RAPID ISOLATION AND DETECTION OF *ESCHERICHIA COLI* O157:H7 BY USE OF RAINBOW AGAR O157™ AND PCR ASSAY

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Abstract. This study has evaluated the use of a commercially available Rainbow agar O157™ and polymerase chain reaction (PCR) assays for the detection of Shiga-like toxin producing *Escherichia coli* and to serotype *E. coli* O157:H7 from raw meat. The Rainbow agar O157™ was found to be selective and sensitive for the screening of the *E. coli* O157 from artificially and naturally contaminated meat samples. Shiga-like toxin producing *E. coli* were identified with two primer pairs that amplified fragments of the SLT-I (384 bp) and SLT-II (584 bp). *E. coli* O157:H7 was serotyped with a primer pair specified for the H7 flagellar gene, which amplify specific DNA fragments (625 bp) from all *E. coli* O157:H7 strains. The use of Rainbow agar O157™ described allows for the presumptive isolation of *E. coli* O157 in 24 hours. Identification and confirmation of the presumptive isolates as *E. coli* O157:H7 by PCR assays require additional 6-8 hours. The above-mentioned screening and identification procedures should prove to be a very useful method since it allows for the specific detection of *E. coli* O157:H7.

INTRODUCTION

Enterohemorrhagic *Escherichia coli* O157:H7 has emerged as an important gastrointestinal pathogen of man which may give rise to serious clinical conditions such as hemolytic uremic syndrome (HUS) and hemorrhagic colitis (Smith and Scotland 1988; Karmali, 1989; Pierard *et al*, 1994). *Escherichia coli* O157:H7 can cause infections through consumption of food and water and by human-to-human transmission. Infection by *E. coli* O157:H7 has now become an important food-borne disease in developed countries (Griffin, 1995). In Malaysia, isolation of *E. coli* O157:H7 has been reported (Son *et al*, 1998). Food industry and public health microbiologists therefore need reliable methods to screen high risk foods for *E. coli* O157:H7. There have been evaluations of various rapid techniques which successfully recovered *E. coli* O157:H7 from naturally contaminated and artificially inoculated samples (Niroomand and Lord, 1994; Bennett *et al*, 1995, 1996). In the present study we combined the use of Rainbow agar with specific PCR to produce a fast and efficient screening procedure which identify the *E. coli* O157:H7 in 30-36 hours.

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MATERIALS AND METHODS

Preparation of artificially and naturally contaminated meat samples

An *Escherichia coli* O157:H7 ATCC culture EDL933 was grown overnight in Luria Bertani broth and spun at 10,000 rpm for 5 minutes. The supernatant was decanted, and the packed cells resuspended in alkaline peptone water (APW) (Oxoid) to give a density of approximately 7 to 8 log CFU/ml. Tenderloin beef, 25 gram each, were placed into individual sterile plastic bag, spiked with 0.1 ml of *E. coli* O157:H7 EDL933, and frozen overnight before analysis. The 25 g of the artificially and naturally contaminated samples were placed into 225 ml of APW in a stomacher bag, homogenized using a stomacher (Colworth 400), and placed in an incubator at 42ºC for 6 hours. A range of dilutions of each sample was made with APW and 0.1 ml volumes were spread on Rainbow agar O157™ and incubated overnight at 37ºC. Rainbow agar O157™ was prepared according to manufacturer's instructions by dissolving 60 g of premixed powder in 100 ml distilled water, boiled until the agar and the other components are completely dissolved and was poured

into petri dishes.

DNA preparation

Single and well isolated presumptive black colony of *E. coli* O157 was transferred into 100 µl of sterile distilled water in a 1.5 ml centrifuge tube and was heated on a water bath at 95ºC for 10 minutes. The heated sample was immediately put on ice. After a 5 minute centrifugation at 10,000 rpm, a 10 µl sample of the supernatant was used in the PCR immediately.

PCR amplification

The oligonucleotide primers used in the study are listed in Table 1. They were synthesized by Genosys Biotechnologies Inc (TX, USA). PCR assays were performed in 25 µl volumes containing 2.5 µl 10x PCR buffer, 2.5 mM $MgCl_2$, 1 mM each (final conc) dNTPs, 5 pmol of respective primer set (SLT-I, SLT-II and flicH7), 1 U *Taq* polymerase and 10 µl of DNA samples extracted from the boiled cells. Amplification conditions were performed as follows: pre-denaturation at 94ºC for 3 minutes, followed by 35 cycles of 1 minute at 94ºC, 1 minute at 65ºC and 2 minutes at 72ºC, and a final elongation at 72ºC for 5 minutes. A negative control without DNA sample was included. The reaction mixtures (15 µl) were fractionated by electrophoresis onto 1.2% agarose gels, and the amplified DNA fragments made visible with *uv* transilluminator after ethidium bromide staining.

