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Characterization of *Escherichia coli* Serotype O157 Strains Isolated in Taiwan by PCR and Multilocus Enzyme Analysis

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ABSTRACT

Escherichia coli serotype O157 strains were isolated from fecal samples of cattle and sheep, as well as raw meat in Taiwan from July 1997 to June 1999. In total, six strains of *E. coli* serotype O157 were collected. Of these, only one strain, isolated from fecal samples of sheep, was characterized as *E. coli* O157:H7. The remaining five strains were determined as H41, H45, H? (non-typeable with 43 O-antisera) and NM (non-motile). All six strains were analyzed for pathogenic genes of Enterohemorrhagic *E. coli* (EHEC) by the Polymerase chain reaction (PCR) method. The *E. coli* O157:H7 was the only strain harbored the virulent genes of *slt2*, *eaeA* and *hlyA*. Meanwhile, this strain was unable to ferment sorbitol and lacked β-D-glucuronidase activity. The five non-H7 serotype of *E. coli* O157 strains were further subjected to PCR analysis for other pathogenic genes, including genes in Enterotoxigenic *E. coli* (ETEC, heat-labile enterotoxin, LT, heat-stable enterotoxin, ST), Enteroinvasive *E. coli* (EIEC, invasive plasmid) and Enteropathogenic *E. coli* (EPEC, adherence factor, EAF). Results revealed that the five non-H7 strains did not carry any of the above mentioned genes. To better understand the genetic relatedness among six isolated strains, ten enzyme loci were delineated using the multilocus enzyme analysis. Six strains were designated to five electrophoretic types. Cluster analysis further revealed that the five non-EHEC O157 and one EHEC *E. coli* O157:H7 strains belong to two distinct clones, respectively. These results suggested that non-EHEC O157 and EHEC *E. coli* O157:H7 strains isolated in the Taiwan area most likely originated from two clones. Furthermore, the genetic relatedness among indigenous *E. coli* O157 strains in Taiwan was elucidated through this multilocus enzyme analysis.

Key words: multilocus enzyme analysis, *E. coli*, clone

INTRODUCTION

Escherichia coli, a Gram negative bacillus, is a normal microbial flora existing in human intestines. Some serotypes of *E. coli* could cause diseases or food poisoning. These types of *E. coli* are generally designated as enterovirulent *E. coli* (EEC) and can be divided into six subgroups: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteroadherent *E. coli* (EAEC) or enteroaggregative *E. coli* (EAggEC), and diffusely adherent *E. coli* (DAEC)[1-3].

The first case report regarding the outbreak of *E. coli* O157:H7 in the USA was traced back to 1982. Since then, similar case reports repeatedly appear around the world. Among them, the incidence of *E. coli* O157:H7 occurred in 1996 in Japan was considered as the largest one[4]. *E. coli* O157:H7 is belonging to EHEC. It is named shiga toxin-producing *E. coli* (STEC) because it can produce shiga-like toxin (SLT). It used to be nominated as verotoxin-producing *E. coli* (VTEC)[5]. So far, most studies in STEC focus on *E. coli* O157:H7, but pay less attention to other serotypes of *E. coli*. The other serotypes related to food poisoning include O157:H$^-$, O26:H11, O104:H21, O111:H8, O111:NM, O48:H21, and O48:H$^-$[5].

In addition to Shiga toxin (SLT I and SLT II), the adherence factor and enterohemolysin are considered to be the pathogenicity factors induced by EHEC[5]. STEC is a pathogen that can be commonly passed between humans and animals. Feces of cattle and sheep are natural reservoirs for this bacterium[5-8]. Children and elders are the most susceptible groups to this pathogen. Some severe complications such as hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) may occur as infected by STEC[5].

The infection dosage of *E. coli* O157:H7 is only 10-100 strains. It lacks β-D-glucuronidase activity and is unable to utilize sorbitol for fermentation. However, some variants possess β-D-glucuronidase activity and are able to do sorbitol fermentation[9]. Foods are frequently contaminated with *E. coli* O157. However, those which non-H7 such as serotypes O157:H3, O157:H12, O157:H16, O157:H38, O157:H43, and O157:H45 are unable to induce food poisoning[10]. Some food poisoning outbreaks which have occurred in Taiwan were caused by *E. coli* O157 rather than non-H7 serotypes, and no shiga-like toxin gene was found in those pathogens[11]. Some O157 serotype *E. coli* such as *E. coli* O157:H8, which belongs to EPEC, can be isolated from specimens of diarrhea patients[12].

