10 ²

Numbers of *L. monocytogenes* ATCC 9525 in the milk samples were convergent after inoculation, while the numbers were different after 24 h of incubation in the samples ranged between (10⁵-10⁶) cfu/ml in cow's milk and buffalo milk and sheep's milk and did not increase numbers in the milk of camels.

The PCR assay, using *LM lip1* primers that amplify a 274 bp fragment of the *prf A* gene of *L. monocytogenes* was performed. Using this method, the contamination rates of the raw milk samples with *L. monocytogenes* were determined as types milk after 0 and 24 h of inoculation (Fig. 1). The results showed that these primers can detect at least 4.4x10² cfu/ml of bacterial cells in samples milk and in specificity test the results clearly indicated that the primers have a high affinity for the correct target sequence and are specific for *L. monocytogenes*.

DISCUSSION

The detection of pathogenic bacteria is a fundamental objective of food microbiology ensuring food quality. The current microbiological culture procedures are laborious and time-consuming. Consequently, there is an increased need for a rapid and reliable detection

method to guarantee food safety. Regarding this, PCR technology has successfully shortened analysis time and has been widely applied for the detection of food borne pathogens (Bruce, 1994). The PCR technology was using for the detection of *L. monocytogenes* bacteria in four types of milk and bacteria numbers ranged between (44x10-55x10) cfu/ml directly after inoculation and (29x10²-65x10⁶) cfu/ml after 24 h at inoculation. The *hlyA* gene of *L. monocytogenes* specific product was reproducibly detected and showed a sensitivity of 10 cfu/ml (Amagliania *et al.*, 2004). Finally the possibility of detection of *L. monocytogenes* bacteria in the four types of milk (cows, buffalo, camel and sheep) by using PCR even if the numbers a few bacteria.

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