RESULTS AND DISCUSSION

Tenderloin beef samples spiked with *E. coli* O157:H7 EDL933 strain were analyzed. Five packages of tenderloin beef were obtained from different retail establishment, and 25 g of spiked and unspiked control samples were prepared. The samples were enriched in APW and the resulting cultures of the artificially and naturally contaminated samples were plated onto Rainbow agar O157™. The results obtained showed that all spiked samples were positive for *E. coli* O157. One unspiked control sample was positive for *E. coli* O157. When the experiment was repeated with the naturally contaminated beef, one of four samples tested was positive for *E. coli* O157.

Well isolated presumptive black colonies of *E. coli* O157 were picked with a sterile toothpick from the marked position areas of the Rainbow agar O157™ plate and transferred into 100 µl of sterile distilled water, boiled and used as source of the template DNA in the PCR assays. Fig 1 shows the results of the PCR amplification of the SLT-I, SLT-II and flicH7 genes of the presumptive culture of *E. coli* O157 from the Rainbow agar O157™ plates. All presumptive cultures of *E. coli* O157 from the Rainbow agar O157™ were positive for the SLT-I, SLT-II and flicH7 genes, and thus were positively identified and confirmed as *E. coli* O157:H7. Elsewhere, other groups of researchers have also described the used of PCR assay employing primer pairs targeting the SLT-I, SLT-II and H7 flagellar genes as a convenient and rapid method for determining *E. coli* O157 serotype H7. The assay provides a more specific and rapid alternative to classical phenotypic methods for detection of *E. coli* O157:H7 (Cebula *et al*, 1995; Fratamico *et al*, 1995; Gannon *et al*, 1997).

Rainbow agar O157™ is a selective and differential medium for isolation and differentiation of *E. coli* O157 from other normal *E. coli* or microflora. Most bacterial species are either inhibited on this medium or grow as white or creamed colored colonies. Typical non-toxigenic *E. coli* could be seen as violet to red, blue or pink colored colonies on Rainbow agar O157™, however, *E. coli* O157 could be observed as black or charcoal grey colony. To

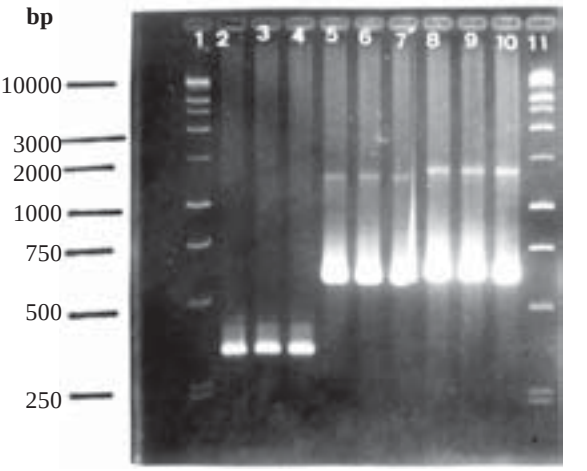

Fig 1–Representative results for the amplification products obtained by PCR assays of selected strains analyzed by agarose (1.2%) gel electrophoresis. Lanes: 1 and 11, 1 kb DNA ladder size marker; 2, 3 and 4, SLT-I fragment (384 bp); 5, 6 and 7, SLT-II fragment (584 bp); 8, 9 and 10, flicH7 fragment (625 bp). Molecular weight size markers in base pairs (bp) are indicated by numbers on the left.

Table 1
Primers used in PCR assays to amplify specific fragments from the genes for SLT-I, SLT-II and the flicH7 gene.

Target	Expected size (bp)	Primer	Sequence	Reference
SLT-I	384	SLTIR SLTIF	5'-CAGTTAATGTGGTGGCGAAGG-3' 5'-CACCAGACAATGTAACCGCTG-3'	Cebula *et al*, 1995
SLT-II	584	SLTIIR SLTIIF	5'-ATCCTATTCCCGGGAGTTTACG-3' 5'-GCGTCATCGTATACACAGGAGC-3'	Cebula *et al*, 1995
FlicH7	625	flicH7R flicH7F	5'-GCGCTGTCGAGTTCTATCGAGC-3' 5'-CAACGGTGACTTTATCGCCATTCC-3'	Gannon *et al*, 1997

resuscitate and amplify *E. coli* O157:H7 in a meat sample, it is usually necessary to enrich the sample in an enrichment broth such as APW, which is not entirely selective for *E. coli* O157 but provides sufficient growth for *E. coli* organism. The short pre-enrichment step will ensure that background microflora will be held to a minimum. Classical methods of detection require selective and confirmational enrichments, biochemical and serological characterization that may take up to 4 days (Johnson *et al*, 1995). From this work, it appears that the Rainbow agar O157™ for *E. coli* O157 in combination with the PCR assays for the SLT-I, SLT-II and H7 genes is a reliable screening confirmation method for this organism.

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