It has been shown that STEC strain involves more than 100 serotypes. This strain is classified into four categories based on Multilocus Enzyme Analysis (MLEA). They are EHEC1 (including O157:H7, O157:NM and non-toxin producing O55:H7), EHEC2 (such as O111:H8, O111:NM,

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O111:H11, and O26:NM), STEC1 (including many O-serotypes such as O113:H21, OX3:H21, and O91:H21, which can be isolated from cattle in north America, Europe, and Asia), and STEC2 (such as O103:H2, O103:H6, and O45:H2). Both EHEC2 and O157:H7 are designated to EHEC; however, the genetic evolution of EHEC2 is significantly different from that of O157:H7. The former is capable of producing SLT1 and enterohemolysin and possesses *eae A* gene. STEC1 usually carries the H21 flagella antigen but does not carry the LEE pathogenicity island, and neither express the Intimin protein. Most of STEC2 carry H2 antigen. Genetically, STEC2 is different from other STEC based on MLEA[13]. Feng *et al.* proposed that *E. coli* O157:H7 is gradually developed from *E. coli* O55:H7[14]. Other studies regarding further classification of *E. coli* O157:H7 were carried out. Using 16 bacteriophages to classify STEC O157:H7, 80 phage types of *E. coli* O157:H7 were categorized according to studies in Canada and England. Among 80 phage types, 24 were found in England and most were types 1, 2, 4, and 49[15]. Whittam selected 20 enzymes to test 369 strains of STEC O157:H7. Results showed that 95% of test strains belonged to the Electrophoretic type (ET) 11. Meanwhile, they found that a close genetic relationship exists between O157:H7 and O55:H7, revealing that *E. coli* O157:H7 could be derived from the same clone[5]. Nonetheless, the STEC O157:H7 found in Wisconsin (USA) was speculated to derive from many clones. Many sources of contamination could possibly lead to this result[16]. A study on the genetic relationship between non-EHEC *E. coli* O157 and EHEC *E. coli* O157:H7 was carried out by Whittam *et al.* MLEA analysis was performed to test 194 strains of *E. coli* O157 for detecting H-antigen including H6, H7, H8, H11, H16, H20, H31, H34, H38, H42, H43, H52, NM, and H?. Results showed that only one strain was belonging to *E. coli* O157:H7 and 99 strains were *E. coli* O157:H43. The genetic analysis of O157 strains isolated from diverse animal and human sources indicated that the above isolated strains originated from five significant clones. The genetic relationship between *E. coli* O157:H7 and other *E. coli* O157 was found to be significantly different since the genetic distance between them was beyond 0.45. The authors concluded that *E. coli* O157:H7 was not derived from *E. coli* O157[17].

The above studies indicate that *E. coli* O157:H7 has received great attention by many countries around the world. Since July 1996, we have been periodically testing samples of raw meat, raw milk, and feces of animals to monitor the contamination of STEC[18-19] as well as to develop the inspection and typing methods[19]. The practicability of several commercial kits has also been evaluated[20]. The work conducted in our laboratory is intended to provide useful information to related authorities. PCR and MLEA were the methods used to analyze the collected strains in this study. MLEA is based on the molecule weight and electric charge difference among proteins that may lead to different migration speed on gel electrophoresis under a fixed electric field. Using this technique, the enzymes with different structures but same functionality are capable of being differentiated and then developed to colors on gel. The different genetic message sent from nucleic acid could lead to different characteristics of protein structure. Thus, using MLEA to differentiate the characteristics of protein helps in understanding the genetic relationship between species[21]. However, MLEA may lead to underestimation of loci variation such as the variation on silent nucleotide substitution[22]. Some amino acid variations occur, but the molecule weight and electric charge still remain the same; therefore the mobility on gel electrophoresis does not change, another drawback of using MLEA[23].

