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Epidemiological analysis of *Escherichia coli* O157 : H7 by pulsed-field gel electrophoresis and multiplex polymerase chain reaction

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Abstract : Twenty three strains of *Escherichia* (*E*) *coli* O157 : H7 isolated from Korea, Japan, USA were analyzed by pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested chromosomal DNA and multiplex polymerase chain reaction. Various PFGE patterns of *E. coli* O157 : H7 were found on the same farm. Most of the *E. coli* O157 : H7 strains had shiga-like toxin (*slt*) II gene only (43.5%) or both *slt* I and *slt* II genes (30.4%). *eaeA* gene was highly conserved in the *E. coli* O157 : H7. There was no correlation between PFGE and *slt* gene patterns. The results indicate that various genotypes of *E. coli* O157 : H7 have spread throughout the country and genomic DNA patterns generated by PFGE are highly specific for different strains and have significant value in epidemiologic investigations of infectious disease outbreaks.

Key words : *E. coli* O157 : H7, pulsed-field gel electrophoresis, multiplex polymerase chain reaction.

Introduction

First implicated in food-borne disease in 1982, *E. coli* O 157 : H7 has since been recognized as a significant human pathogen causing abdominal cramps, diarrhea, hemorrhagic colitis, and hemorrhagic uremic syndrome[1,2]. Although the outbreak of food-borne disease associated with *E. coli* O157 : H7 has not been reported in Korea, the pathogen was detected from hamburger and human feces[3,4]. Most of the *E. coli* O157 : H7 infection has been epidemiologically linked to undercooked ground beef[5], water[6] and other foods[7]. The infection with *E. coli* O157 : H7 can be also transmitted by person-to-person interaction[5].

The study on characterization of genetic relatedness among bacterial isolates involved in food-borne disease outbreak is a prerequisite for epidemiologic investigations. During the last decade, traditional methods of strain typing, such as serotyping and bacteriophage typing, have been supplemented or replaced with plasmid fringerprinting[5], ri-

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botyping[8], PCR-based method[9], multilocus enzyme electrophoresis[10] and pulsed-field gel electrophoresis (PFGE)[11]. Among these methods, PFGE is a highly reproducible and discriminating tool for the molecular typing of bacteria and has been successfully applied to a broad range of bacterial species in epidemiological studies of populations where diseases are endemic, as well as in outbreaks[12]. Genetic characterization with restriction enzyme *Xba*I provided the best discrimination with the most easily interpreted patterns and used widely in PFGE of *E. coli* O157 : H7[7].

The present study was performed to evaluate PFGE as a method for subtyping *E. coli* O157 : H7 isolates and to compare PFGE results with virulence genotypes generated by multiplex PCR.

Materials and Methods

Bacterial strains : A total of 23 *E. coli* O157 : H7 isolates were analyzed ; 6 of these were isolated in Korea (5 isolates from cattle feces and 1 from imported beef) and 2 Japanese isolates and 15 cattle feces isolates were kindly provided by Public Health Research Institute in Japan and Cornell university in USA, respectively.

