Detection of Shiga Toxin 1 and 2 (stxl and stx2) genes in Escherichia coli 0157:H7 isolated from retail beef in Malaysia by multiplex polymerase chain reaction (PCR).

ABSTRACT

Twenty (n=20) beef isolates of Escherichia coli O157:H7 were examined for the detection of Shiga- toxin 1 and 2 (stx1 and stx2) genes by multiplex polymerase chain reaction (PCR) and characterized using Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) fingerprinting. All isolates were obtained from the laboratory of Food Science and Biotechnology, University Putra Malaysia, Serdang, Selangor. In the detection of stx1 and stx2 genes, 14 of isolates (14/20) were positive to stx1 and stx2. 5 isolates (5/20) were positive to stx1 and 1 isolate (1/20) was negative by either of stx1 or stx2 genes. Using RAPD-PCR analysis, two oligonucleotides were chosen because they yielded clearly and reproducible band. There were OPAR8 (5'-TGGGGCTGTC-3') and OPAR20 (5' ACGGCAAGGA-3'). Subsequently, all 20 isolates of E.coli O157:H7 were subtyped using OPAR8 and OPAR20. Primer OPAR8 produced 8 RAPD-PCR fingerprinting namely P1 to P11. Whereas, OPAR20 produced 16 RAPD-PCR fingerprinting of Q1-Q18. Combination of two primers was analyzed using Unweighted Pair Group Method with Arithmetic mean (UPGMA). Dendogram performed from cluster analysis showed that all the 20 isolates of E.coli O157:H7 differentiated into 20 individual isolates which may suggest the high level of local geographical genetic variation.

Keyword: Escherichia coli O157:H7; Multiplex PCR; Retail beef; Stx1 and stx2 genes.