STX GENE IN ESCHERICHIA COLI ISOLATES OF PORK AND BUFFALO MEAT ORIGIN

Daljeet Chhabra, K.K. Kunal, S. Chhabil and S.B. Barbuddhe
Department of Veterinary Microbiology
College of Veterinary Science and A.H., Mhow (M.P.)
Corresponding author : daljeetchhabra@rediffmail.com
Received : 8-11-2012 Accepted : 25-12-2012

ABSTRACT

Thirty six *E. coli* isolates recovered from pork meat and twenty nine *E. coli* isolates recovered from buffalo meat were characterized for *Stx* gene. The PCR assay was standardized and the primer set for this gene allowed for amplification of 584 bp. Out of 36 isolates 15 (41.7%) were positive for *Stx* gene from pork samples and out of 29 isolates 15 (51.7%) for *Stx* gene from buffalo meat samples.

KEYWORDS : *E. coli, Stx* gene, buffalo meat, pork

INTRODUCTION

E. coli is a frequent cause of intestinal and extra-intestinal diseases in animals and humans. The carriage of microorganisms and dissemination of enteric contaminants associated with meat continues to represent a major food safety issue. *E. coli* occupies a key position causing intestinal disorders and septicemia following consumption of contaminated meat.

Meat and meat products often have a very complex microflora, which makes isolation procedure even more difficult. Therefore, the molecular technique like PCR is adopted for specific detection of organisms in short time and characterization of their virulent associated genes.

MATERIALS AND METHODS

The study was conducted on thirty six *E. coli* isolates belonging to pork meat and twenty nine *E. coli* isolates belonging to buffalo meat which were maintained at the Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Mhow.

The culture was prepared by inoculating the isolates in Brain heart infusion (Hi Media, Mumbai) and incubating at 37°C for 12-18 hrs (overnight grown culture).

Preparation of material for nucleic acid extraction

Readymade Kit was used for extraction of nucleic acid (Chromous Biotech Pvt. Ltd., Bangalore).

The PCR was standardized for the detection of the virulent gene (*Stx*) of *E. coli* using suitable primer (Table 1) as described by Ewers *et al.* (2003) with suitable modifications.

Table 1: Details of primers used for PCR reaction

Primers	Sequencec (5'-3')	Target gene	Size of amplified product(bp)	Reference
Stx (F)	5'-ATCCTATTCCCGGGAGTTTACG-3'			Bonnet *et*
Stx (R)	5'-GCGTCATCGTATACACAGGAGC-3'	*Stx*	584	*al.* (1998)

PCR conditions were same as described in our previous experiment (kunal et al., 2013).

The PCR tube containing the reaction mixture was tapped thoroughly with finger and then flash spun on a microcentrifuge (Remi, India) to get reactants at the bottom. The DNA amplification reaction was performed in Thermocycler (Eppendorf Research, Germany) with a pre-heated lid. The steps and cycling conditions of thermo cycling for *Stx* gene by PCR were set as Initial Denaturation at 94 ºC for 5 min followed by another Denaturation at 94 ºC for 1 min, Annealing at 55 ºC for 1 min, Extension at 72 ºC for 2 min and Final Extension at 72 ºC for 10 min. PCR products were kept at -20ºC until further analysis by agarose gel electrophoresis. Steps 2 to 4 were set for 30 cycles.

Agarose Gel Electrophoresis : The PCR product was analyzed by submersive agarose gel electrophoresis to resolve the amplified DNA fragment of the target gene.

RESULTS AND DISCUSSION

E. coli producing *Stx* is an important food borne pathogen responsible for sporadic cases to serious outbreaks worldwide. STEC produce potent cytotoxin and causes watery / bloody diarrhea, thrombocytopenic purpura and death (Kaper *et al.*, 2004).

An amplification of 584 bp of *Stx* gene was obtained in present study from *E. coli* isolates as reported by Bonnet *et al.* (1998). In the persent study out of 29 isolates from buffalo meat, 15 (51.7%) were found positive for *Stx* gene. Contrary to our findings various workers (Aslani and Bauzari, 2003; Mora *et al.*, 2007; Zhang *et al.*, 2007 and Vu-Khac and Cornick, 2008) recorded low frequency (10 two 17 per cent) of *Stx* gene. On the other hand Barua *et al.* (2007) reported very low (0.9%) frequency of *Stx* gene.

Out of total 36 isolates from pork samples, 15 (41.7%) isolates showed amplification product size of 584bp for *Stx* gene. Whereas Heuvenlink *et al*. (1999) reported very low per cent (1.4%) from slaughtered pig.

Specificity of single-plex PCR:

Specificity of all the primer sets employed for individual detection of virulence associated genes *Stx* of *E. coli* was evaluated. The PCR primer pairs used in this study were found to be specific to the virulence-associated gene i.e *Stx* (584 bp) of *E. coli*. The primer was found to be specific to the target gene as it specifically amplified the designed product of that gene by PCR.

REFERENCES:

Aslani, M. and S. Bouzari (2003). An epidemiological study on verotoxin producing *E. coli* infection among population of northern region of Iran. *Eur. J. Epidemiol*., **18:** 345-349.

Barua, R., K.N. Bhilegaonkar, R.K. Agarwal, D.K. Singh, S.V.S. Malik, R.S. Rathore, H. Rahman, V.M. Vaidya and B. Sunil (2007). Prevalence and rapid detection of shiga toxin producing *E. coli* in water and ready to eat foods. *J. Vet. Pub. Hlth*., **5**: 75-83.

Bonnet, R., B. Souweine, G. Gautheir, C. Rich, V. Livrelli, J. Sirot, B. Joly and C. Forestier (1998). Non O157:H7 Stx2 producing *E. coli* strains associated with sporadic cases of haemolytic uremic syndrome in adults. *J. Clin. Microbiol*., **36**: 1777-1780.

Ewers,T., T. Janseen and L.H. Wieler (2003). Avian pathogenic *E. coli* (APEC) *Beri Munch Tierarztl Wochenschr*., **116** : 381-395.

Heuvelink, A.E., J.T. Zwartkruis-Nahuis, R.R. Beumer and E. D. Boer (1999). Occurrence and survival of verocytotoxin-producing *E. coli* O157 in meats obtained from retail outlets in The Netherland. *J. Food Prot*., **62**: 1115-1122.

Kaper, J.B., J.P. Nararo and H.L. Mobley (2004). Pathogenic *E. coli*. *Nat. Rev. Microbiol.*, **2**: 123-140.

Kunal, K.K., chhabra, Daljeet and barbuddhe, S.B. (2013) Characterization of E. coli isolates of buffalo meat origin for papc virulence gene. Indian J. Field Vet, **8**-3: 24-26

Mora, A., M. Blanco, J.E. Blanco, G. Dahbi, C. Lopez, P. Justel, M.P. Alonso, and J. Blanco (2007). Serotypes, virulence genes and intimin types shiga toxin producing *E. coli* isolates from minced beef in Lugo (spain) from 1995 through 2003. *BMC Microbiol*., **117**: 13.

Vu-Khac, H. and N.A. Cornick (2008). Prevalence and genetic profiles of Shiga toxin producing *E. coli* strains isolated from buffalo, cattle and goats in central Vietnam. *Vet. Microbiol*., **126**: 356-363.

Zhang, W., M. Zhao, L. Ruesch, A. Omot and D. Francis (2007). Prevalence of virulence genes in *E. coli* strains recently isolated from young pigs. *Veterinary Microbiology*, **123**: 145-152.

❑