Until present, we have collected 6 strains of *E. coli* O157. One is serotype O157:H7 and the others are O157:NM, O157:H41, O157:H45, and O157:H?. In Taiwan, an outbreak of *E. coli* O157:H7 has not yet occurred. In this study, for better understanding of the genetic relationship among strains of *E. coli* O157, the PCR method was used to confirm if the pathogenic gene exists in isolated strains and MLEA was performed to analyze the correlation among strains.

MATERIALS AND METHODS

I. *Materials*

(I) *Chemicals*

The following chemicals were used in this study. Novobiocin, isocitrate, inosine, glucose-6-phosphate, NADP, NAD, DL-malic acid, KCN, fructose-6-phosphate, mannitol-1-phosphate, pyridoxal-5-phosphate, O-dianisidine tetrazotized, α-naphthyl acid phosphate, β-naphthyl acid phosphate, Fast black K salt, 6-phosphogluconic acid, N-methylphenazonium methosulfate (PMS), nitro-blue tetrazolium (NBT), and DL-isocitric acid trisodium salt were purchased from Sigma (St. Louis, Missouri, USA). Glycerol, ethanol, isopropanol, citric acid monohydrate, EDTA, sodium dihydrogenphosphate, magnesium chloride hexahydrate, and sodium acetate were obtained from Merck (Darmstadt, Germany). 2-Oxoglutaric acid was purchased from Fluka (Switzerland) and agarose was from Amresco (Solon, Ohio, USA). Xanthine oxidase, and glucose-6-phosphate dehydrogenase were obtained from Boehringer (Mannheim, Germany).

(II) *Instruments*

PCR reactor: equipped with programmable thermal controller (Model PTC-100, MJ Research, Water Town, Mass., USA). Electrophoresis chamber and cooling system: Model Multiphor II (Pharmacia Biotech, Uppsala, Sweden); Electrophoresis power supply: Model EPS 3500 (Pharmacia Biotech, Uppsala, Sweden).

(III) *PCR Primers and Reagents*

Ten pairs of primers (Table 1), which were synthesized

by TIB Molbiol Company (Berlin, Germany), were used in this study. These selected primers were specific to the following *E. coli*. EHEC: *slt*, *hlyA*, *eaeA*; EIEC: invasive plasmid; EPEC: adherence factor; ETEC: heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST). PCR reaction was conducted by using DynaZyme DNA Polymerase kit (Finnzymes, Espoo, Finland).

(IV) *Culture Media*

Eosin methylene blue (EMB) agar and tryptic soy agar (TSA) were purchased from Difo. (Detroit, Mich., USA). Sorbitol MacConkey agar and Fluorocult *E. coli* O157:H7 agar were obtained from Oxoid. (Hampshire, England) and Merck (Darmstadt, Germany), respectively.

(V) *Bacterial Strains*

The reference strains listed below were purchased from the Culture Collect & Research Center at Food Industry Research and Development Institute. *E. coli* CCRC 14824 capable of producing SLT1 and SLT2 and possessing *eaeA* and *hlyA* genes was used as reference strain of EHEC. *E. coli* CCRC 15375 containing invasive plasmid was the reference strain of EIEC. *E. coli* CCRC 15536 having adherence factor gene was the reference strain of EPEC. *E. coli* CCRC 15372 possessing ST and LT genes was the reference strain of ETEC (Table 1). The following six native strains of *E. coli* O157 were isolated and collected by our laboratory during the years 1997 and 1999. CLC P7-1 (*E. coli* O157: H41) was isolated from marketed raw pork. CLC C14-3 (*E. coli* O157: NM) and CLC C35-3 (*E. coli* O157: H?) were isolated from marketed raw chicken meat. CLC 41-3 (*E. coli* O157: NM) and CLC 136B-2 (*E. coli* O157: H7) were isolated from feces of sheep; while CLC 159B-1 (*E. coli* O157: H45) was isolated from cattle feces.

II. *Methods*

(I) *Biochemical Tests*

1. Sorbitol fermentation test

E. coli strains were inoculated on Sorbitol MacConkey Agar and cultured at 37˚C for 18-24 hrs. The strain colonies capable of utilizing sorbitol for fermentation appeared to be pink color; while the strain colonies not able to ferment sorbitol appeared gray-white in color.

2. Test for β-D-glucuronidase activity

The strains were inoculated on Fluorocult *E. coli* O157: H7 agar and cultured at 37˚C for 18-24 hrs. The *E. coli* strains with β-D-glucuronidase activity showed a blue florescence under UV 365 nm.

