Detection of Salmonella Typhimurium in Artificially Inoculated Chicken Meat by PCR Technique

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ABSTRACT

A set of primers derived from fli C gene was employed to standardize the PCR assay for detection of Salmonella Typhimurium in artificially inoculated chicken meat. In the present study, the minimum detection level of Salmonella Typhimurium was found to be 3.7 cfu/ml or 0.01 cfu per PCR reaction and enrichment in tetrathionate broth was observed to be a suitable broth to detect very low levels of Salmonella.

Keywords: fli C gene, PCR technique, Salmonella Typhimurium, sensitivity, specificity

Introduction

Salmonella enterica serovar Typhimurium causes outbreaks of salmonellosis in a wide variety of animals including human, mouse and chicken (Lim et al., 2003). Salmonella is also one of the most important pathogens involved in human foodborne illnesses both in developed and developing countries. Food safety hazards caused by foodborne Salmonella Typhimurium remain a major problem for the food industry, particularly poultry processors (USDA, 2003) and it is the most frequently isolated serovar from global foodborne outbreaks (Lim et al., 2003). The rapid, cost-effective, and automated diagnosis of foodborne pathogens throughout the food chain continues to be a major concern for the industry and public health. PCR has become a powerful tool in microbiological diagnostics during last decade (Sachse, 2003). The present study was envisaged to develop a rapid, sensitive and specific PCR assay to detect Salmonella Typhimurium from artificially inoculated chicken meat and to evaluate the threshold sensitivity of PCR assay.

Material and Methods

Bacterial strains

The various Salmonella serotypes viz., S. Typhimurium, S. Enteritidis, S. Virchow were obtained from Department of Veterinary Microbiology, C.V.Sc, Rajendranagar. Other cultures like S. Typhi and S. Gallinarum maintained in the Department of Veterinary Public Health, C.V.Sc, Rajendranagar were utilized.

Template preparation

About 1000 µl of the 24 h old broth culture was centrifuged at 6000 rpm for 5 min and resuspended in 50 µl of molecular grade water. The suspension was then kept in a boiling water bath for 10 min and immediately transferred onto ice and was centrifuged with ultracentrifuge (Beckman- Optima TLX 120) at 13000 rpm for 5 min. For PCR technique, 5 µl of supernatant was used as template.

PCR Protocol

PCR assay was performed in MC gradient (Eppendorf) thermal cycler. The nucleotide sequence of the primers used were Fli 15 (22): 5’-CGG TGT TGC CCA GGT TGG TAA T-3’ and Typ

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(16): 5’- ACT GGT AAA GAT GGC T-3’ custom synthesized by Integrated DNA Technologies (IDT). The reaction mixture consisted of 5 µl of the bacterial lysate, 2.5 µl of 10x assay buffer for Taq polymerase, 1.5 mM MgCl₂, 1 µl of 25 µM each dNTP mix, 1 µl each of forward and reverse primer (4 pmol) and 0.9 U/µl of Taq DNA polymerase made up to 25 µl using molecular grade water. Routinely, master mix was prepared and 20 µl each was distributed to the PCR tubes, to which 5 µl of the template was added. Amplification was carried out with initial denaturation at 94°C for 5 min, followed by 35 cycles each of denaturation at 94°C for 1 min, annealing at 45.1°C for 30 sec and extension at 72°C for 38 sec with a final extension period of 72°C at 7 min. The products of PCR amplification were analyzed by agarose gel electrophoresis using 1.5% agarose gel containing 0.5 µg/ml ethidium bromide (Biogene, USA) at constant voltage 80 V for 30 min in 1X TAE.

Specificity of the PCR assay
The specificity of the PCR assay for primers (Fli 15 and Typ 04) from fli C gene was validated by subjecting various serotypes of Salmonella cultures mentioned earlier.

Evaluation of threshold sensitivity of PCR assay
The sensitivity of the PCR assay was evaluated by subjecting serial ten fold dilutions of a pure culture of S. Typhimurium, ranging from 3.7x10⁷ cfu/ml to 0.37 cfu/ml to PCR.

Artificial inoculation studies
Artificial inoculation studies in chicken meat were done to determine the ideal enrichment protocol, which could detect the least concentration of inoculum at the earliest by PCR. Homogenized chicken meat was artificially inoculated with S. Typhimurium at the rate of 370 cfu, 37 cfu, 3.7 cfu and 0.37 cfu per 10 g of homogenized meat with a negative control also included in the study. Ninety millilitres of buffered peptone water (BPW) was added to each meat portion and incubated at 37°C. After 8 h of incubation, subcultures were made into four different selective broths, namely Rappaport vasiliadis (RV), tetrethionate (TT), selinite cysteine (SC) and selinite F (SF) broths. The same procedure was repeated at the end of 16 h incubation of BPW. All the broths except SC (37°C) were incubated at 42°C. Aliquots were collected from the enrichment broths at 12 h and 18 h post incubation for PCR assay.

Results and Discussion
Upon standardization, the PCR yielded an amplicon of 620 bp without any spurious product. This was in agreement with the findings of Oliviera et al. (2002). The specificity of standardized PCR assay for primers targeted to fli C gene yielded a
specific PCR product of desired length (620 bp) only for *S. Typhimurium*, which also was in agreement with the results of Soumet *et al.* (1999) and Oliveira *et al.* (2002). The PCR assay exhibited a minimum detection level of 3.7 cfu/ml or 0.01 cfu per PCR reaction. This finding is similar to that reported by Oliveira *et al.* (2002) with the same set of primers. Spiked studies in the present study revealed that 16 h pre-enrichment was sufficient to detect *S. Typhimurium* at all contamination levels by PCR method. These findings are in accordance with the findings of Szabo and Mackey (1999) and Surendran *et al.* (2003). The results of PCR based detection of *S. Typhimurium* from spiked chicken meat revealed that 26 h enrichment (8 h pre-enrichment + 18 h selective enrichment) in tetrathionate (TT) and selenite-F (SF) broths were able to detect the lowest inoculation level (0.37 cfu/ml). This is in concurrence with the results of Schrank *et al.* (2001), who observed the superiority of TT broth from both food and clinical samples. Earlier studies on PCR based detection of *Salmonella* from spiked chicken meat showed that a two step enrichment gave a minimum detection level in the range of 1.7 cfu to 10 cfu per 26 g (Miyamoto *et al.*, 1999, Gado *et al.*, 2000), whereas a single step pre enrichment gave a minimum detection level in the range of 10 cfu to 25 cfu/25 g (Szabo and Mackey, 1999, Lampel *et al.*, 1996). Cocolin *et al.* (1998) and Schrank *et al.* (2001) obtained a threshold sensitivity of 1-10 cells/25 g by using single step selective enrichment.

The results of the present study indicated that the standardized PCR assay for primers from *fliC* gene could be specifically useful for the detection of *S. Typhimurium* with a minimum detection level of 3.7 cfu/ml or 0.01 cfu per PCR reaction. Further, tetrathionate broth would be a suitable broth to detect very low levels of *Salmonella*.

**References**