PFGE procedure : *E. coli* O157 : H7 isolates were subtyped using pulsed field gel electrophoresis patterns of *Xba*I-digested chromosomal DNA. Chromosomal DNA of each *E. coli* O157 : H7 isolate was prepared as follows : Isolates were grown in Lauryls broth (Difco, Detroit, USA) to an optical density, 0.3 at 560nm. One ml of the bacterial culture was centrifuged at 14,000×g for 3 min, the supernatant was discarded and the bacterial pellet was suspended in 250μl EET (100mM EDTA ; 10mM EGTA ; 10mM Tris, pH 8.0). The bacterial suspension was then mixed with 350μl of 1.6% chromosomal grade agarose (Bio-Rad, Hercules, CA, USA) in EET, placed into agarose plug molds (Bio-Rad, Hercules, CA, USA) and cooled for 30 min at 4℃. Agarose plugs for each isolate were then placed into 50ml centrifuge tubes containing 1ml of EET with 200μg/ml of lysozyme and 0.05% N-laurylsarcosine sodium (EET-LS) and incubated four hours in a water bath at 30℃. The EET-LS was removed and 1ml of EET with 1mg/ml of proteinase K and 1.0% lauryl sulfate sodium (EET-SP) was added to each tube and incubated overnight in a water bath at 50℃. The EET-SP was then removed, the plugs were rinsed four times by a 30 min soak in 40ml of TE buffer (1mM EDTA ; 10mM Tris, pH 8.0) and then stored at 4℃ in TE buffer. Two slices of agarose plugs were preincubated with 100μl of 1× *Xba*I buffer (Promega, Madison, WI, USA) for 10 min. The buffer was then removed and replaced with a fresh mixture containing 20U of *Xba*I restriction enzyme (Promega, Madison, WI, USA) in 1× restriction buffer and incubated at 37℃ for 16 to 20h. The plugs were then briefly soaked in 0.5× Tris-borate-EDTA (TBE) prior to electrophoresis or, if necessary, stored at 4℃ in 0.5ml of TE buffer for several hours. Electrophoresis of the prepared samples was performed on the GenePath system (Bio-Rad, Hercules, CA, USA) by using pulsed-field certified agarose (Bio-Rad, Hercules, CA, USA) with 2 liters of 0.5× TBE running buffer. The electrophoretic conditions used were as follows : initial switch time, 2.2s ; final switch time, 54.2s ; run time, 22h ; gradient, 6.0V/cm ; temperature, 14℃ ; ramping factor, linear. After electrophoresis the gels were stained for 15 min in 1 liter of distilled water containing 100μl of ethidium bromide (10mg/ml) and destained for 15 min by using 1 liter of distilled water.

PFGE patterns for each isolate were compared using scanned images of photographs of agarose gels. The presence, absence or apparent mobility of a band was considered one difference from the pattern of the strain. Variations in band intensity were not counted as a difference. A Bio-profile-Bio-1D program (Bioprofil-Vilber Lourmat, France) was used to compare the DNA bands more than 200kb for each isolate. This comparison allowed for a 3% margin of error in DNA bands.

***slt* and *eaeA* gene profile** : The presence of the *slt* and *eaeA* genes in the *E. coli* O157 : H7 isolates was investigated by multiplex PCR as described previously[13].

Results

The PFGE patterns of *E. coli* O157 : H7 isolates are shown in Figure 1A and 2A. A total of 20 of the 23 *E. coli*

O157 : H7 isolates had common DNA fragments in about 300kb and 360kb. *E. coli* O157 : Hund (H serotype undetermined) isolate that was sorbitol-positive and *slt* gene-negative phenotype was shown 〉50% differences of the well-resolved fragments in the PFGE patterns from *E. coli* O157 : H7 (data not shown).

W1 and W2 strains were separately isolated from each cattle farm but their PFGE patterns were indistinguishable. Although J18 and J19 strains were isolated in the same cattle farm, they had different PFGE patterns more than 7 fragments (Fig 1A). KQS12 strain was shown 94% similarity with C96 strain by dentrogram (Fig 1B).

Seven of the fifteen strains that were isolated from bovine fecal samples in New York State were very closely related genetically (Fig 2A, Fig 2B). Although some isolates had the same *slt* gene patterns, they were shown various PFGE patterns. On the other hand isolates with different *slt* gene types were shown indistinguishable PFGE patterns (Fig 1A, Fig 1B).

