

## Analysis of *Escherichia coli* O157:H7 Isolates from Beef by RAPD-PCR and Plasmid Profiles

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**Abstract:** A total of 70 isolates of *Escherichia coli* O157:H7 isolated from beef samples were examined with respect to plasmid profiles and random amplified polymorphic DNA (RAPD) patterns. All isolates carried the 90 kb pO157 plasmid alone or in combination with other smaller plasmids. Using Gen1-50-02 (5'-CAATGCGTCT-3'), Gen1-50-09 (5'-AGAGGCGATG-3') and Gen1-50-10 (5'-CCATTTACGC-3') as primers, respectively, we obtained DNA polymorphisms which allowed us to discriminate the *E. coli* O157:H7 isolates into one, six and five RAPD patterns; providing bands ranging in size from 0.25 to 4.0 kb. Our results demonstrate that both plasmid profiling and RAPD-PCR fingerprinting methods are suitable tools for a fast and reliable molecular typing of *E. coli* O157:H7. The RAPD-PCR method is more sensitive with respect to the individualization of isolates and that RAPD-PCR assay could be a valuable technique for epidemiological studies.

**Key words:** *Escherichia coli* O157:H7, beef, plasmid, RAPD-PCR.

### Introduction

Interest in the clinical and ecological significance of enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 has increased tremendously during the past decade as this organism has been recognized as agent of haemorrhagic colitis, which can progress to the hemolytic-uremic syndrome or thrombotic thrombocytopenic purpura (Griffin and Tauxe, 1991). The important determinants of disease include the production of a Shiga-like toxin (SLT) or Verotoxin (VT), the ability to cause attaching-and-effacing lesions, and the possession of a large plasmids encoding adhesins and hemolysis (Tesh and O'Brien, 1992; Griffin, 1995). The majority of infections in humans are associated with the consumption of contaminated and improperly cooked beef. In addition, unpasteurized milk, faeces-contaminated vegetables, water, apple cider and other more novel foods have become contaminated and were implicated with *E. coli* O157:H7 infections (Karmali, 1989; Griffin and Tauxe, 1991; Swerdlow *et al.*, 1992; Besser *et al.*, 1993; Feng, 1995; Cody *et al.*, 1999). Identification of the source of contamination of foods with *E. coli* O157:H7 is important to the understanding of the epidemiology of human infection and devising strategies for its control. Thus, this study was undertaken to determine the prevalence of *E. coli* O157:H7 from imported frozen beef. The strains were characterized by the determination of their plasmid profiles and randomly amplified polymorphic DNA (RAPD) patterns.

### Materials and Methods

**Samples, selective agar and immunomagnetic separation reagents:** The beef samples (tenderloins) were taken from different retail outlets in Selangor and Kuala Lumpur. CT-SMAC (Sorbitol MacConkey agar) was used for the isolation of *E. coli* O157:H7 following enrichment and Dynabeads coated with antibodies to *E. coli* O157 (Dynal UK Ltd) were used for the immunomagnetic separation technique.

**IMS procedure:** Forty samples of frozen beef were tested. Twenty-five grams of meat sample were added to 225 ml of modified EC (mEC) broth containing novobiocin (20 mg/ml) (Kyokuto, Japan), in a stomacher bag containing a nylon filter, and homogenized using stomacher. The homogenates were incubated at 37°C without agitation for 6 h. The immunomagnetic separation (IMS) procedure was conducted according to the manufacturer's instructions using 1 ml of the 6 h enrichment culture added to 20 µl of Dynabeads anti-*E. coli* O157:H7. A volume of 50 µl of the resuspended Dynabeads was then inoculated onto CT-SMAC (Zadik *et al.*, 1993) and plated to produce discrete colonies after incubation at 37°C for 18-24 h.

**Confirmation and characterization of isolates:** A maximum of 10 non-sorbitol fermenting (NSF) colonies were randomly selected, and were inoculated onto the surface of fresh CT-SMAC agar. Non-sorbitol-fermenting colonies were presumptively tested for agglutination with a latex test kit (Oxoid DR622) for detection of *E. coli* O157:H7. Latex agglutinating isolates were confirmed biochemically as *E. coli* by API 20E (Biomeriux) and as serogroup O157 by agglutination with *E. coli* O157 latex kit (Oxoid DR620). The isolates were further confirmed as serotype O157:H7 with a primer pair (flicH7R, 5'-GCGCTGTCGAGTTCTATCGAGC-3' and flicH7F, 5'-CAACGGTGACTTTATCGCCATTCC-3') specific for the H7 flagellar gene as described elsewhere by Gannon *et al.* (1997).

**DNA extraction:** Genomic DNA were extracted by the mini-preparation method of Ausubel *et al.* (1987). Extraction of plasmid DNA from *E. coli* O157:H7 isolates, followed by electrophoresis were performed essentially as described by Sambrook *et al.* (1989). The approximate molecular mass of each plasmid was determined by comparison with plasmids of known molecular mass of *E. coli* V517 (Macrina *et al.*, 1978).

**PCR-based fingerprinting and identification:** PCR-based fingerprinting was carried out in a volume of 25 µl containing 20-30 ng of *E. coli* O157:H7 total DNA, 2.5 mM MgCl<sub>2</sub>, 20 pmol of primer, 1 U of *Taq* DNA polymerase (Promega), 250 µM each of dNTPs (Promega). A thermal cycler (Perkin Elmer 2400) was used for amplification. The program for amplification was 30 cycles at 94°C for 2 min, 36°C for 1 min and 72°C for 2 min. A final elongation step of 72°C for 5 min was included. The PCR assays for specific identification of the isolates by their flagellar H7 genes were performed in 25 µl volumes containing 2.5 µl 10x PCR buffer, 2.5 mM MgCl<sub>2</sub>, 1 mM each (final conc.) dNTPs, 5 pmol of the primer H7 set, 1 U *Taq* polymerase and 10 µl of DNA samples extracted from boiled cells. Thirty five cycles were performed, each consisting of 1 min at 94°C, 1 min at 65°C and 2 min at 72°C, followed by one cycle consisting of 5 min at 72°C. After PCR, 10-20 µl aliquots of products were electrophoresed in 1.2% agarose gels, followed by ethidium bromide staining and photography under uv light.

### Results and Discussion

Of recent years, Malaysia has increasingly obtained its supply of beef meat from other countries and this has prompted this investigation since beef meat infected by strains of *E. coli* O157:H7 potentially may serve as reservoirs for these bacteria and aid in their dissemination. Of the 40 samples examined, 70 strains of *E. coli* O157:H7 were isolated from 19 (47.5%) of the beef