Listeria monocytogenes are a relatively new emerging pathogen. Listeria monocytogenes has also been found in raw or processed foods like meat and seafood\(^1\)-\(^2\). Listeriosis is a food borne infection with flu-like symptoms in healthy people and severe complications in immuno-compromised children, pregnant women and elderly person\(^3\). It is a very persistent microorganism that survives on surfaces and equipment of food processing units in conditions of insufficient cleaning. Post-processing contamination is the major source and cross-contamination which may also occur at the retail shop due to improper hygienic practices.

The conventional culture methods used for the isolation of bacteria are laborious and time-consuming. Hence, there is a thrusting need to develop a rapid and reliable method to detect the emerging food pathogens in the meat while processing to deliver safe and quality meat and meat products to the consumers. Polymerase chain reaction (PCR) is a rapid method with both high sensitivity and specificity for rapid detection and identification of pathogenic bacteria from different food matrix. Hence, the present study was undertaken to develop PCR protocol for detection of Listeria monocytogenes from chicken.

**MATERIAL AND METHODS**

The reference strain of Listeria monocytogenes (MTCC 657) was obtained from Institute of Microbial Technology (IMTECH), Chandigarh. The DNA was extracted by three methods - Bacterial DNA extraction kit method (Qiagen), Phenol-Chloroform-Isoamyl alcohol (25:24:1) extraction method and Boiling method.

The genus specific primer was designed targeting prfA gene: L-Forward: 5' GAGCTATGTGCGATGCCACTT 3' and L-Reverse : 5'ATTAGCGAGCAGGCTACCGCAT 3', were designed. A 20 µl of the reaction mixture was set up in 0.2 ml PCR tube with following components such as master mix - 10µl, forward primer-1 µl, reverse primer-1 µl, template DNA-1 µl and nuclease free water-7 µl. The PCR amplification was carried out in Master Cycler Gradient Thermo cycler (M/s. Eppendorf, Germany) with the following cycling conditions of initial denaturation at 94ºC for 5 minutes, followed by 30 cycles of denaturation (94ºC for 30 seconds), annealing (52ºC for 30 seconds) and extension (72ºC for 30 seconds) and subsequently a final extension at 72ºC for 7 minutes. The PCR product obtained was subjected to electrophoresis in 2% Agarose gel incorporated with Ethidium bromide (10mg/ml was added at the rate of 5µl / 100 ml of Agarose) and electrophoresis was carried out using 1X TAE buffer at 100 volts for 30 minutes. The gel was viewed under UV illuminator and documented using gel documentation system (BioRad, USA).

DNA from reference strain was extracted and quantified using Biophotometer plus (M/s Eppendorf, Germany). The quantified DNA was serially 10 fold diluted in sterile nuclease-free water and for each dilution, PCR amplification was carried out. The sensitivity of PCR in terms of DNA concentration was determined. The highest dilution of the DNA showing a visible band in the gel was taken as the detection limit for Listeria monocytogenes. The chicken meat sample which was negative by culture was taken as the detection limit for Listeria monocytogenes. The chicken meat sample which was negative by culture was used as the positive control.
Table 1: Purity and concentration of DNA extracted by different protocols.

<table>
<thead>
<tr>
<th>DNA Extraction Method</th>
<th>Purity (OD ratio)</th>
<th>Concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qiagen Bacterial DNA extraction kit</td>
<td>1.85 ± 0.02</td>
<td>653.83 ± 0.67</td>
</tr>
<tr>
<td>Phenol-Chloroform-Isoamyl alcohol</td>
<td>1.85 ± 0.02</td>
<td>593.83 ± 0.79</td>
</tr>
<tr>
<td>Boiling</td>
<td>1.08 ± 0.01</td>
<td>800.83 ± 0.60</td>
</tr>
</tbody>
</table>

for *Listeria monocytogenes* as confirmed by culture methods was used for artificial inoculation study. The bacteria in respective tenfold dilutions from $3 \times 10^8$ CFU/ml to $3 \times 10^2$ CFU/ml (0.2 ml of each dilution) were inoculated together in 1.8 ml of meat homogenate obtained by homogenizing 25 gram of chicken meat in 225 ml of BPW. The minimum detection level (sensitivity) for PCR in terms of the number of organisms was determined.