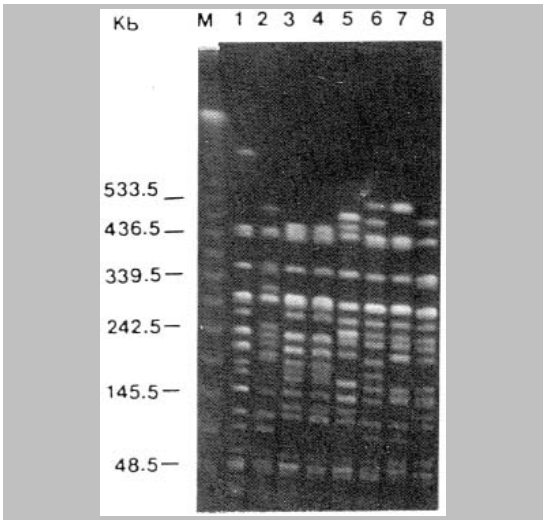

Fig 1A. PFGE of *E. coli* O157 : H7 isolates showing *XbaI*-digested genomic DNA. Lane M, lambda concatemers used as molecular size marker (Bio-Rad, Hercules, CA, USA); lane 1, J18 : lane 2, J19 ; lane 3, W1 ; lane 4, W2 ; lane 5, KQS12 ; lane 6, KSC109 ; lane 7, C73 ; lane 8, C96. Lane 1 to 4 and 6, cattle feces isolates from Korea ; lane 5, isolate from Nebraska beef ; lane 7 and 8, patient isolates from Japan.

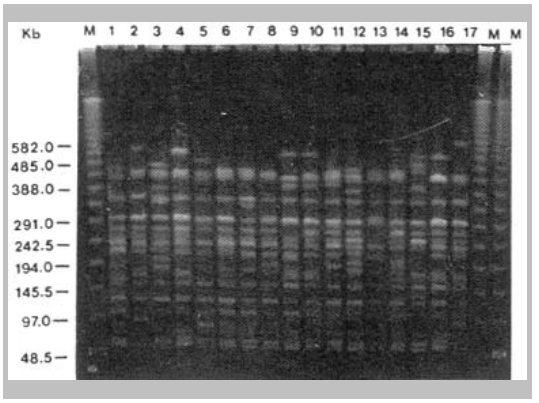

Fig 2A. PFGE of *E. coli* O157 : H7 isolated from New York State dairy cattle. Lane M, lambda concatemers used as molecular size marker (Bio-Rad, Hercules, CA, USA) ; lane 1 to 17 : MISC#8, 4-FS, 897, 516, 93, 82B, 225, 137, 1398, 973, 75, 796, 1489, 211, 529, ATCC 35150, ATCC 43894.

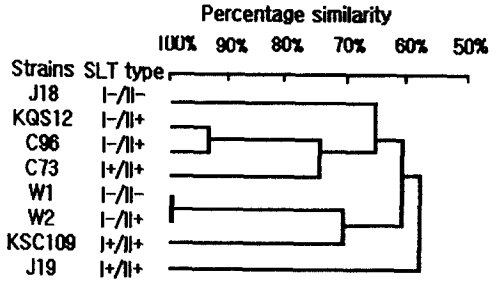

Fig 1B. Dendrogram of cluster analysis based on PFGE of *E. coli* O157 : H7 digested with *XbaI*. The horizontal axis indicates the percentage similarity as determined by the Coincidence Index of Dice. Shiga-like toxin typing was determined on polymerase chain reaction as previously described (Jung *et al*, 1998).

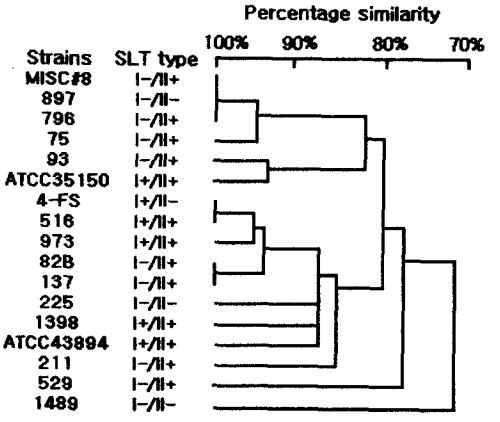

Fig 2B. Dendrogram *E. coli* O157 : H7 isolated from New York State dairy cattle feces according to PFGE in Fig 2A.