(II) *Confirmation of EHEC, EIEC, EPEC, and ETEC Pathogenic Genes*

Confirmation of pathogenic genes was conducted using the PCR method[24-33]. Table 1 lists the primers used in this study. The PCR reagent comprised 63.8 μL of water and a DynaZyme DNA Polymerase kit, which was composed of 10 μL of 10-fold buffer with 1.5 mM Mg^{2+}, 1.5 μL of 200 μM dNTP, 0.5 unit DNA polymerase, and 1 μL of 100 μM primer. The PCR reaction was performed as follows. One loop of strain colony was transferred into a centrifugation tube containing 300 μL of sterilized water. The tube was shook vigorously and placed in boiling water for 10 min. After cooling, 20 μL of strain suspension was transferred into a micro-cen-

Table 1. Primers used in PCR of EHEC, ETEC, EIEC and EPEC

Primer	Sequence 5'-3'	Gene	Amplicon (bp)	Reference
SLT1R	CTG AAT CCC CCT CCA TTA TG			
SLT1F	ACA CTG GAT GAT CTC AGT GG	*slt1*	614	(24)
SLT2R	CCT GTC AAC TGA GCA CTT TG			
SLT2F	CCA TGA CAA CGG ACA GCA GTT	*slt2*	779	(24)
LP30	CAG TTA ATG GGT GGC GAA GG			
LP31	CAC CAG ACA ATG TAA CCG CTG	*slt1*	348	(25)
LP43	ATC CTA TTC CCG GGA GTT TAC G			
LP44	GCG TCA TCG TAT ACA CAG GAG C	*slt2*	584	(25)
hlyAR	AAT GAG CCA AGC TGG TTA AGC T			
hlyAF	GCA TCA TCA AGC GTA CGT TCC	*hlyA*	534	(26)
eaeAF	GAC CCG GCA CAA GCA TAA GC			
eaeAR	CCA CCT GCA GCA ACA AGA GG	*eaeA*	384	(27)
KL1	TAA TAC TCC TGA ACG GCG			
KL8	TTA GGT GTC GGC TTT TCT G	Invasive plasmid	760	(28)
EAF1	TAA TAC TCC TGA ACG GCG			
EAF25	TTA GGT GTC GGC TTT TCT G	EAF	397	(29)
LTIa-1	TCT CTA TAT GCA CAC GGA GC			
LTIb-1	TCT CTA TGT GCA TAC GGA GC	LT	322	(30,31)
LTI-2	CCA TAC TGA TTG CCG CAA T			
STI-1	TTA ATA GCA CCC GGT ACA AGC AGG	ST	140	(32,33)
STI-2	CTT GAC TCT TCA AAA GAG AAA ATT AC			

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trifugation tube where the PCR reagent and one drop of mineral oil were then added. The above mixture was then placed in a PCR reactor and thermally programmed at 94˚C for 4 min, 94˚C for another 1 min, 60˚C for 2 min, and 72˚C for 2 min, with 35 cycles in total. The PCR products were analyzed by gel electrophoresis on 2% agarose gel.

(III) *Preparation of Crude Enzyme Liquid*

One colony was picked from EMB culture medium and inoculated on TSA medium at 37˚C overnight. The strain was then transferred to a 5-mL Tris-buffer solution (0.01 M, pH 6.8) using a cotton bud. The strains in suspension were ultrasonically broken while kept at low temperature to prevent enzyme activity from being destroyed. After centrifuged at 14,000 rpm/min for 15 min while kept at 4˚C, the supernatant (crude enzyme liquid) was stored at –70˚C until use[21].

