ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 9, September 2017

Detection of Toxin Genes in E. Coliisolate PE74 by PCR analysis

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***Abstract:* — E.coliisolatepe74 culture which showed positive for cytotoxic effect was selected for PCR analysis. On genomic DNA isolation, isolate pe74 showed prominent band on agarose gel electrophoresis. Isolatepe74 was analyzed for the presence of stx, stx2f, stx2, stx2c, stx2e, eaeA, stx2dandhlyA using different primers. On PCR amplification isolate pe74 showed no amplified product for Stx1 however only primer dimmers were observed in sample. PCR analysis of isolate pe74 showed positive to stx2f with a band close to 150 bp and a band between 100 and 200 bp for stx2c.**

***Key words*: E. coli, PCR analysis, toxin production**

I. INTRODUCTION

Escherichia coli cells are rod shaped,gram negative, facultative anaerobic bacteria found in lower intestine of human and animals that grows at pH 7, temperature 37ºC. Many strains of E. coli produce a variety of toxins, including Shiga-like toxins (stx). These strains are often referred to as Verotoxin-producing E. coli. Such toxigenic isolates have been identified to cause serious human gastrointestinal disease, often with severe complicating problems that include bloody diarrhea, life threatening condition hemolytic-uremic syndrome (Xiaodonget al., 2010). In the present study eight genes of E. coli are targeted for PCR confirmation, namely stx, stx2f, stx2, stx2c, stx2e, eaeA, stx2dandhlyA. The first major outbreak of STEC O157:H7 infection was first reported in the United States in 1982 and was linked to eating undercooked ground beef from fast-food restaurant chain (Riley et al., 1983). Based on the results obtained, isolate pe74 which showed positive to stx2fand stx2was further subjected to purification method. Hence the study of toxins gene in E. coli isolate was carried out.

II. MATERIALS AND METHODS

2.1*E. coli*CULTURE

*E. coli*isolate pe74 was sub-cultured on Eosin Methylene Blue agar media. Colonies with green metallic sheen on EMB were selected for present study. *E. coli* culture pe74was inoculated into 250 ml nutrient broth and was incubated over night at room temperature with gentle shaking was subjected to Genomic DNA procedure.

2.2GENOMIC DNA EXTRACTION

The pellet from 1.5 ml of overnight culture was resuspended in 500 µl of lysis buffer and incubated at 37°C for 1hour to lyse the cells. Genomic DNA was then extracted by Chloroform. DNA from the aqueous phase was precipitated with isopropanol, washed with 70% Ethanol and air dried. The DNA pellet was dissolved in 50 µl of TE buffer. 1µl of genomic DNA was used to analyze on 0.8% Agarose gel electrophoresis (Sambrook and Russell 1989).

2.3 POLYMERASE CHAIN REACTION (PCR)

Amplification of the *stx*$_1$, *stx*$_{2f}$, *stx*$_2$, *stx*$_{2c}$, *stx*$_{2e}$, *eaeA*, *stx*$_{2d}$ and *hlyA* gene was performed using primer pairs (Wang *et al*., 2002).

PCR was performed as follows in a 0.2 ml thin walled tube.

Components	Quantity
Nuclease free water	15.75 µl
Genomic DNA (0.2 µl/ µl)	2.25 µl
Forward Primer (10 µM)	0.75 µl
Reverse Primer (10 µM)	0.75 µl
10X Reaction Buffer	2.5 µl
dNTP Mix	2.25 µl
Taq DNA polymerase (l µ/µl)	0.75 µl
Total Volume	**25 µl**

The amplification was carried out in thermal cycler by setting the following temperatures and time periods for each step. Initial denaturation of 94°C for 3 minutes followed by

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 9, September 2017

30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 1 minute. Final extension was carried out at 72°C for 5 minutes. 10 µl of PCR products on 1.6% agarose gel along with 1 kb ladder DNA as a standard and run at 100 V for 60 min. The bands were stained with ethidium bromide and visualized under UV-transilluminator. (Sawhney and Singh 2002)

III. RESULTS

The isolate pe74 showed metallic green sheath on Eosin Methylene blue agar (figure 1). On genomic DNA isolation, isolate pe74 showed prominent band on agarose gel electrophoresis (figure 2). On PCR amplification isolate pe74 showed no amplified product for *Stx1* however only primer dimmer were observed in sample (figure 3). PCR analysis of isolate pe74 showed positive to stx_{2f} with a band close to 150 bp and a band between 100 and 200 bp for stx_{2c}(figure 4).

