DETECTION OF ESCHERICHIA COLI O157: H7 VT AND RFB O157 BY MULTIPLEX POLYMERASE CHAIN REACTION

Apirak Visetsripong¹, Kobchai Pattaragulwanit¹, Jiraporn Thaniyavarn¹, Ryosuke Matsuura², Akio Kuroda³ and Orasa Sutheinkul⁴

¹Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand; ²Kyoto-Biseibutsu-Kenkyusyo, Kyoto, Japan; ³Kyoto City Institute of Health, Kyoto, Japan; ⁴Department of Microbiology, Faculty of Public Health, Mahidol University, Bangkok, Thailand

Abstract. A rapid method for detection of Escherichia coli O157: H7 using multiplex PCR was developed. Two oligonucleotide primer pairs were used for simultaneously detection of vt encoding verotoxin genes for virulence factor and rfb O157 encoding the O-antigen specific for E. coli O157: H7. Multiplex PCR generated two products of 215 bp and 420 bp for vt and rfb O157, respectively. Multiplex PCR detected reference strain O157: H7 (NF-7777) with a sensitivity of 10⁵ CFU per ml with no amplification of other 15 pathogenic bacteria. After incubation of 10² CFU/25 gram raw meat in tryptic soy broth at 37°C for 8 hours, multiplex PCR conducted with the addition of 100 mg bovine serum albumin produced the two specific PCR products for E. coli O157: H7. This modified multiplex PCR is a rapid, sensitive, and specific technique for detecting and differentiating E. coli O157: H7 and has the potential to be used as an alternative to conventional methods for the screening of O157: H7 strains isolated from raw meat.