(IV) *Electrophoresis and Enzyme Coloring Reaction*

Tris-citrate buffer (pH 8.0) composed of 0.68 M Tris and 0.16 M citrate was used as electrophoresis buffer solution. A 0.8% agarose gel, which was prepared with 30-fold diluted electrophoresis buffer, was used as an electrophoresis gel. The operation procedures for electrophoresis are as follows. A Whatman No. 3 filter paper was cut into 2×7 mm pieces, which were then wetted with crude enzyme liquid and placed on the gel top. The amaranth was located at the front and back of the electrophoresis chamber for observation of electrophoresis processing. The electrophoresis was conducted under 150 V for 1~1.5 hrs while controlled at 4~8˚C to keep the enzyme activity from destroying. After performing electrophoresis, the gel was placed in a 37˚C oven for about 20 min to recover gel temperature for enzyme color developing. The method reported by Selander *et al*.[21] for enzyme color developing was used in this study. Various color-developing substrates specific to the following enzymes were prepared and placed on the gel top. The color was thus developed after a few minutes or hours. Ten enzymes were tested: isocitrate dehydrogenase (ICD, E. C. No. 1.1.1.42), malate dehydrogenase (MDH, E. C. No. 1.1.1.37), alcohol dehydrogenase (ADH, E. C. No. 1.1.1.1), glucose-6-phosphate dehydrogenase (G6P, E. C. No. 1.1.1.49), 6-phosphogluconate dehydrogenase (6PG, E. C. No. 1.1.1.44), nucleoside phosphorylase (NSP, E. C. No. 2.4.2.1), glutamic-oxalacetic transaminase (GOT, E. C. No. 2.6.1.1), mannitol-1-phosphate dehydrogenase (M1P, E. C. No. 1.1.1.17), acid phosphatase (ACP, E. C. No. 3.1.3.2), and phosphoglucose isomerase (PGI, E. C. No. 5.3.1.9).

(V) *Statistical Analysis*

The allele and frequency of enzyme locus in enzymes were calculated. The genetic diversity was calculated based on the formula $(1-\varepsilon Xi^2)(n/n-1)$; where Xi is the frequency in *i* allele and n is the ET number[35]. The cluster analysis was accomplished using Nei genetic distance and unweighted pair groups with moving averages (UPGMA)[35].

RESULTS AND DISCUSSION

I. *Characteristics of Biochemistry and Pathogenic Genes of Native E. coli O157*

Up to June 1999, we had inspected the contamination of STEC in marketed beef, sheep, pork, raw chicken meat, egg products, cattle and sheep raw milk, and their feces with 1623 specimens in total. Six serotypes of *E. coli* O157 were isolated from the above samples. They were *E. coli* O157: H41 (from raw pork), *E. coli* O157: NM (from raw chicken meat and sheep feces), *E. coli* O157: H? (from raw chicken meat), *E. coli* O157: H45 (from cattle feces), and *E. coli* O157: H7 (from sheep feces) as listed in Table 2. These 6 strains all possess O157-antigen but with different H-antigen. To better understand if they contain the pathogenic genes, 3 major pathogenic genes in EHEC were analyzed by the PCR method. Results showed that CLC 136B-2 (*E. coli* O157: H7) includes all 3 pathogenic genes, *slt2*, *hlyA*, and *eaeA*, which were not found in other 5 non-O157: H7 strains. Biochemical tests using Sorbitol MacConkey and Fluorocult *E. coli* O157: H7 agars revealed that only *E. coli* O157: H7 strain is not capable of performing sorbitol fermentation and lacks of β-glucuronidase activity.

To further study if the 5 non-STEC O157 strains belong to other pathogenic *E. coli*, the PCR method was employed to check the ST and LT genes in ETEC, EAF gene in EPEC, and invasive plasmid gene in EIEC. Negative results were obtained from the above PCR test.

Table 2. Characteristics of *E. coli* O157 strains isolated in Taiwan

Strain	Source	Serotype	Pathogenic genes				Biochemical reaction	
			slt1	*slt2*	*hlyA*	*eaeA*	SOR	MUG
P7-1	Pork	O157:H41	–[a]	–[a]	–[a]	–[a]	+[b]	+[c]
C14-3	Chicken	O157:NM	–	–	–	–	+	+
C35-3	Chicken	O157:H?	–	–	–	–	+	+
41-3	Sheep	O157: NM	–	–	–	–	+	+
159B-1	Cattle	O157: H45	–	–	–	–	+	+
136B-2	Sheep	O157:H7	–	+[a]	+[a]	+[a]	–[b]	–[c]
CCRC 14824	Reference strain	O157:H7	+[a]	+	+	+	–	–

[a] +/–: with / without this gene.

[b] +/–: ability / inability of sorbitol-fermenting.

[c] +/–: with / without β-D-glucuronidase activity.

