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