Figure 1: Growth of isolate pe74 on Eosin Methylene Blue agar medium

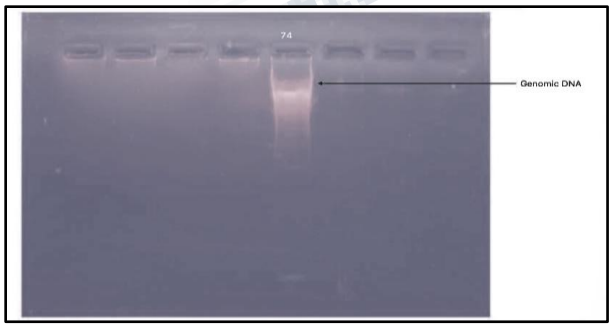

Figure 2: Agarose gel electrophoresis of genomic DNA from E. coli isolate pe74

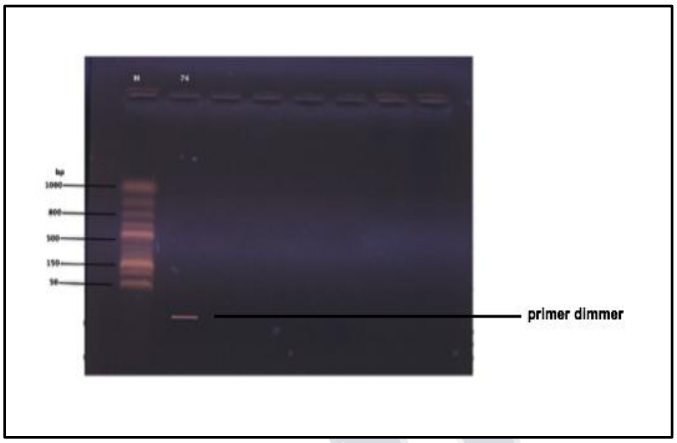

Figure 3: Agarose gel electrophoresis of PCR products of stx_1gene from E. coli isolate pe74Lane M: DNA molecular weight marker, Lane 1: pe74

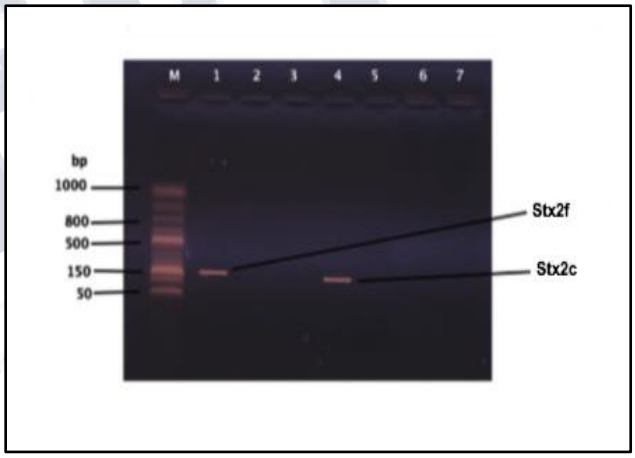

Figure 4: Agarose gel electrophoresis of PCR products of isolate pe74 Lane M: DNA molecular weight marker, Lane 1: stx_{2f}, Lane 2: EHEC hlyA,Lane 3: stx_2, Lane 4: stx_{2c}, Lane 5: eaeA, Lane 6: stx_{2d}, Lane 7: stx_{2e}

IV. DISCUSSION

PCR analysis of isolate pe74 confirmed that *Stx2f* and *Stx2c* were detected. Among Shiga-toxin producing *Escherichia coliStx2f* was recently reported to cause illness among human and difficult to detect immunologically (Skinner *et al.*, 2013).

V. ACKNOWLEDGEMENT

Authors acknowledge their thanks to Jain University for carrying out this present research work. Special thanks to Shri Peter Mathew.

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