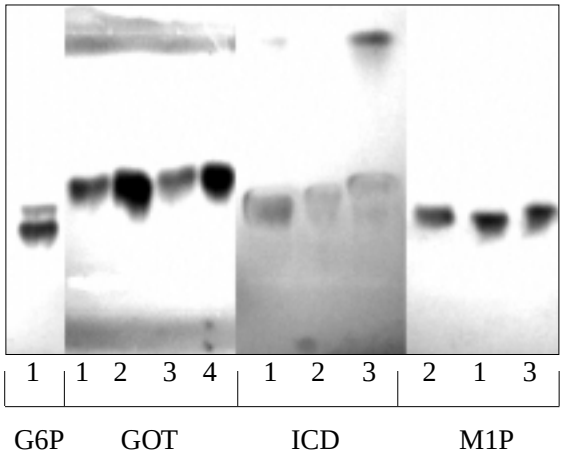
II. *Multilocus Enzyme Analysis (MLEA) on Native Strain of E. coli O157*

STEC comprises more than 100 serotypes. Using MLEA, Whittam divided STEC into four categories: EHEC1, EHEC2, STEC1, and STEC2[13]. However, an argument regarding if *E. coli* O157: H7 comes from a single clone[5] or from multiple clones[16] still exists in terms of the evolution of *E. coli* O157: H7. In this study, we found that 5 non-O157: H7 strains isolated in our laboratory did not contain pathogenic genes. This result may raise some questions. What is the genetic relationship among them? What is their genetic correlation to the serotype O157: H7. To answer these questions, the isolated strains and a reference strain *E. coli* CCRC 14824 were analyzed using MLEA to compare their genetic relations. After preliminary screening of 20 enzymes, 10 of them including ICD, MDH, ADH, G6P, 6PG, ACP, NSP, GOT, M1P, and PGI were selected for MLEA. Eight out of 10 enzyme loci in 7 test strains showed polymorphism including 3 allele in ICD, 2 in MDH, 2 in ADH, 2 in 6PG, 2 in ACP, 4 in GOT, 3 in M1P, 2 in PGI, 1 in G6P, and 1 in NSP as shown in Figure 1 A and B. Average allele was 2.2. The reference strain *E. coli* CCRC 14824 was short of ADH activity indicating this strain does not carry this locus or a gene variation occurred. Frozen storage of the strain could also lead to this result[13]. Based on the allele profiles of 10 enzyme loci, 7 test strains were categorized into 6 ETs (Table 3). Among them, a strain of O157: NM isolated from 2 different specimens showed the same ET.

The genetic diversity was calculated according to a formula involving allele frequency, which was calculated based on the allele number of locus. Basically, G6P and NSP possessed single type of allele with a diversity value of 0. The diversity value of other enzyme loci were found to be 0.34 in MDH, 6PG, and PGI, 0.65 in ACP, 0.60 in ADH, 0.63 in both M1P and ICD, and 0.87 in GOT. The average diversity value of 10 enzyme loci in 7 strains was 0.44. In other words, 44% loci diversity could occur as randomly selects 2 from 7 strains. The result also showed that the diversity of GOT loci was the biggest among 10 test enzyme loci. Ochman *et al.* (1983) performed the same test on 1600 strains of *E. coli* isolated from human and animal and found that only 0.05 genetic diversity indicating 95% of test strains contained the same GOT loci. They also found that the GOT migration speed of *E. coli* O157: H7 on gel electrophoresis was significantly different from that of other *E. coli* strains suggesting this method could be used to screen sorbitol-negative strains[36]. In this study, 10 loci in 121 STEC strains were simultaneously analyzed. We found that the M1P gene in *E. coli* O157: H7 isolated in Taiwan was different from that in most STEC strains, except for 2 STEC strains which showed the same M1P migration speed as *E. coli* O157: H7[19]. Results in this study also showed that 6PG loci of reference strain CCRC 14824 was completely different from that of native STEC and serotype O157 strains.