Ten (43.5%) of the twenty three *E. coli* O157 : H7 isolates had only *slt* II gene ; seven (30.4%) had both *slt* I and *slt* II genes. Few *E. coli* O157 : H7 isolates possessed only *slt* I gene (4.3%). *eaeA* gene was highly conserved in the *E. coli* O157 : H7 (Fig 3).

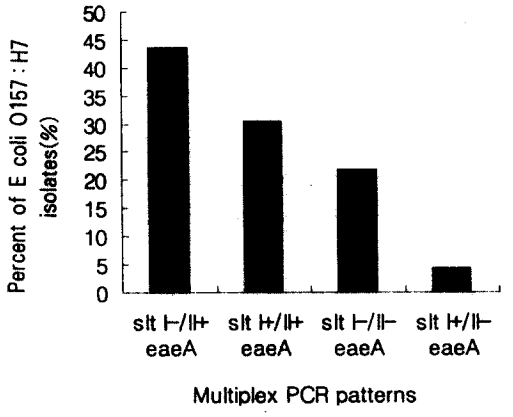

Fig 3. Multiplex PCR analysis of *E. coli* O157 : H7 isolates with primers specific for genes of *slt*I, *slt*II and *eaeA*.

Discussion

Although healthy cattle may carry *E. coli* O157 : H7, other animals may also be a natural reservoir for *E. coli* O157 : H7[14]. The goal of bacterial subtyping is to provide laboratory evidence that epidemiologically related isolates collected during an outbreak of disease are also genetically related. This information is helpful for understanding and controlling the spread of disease.

Currently, there are no standardized criteria for analyzing PFGE. Consequently, different investigators interpret the same PFGE results with different conclusions on isolates which should be designated as outbreak-related and those which should be designated as non-outbreak-related. Barrett *et al*[12] has suggested that isolates with PFGE patterns that differed from those of the outbreak strain by more than one band didn't appear to be outbreak-related. However strains differed from the outbreak strain by a single band couldn't be classified on the basis of PFGE pattern alone. Since a single genetic event can result in as many as three differences, Goering[15] has suggested that isolates with PFGE patterns differed less than four bands should be considered subtypes of the same strain. Tenover *et al*[16] has divided PFGE patterns with following four categories. First, if their restriction patterns have the same numbers of bands and the same apparent size, isolates are designated genetically indistinguishable. Isolates that are indistinguishable by PFGE are unlikely to demonstrate substantial differences by other molecular typing technique. Second, a spontaneous mutation that creates a new chromosomal restriction site will split one restriction fragment into two smaller fragments. Especially variations of two to three bands have been observed in strains of some species when they are cultured repeatedly over time[17]. These kinds of isolates are considered to be closely related. Third, isolates with two independent genetic events, i.e. four to six bands difference, may have the same genetic lineage as that of the outbreak strain. Consequently, they are not as closely related genetically and less likely to be related epidemiologically. Such variation has been observed among isolates collected over longer periods or taken from large numbers of animals involved in extended outbreaks[18]. Finally, isolates with three or more independent genetic events, generally seven or more bands difference, are considered unrelated to the outbreak strains.

Although J18 and J19 strains were isolated from the same cattle farm, they had different PFGE patterns more than 7 fragments (Fig 1A). Daniel Rice observed that there were various PFGE patterns of the *E. coli* O157 : H7 on the same farm and various PFGE subtypes according to the seasonal isolation in the same cattle (personal communication). These results indicate that various genotypes of *E. coli* O157 : H7 are on the same cattle farm. Most of the *E. coli* O157 : H7 isolates (73.5%) had *slt* II gene or both *slt* I and *slt* II genes (Fig 3). These results were similar with a study of Izumiya *et al*[11].

In this study we suggest that various genotypes of *E. coli* O157 : H7 have spread throughout the country. To prevent outbreaks of illness by contaminated food in Korea, coordination of field and molecular epidemiological studies of *E. coli* O157 : H7 outbreaks are essential. Genomic DNA patterns generated by PFGE were highly specific for different strains and had significant value in epidemiologic in-

vestigations of infectious disease outbreaks.

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