**RESULTS AND DISCUSSION**

The purity of DNA (OD ratio 260nm: 280 nm) extracted by three different methods are presented in Table-1. The OD ratio between 1.7 and 1.9 was considered good. For the boiling method, the purity of DNA was 1.06 for *Listeria monocytogenes* and it was not considered good. The reason for not getting good purity may be due to the presence of other compounds than DNA and the other materials are not properly separated or removed during extraction procedure. Among the two extraction methods, Qiagen kit method was best because of higher concentration and purity of DNA which may be due to the presence of spin column which can easily remove or filter the other impurities leaving the DNA. Even though the kit method was considered as best, Phenol-Chloroform-Isoamyl alcohol (25:24:1) extraction method was cost-effective. Hence Phenol-Chloroform-Isoamyl alcohol method was standardized and used for further screening of retail chicken meat.

**Standardization of PCR:** Standardization was performed using DNA extracted from standard culture (MTCC 657) using the genus specific primers designed for this study targeting the *prfA* gene. DNA of *Listeria monocytogenes* was amplified which gave the product size of 290 bp (Fig.-1). The amplified PCR products of *prfA* gene revealed 290 bp when examined in 2% agarose gel. Similarly, a PCR assay was carried out for detection of *Listeria monocytogenes* by amplification of *prfA* gene in cold smoked salmon and also detected *Listeria monocytogenes* from milk by amplifying *prfA* gene.

**Table 1 : Purity and concentration of DNA extracted by different protocols.**

<table>
<thead>
<tr>
<th>DNA EXTRACTION METHOD</th>
<th>Purity (OD ratio)</th>
<th>Concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qiagen Bacterial DNA extraction kit</td>
<td>1.85 ± 0.02</td>
<td>653.83 ± 0.67</td>
</tr>
<tr>
<td>Phenol-Chloroform-Isoamyl alcohol</td>
<td>1.85 ± 0.02</td>
<td>593.83 ± 0.79</td>
</tr>
<tr>
<td>Boiling</td>
<td>1.08 ± 0.01</td>
<td>800.83 ± 0.60</td>
</tr>
</tbody>
</table>
The samples were tested using Hunter Lab Mini Scan XE plus Spectro Co.

Minimum detection level: The sensitivity (detection limit) of PCR for *Listeria monocytogenes* was found to be 0.1 ng/µl of DNA in a reaction mixture (20 µl) (Fig.-2). The sensitivity of primers was determined by assessing the minimum amount of DNA required for PCR to detect *L. monocytogenes*. The sensitivity of *L. monocytogenes* was 0.5 µg of DNA per 25 µl of reaction mixture. The detection limit of *Listeria monocytogenes* was found to be 10 copies/µl from food samples like ground meat, beef, pork, etc.

Sensitivity of artificially inoculated *Listeria monocytogenes* in chicken meat by PCR: The meat sample artificially inoculated with the reference strain of *Listeria monocytogenes* (MTCC 657) from 3 x 10^8 CFU/ml to 3 x 10^3 CFU/ml. The DNA was extracted and the developed PCR technique was able to detect *Listeria monocytogenes* up to 3 x 10^4 CFU/ml of meat homogenate (Fig.-3). A study reported a detection level of 10^5 CFU/ml in 500 µl of pre-enrichment broth without incubation and 3 x 10^1 CFU/ml after incubation. The detection levels are higher than other who detected *Listeria monocytogenes* from pork sausage and mozzarella cheese at a contamination level of 1 CFU/g before culture enrichment.

**CONCLUSION**

The PCR assay developed was found to be highly sensitive and specific for detection of *L. Monocytogenes* and can thus be employed for rapid detection in the food matrix.

**ACKNOWLEDGEMENTS**

The authors are thankful to the Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 051, India for providing the financial support and facilities and the Dean, Madras Veterinary College for providing the necessary facilities to carry out the research work and are duly acknowledged.

**REFERENCES**