III. *Genetic Relationships among Native E. coli Serotype O157*

On the basis of Nei's principle and UPGMA method, the genetic distance and a cluster were calculated and plotted, respectively. Seven strains from different sources were classified into 3 clones. Clone I was composed of ET1, ET2, ET3, and ET4. ET5 and ET6 constituted Clone II and III, respectively (Figure 2). Five non-STEC O157 strains all belonged to Clone I with genetic distance less than 0.22 and 1~3 loci variation. The strains in this category were isolated from raw meat or feces of animals without carrying the pathogenic



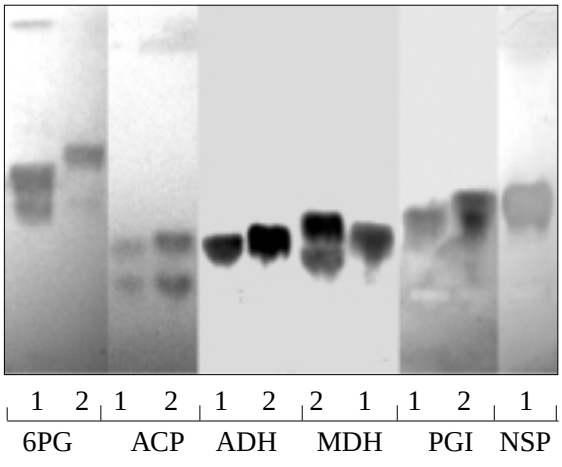


Figure 1 A. Agarose gel electrophoretic morphism of four enzymes.
G6P=glucose-6-phosphate dehydrogenase (pattern 1: CLC136B-2); GOT=glutamic-oxalacetic transaminase (pattern 1: CLC159B-1, pattern 2: CLC P7-1, pattern 3: CLC C14-3, pattern 4: CLC 136B-2); ICD=isocitrate dehydrogenase (pattern 1: CCRC 14824, pattern 2: CLC 136B-2, pattern 3: CLC C14-3); M1P=mannitol-1-phosphate dehydrogenase (pattern 1: CCRC 14824, pattern 2: CLC C14-3, pattern 3:CLC 136B-2).
Figure 1 B. Agarose gel electrophoretic morphism of six enzymes.
6PG=6-phosphogluconate dehydrogenase (pattern 1: CLC 136B-2, pattern 2: CCRC 14824); ACP=acid phosphatase (pattern 1: CCRC 14824, pattern 2: CLC 136B-2); ADH=alcohol dehydrogenase (pattern 1: CLC 136B-2, pattern 2: CLC C14-3); MDH-malate dehydrogenase (pattern 1: CCRC 14824, pattern 2: CLC 136B-2); PGI=phosphoglucose isomerase (pattern 1: CCRC 14824, pattern 2: CLC C14-3); NSP=nucleoside phosphorylase (pattern 1: CLC 136B-2).

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genes of EEC. Feng also reported that O157 serotype of *E. coli* can be isolated from food, but it would not cause food poisoning[10]. The same result was found in our laboratory. The O157 type of *E. coli* isolated from food poisoning-like cases in our laboratory did not carry the *slt* gene[11]. The results of this study revealed that a close genetic relationship might exist among O157 types of *E. coli* without carrying pathogenic genes. Furthermore, 2 strains of *E. coli* O157: H7 (CLC136B-2 isolated from sheep feces in Taiwan and CCRC 14824 isolated from the hamburger of a food poisoning outbreak that occurred in the USA) can be designated to Clone II and III, respectively. The genetic distance between Clone I and Clone II or III was beyond 0.50 indicating the gene message of STEC O157: H7 is indeed different from that of other O157 types of *E. coli.* The same result was found by Whittam *et al*.[17] ensuring that *E. coli* O157: H7 does not originate from *E. coli* O157. *E. coli* O157: H7 isolated in Taiwan and from the USA belonged to different clones according to the results of this study. Wittman[5] and Faith[16] have proposed that *E. coli* O157: H7 could be from a single clone or multiple clones. Further study regarding genetic correlation between native *E. coli* O157: H7 and the *E. coli* O157: H7 isolated from other countries needs to be carried out.

Based on the results of this study, we have obtained a preliminary understanding of the characteristics of native *E. coli* serotype O157 strains. STEC *E. coli* O157: H7 and non-STEC O157 strains originate from different clones. Non-STEC O157 strains isolated from raw meat or animal feces do not carry pathogenic genes tested in this study and show a very close genetic relationship. However, the only *E. coli* O157: H7 isolated in this study contains 3 key pathogenic genes. Future work in our laboratory will continue to isolate and collect native STEC and establish a typing method and further study the pathogenicity of collected strains.

