P12

Characterization of Escherichia coli O157 Isolated from Slaughterhouses and Retail Stores in Korea

Ji Yeon Kim, W. K Bae, S. H. Kim, N. H. Kwon,
J. Y. Lim, J. M. Kim, K. M. Noh, W. K. Jung, and Y. H. Park

Department of Microbiology, College of Veterinary Medicine and
School of Agricultural Biotechnology, Seoul National University,
Seodoon-Dong 103, Kwonsun-Gu, Suwon, Gyunggi, 441-744, KOREA

Introduction

Shiga-like toxin-producing *Escherichia coli* (STEC) is one of the most important recently emerged group of food-borne pathogens. Apart from these shiga-like toxins, *E. coli* O157 group may other virulence factors those associated with the attaching and effacing lesions: intimin encoded by *eae and* hemolysin.

Various genotypic methods have been established useful for species identification, epidemiological typing and determining the genetic relation among these isolates.

There is little known about *E. coli* O157 serotypes in meat and fecal samples in Korea yet, thus we were trying to establish procedure for detecting and epidemiological tracking of this organism, which will be helpful in a national monitoring program such as HACCP. In this study, we use the immunomagnetic separation technique in combination with PCR assay using specific primers targeting the *stxl*, *stx2*, *eae* and *hly* genes. The isolates were characterized by their antibiotic resistance, plamid profiling and randomly amplified polymorphic DNA (RAPD).

Materials and Methods

1. *Samples, selective agar and immunomagnetic separation reagents*

The isolates were isolated from fecal samples and meat samples at random slaughterhouses and retail houses. An ATCC culture, *E. coli* O157:H7 43894 was used as positive control. CT-SMAC agar and Fluorocult agar were used for the isolation of *E. coli* O157 serotype following enrichment and Dynabeads coated with antibodies to *E. coli* O157:H7 (Dynal, UK) were used for the immunomagnetic separation technique.

2. *Immunomagnetic procedure*

Twenty-five grams of meat and fecal samples were added to 225 ml of modified EC (mEC) broth containing novobiocin

(20 mg/l), in a stomacher bag with a nylon filter, and homogenized using stomacher. The homogenates were incubated at 37℃ without agitation 6h. Then, this IMS procesure was conducted according to the manufacturer's instructions. A 50 ul of resuspended Dynabeads was then inoculated onto CT-SMAC agar and Fluorocult agar, and then incubated at 37℃ 20-24 hours. This overnight culture were used as the source of the template preparation in the PCR.

3. PCR assay for stx1, stx2, eae, hly genes amplification

Template was carried out by the boiling method. The PCR primers for *stx1*, *stx2*, *eae*, and *hly* and their PCR amplified products are 614, 779, 890 and 165 bp respectively.

4. Antimicrobial susceptibility tests

Disc diffusion tests were performed with antibiotic-containing disks from BBL Microbiology system. The isolates were tested against the 23 antibiotic disks.

5. RAPD fingerprinting

In this experiments, ten 10-mer random primers with 50% GC content were investigated for RAPD-PCR of isolates. Based on the results obtained primer GENI 5001 (5'-GTGCAATGAG-3') was used for analysis of all *E. coli* O157 isolates.

Results and Discussion

In the PCR assay, the results showed that they all carried stx1 gene alone or in combination with the stx2 gene. Antimicrobial susceptibility to 23 antibiotics showed that all isolates were resistant or intermediately resistant to two or more antibiotics tested. For plasmid profiling, they all had the most common plasmid of 60 MDa. Further, we used DNA fingerprinting by randomly amplified polymorphic DNA (RAPD) to compare the identified *E. coli* O157 strains and we observed these strains had relatively high similarity.

The result has concluded the identification and typing methods used in the study for *E. coli* O157 might be useful for the epidemiological analysis of *E. coli* O157 outbreaks.

Acknowledgments

This study was supported by the Brain Korea 21 Projects.

References

1. Son R., Ooi W. L., et al. J. Microbiol. Methods 46:131-139.
2. Varaporn, V., Nuanjira, P., et al. 2000. FEMS Microbiol. Lett. 182:343-347.
3. Yung-Bu, K. 2001. J. Bacteriol. 31:123-131.