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Table 3. Allele profiles of 10 enzyme loci for 6 electrophoretic types (ETs) of *E. coli* O157 isolates

ET	Allele profile										No. of isolates
	ICD	MDH	ADH	G6P	6PG	ACP	NSP	GOT	M1P	PGI	
1	3	1	2	1	1	2	1	2	2	2	1
2	3	1	2	1	1	2	1	3	2	2	2
3	3	1	1	1	1	1	1	3	2	2	1
4	3	1	1	1	1	2	1	1	2	2	1
5	2	2	1	1	1	2	1	4	3	2	1
6	1	1	0	1	2	1	1	2	1	1	1

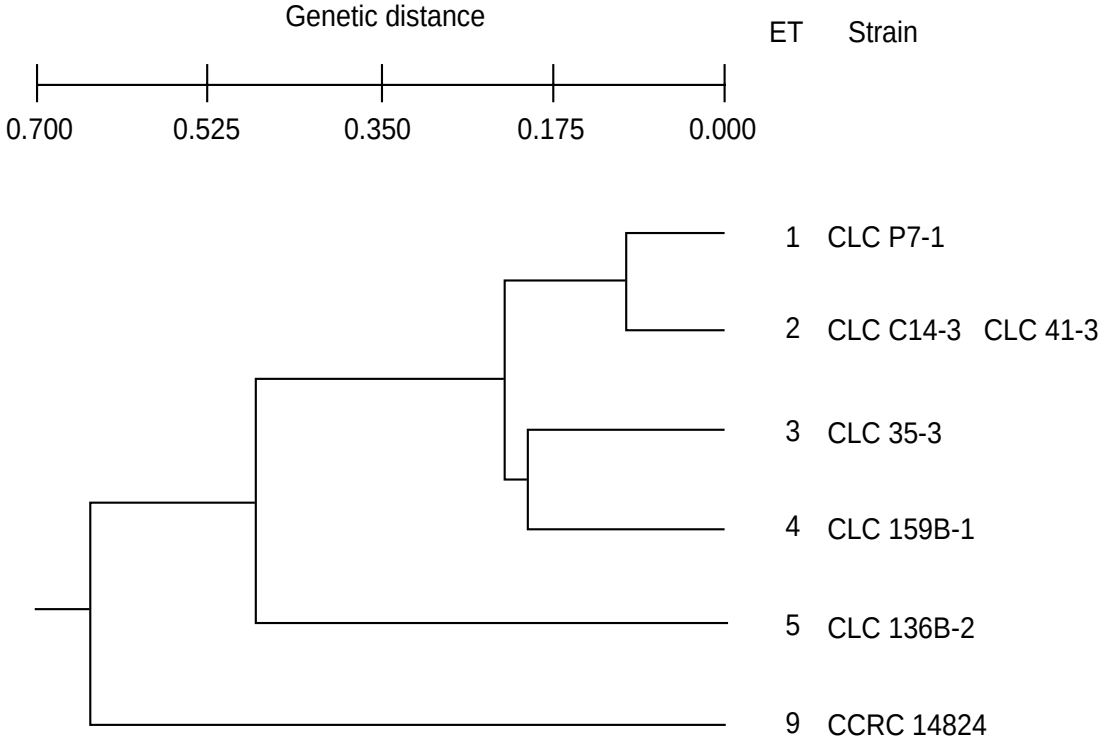

Figure 2. Genetic relationships among 6 electrophoretic types of *E. coli* O157 strains. The dendrogram was generated by the UPGMA method of clustering from a matrix of coefficients of Nei's genetic distance, based on 10 enzyme loci.

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以PCR及多位點酵素分析法探討自台灣地區分離之0157型大腸桿菌特性

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摘　要

本研究自1997年7月至1999年6月，從台灣地區牛羊糞便或生肉檢體分離、蒐集O157型大腸桿菌（*Escherichia coli*），共六株，僅有一株分離自羊糞檢體之菌株屬*E. coli* O157:H7，其餘為H41、H45、H?（以市售43種O型血清無法鑑別出）、或不具運動性（non-motile, NM）。進一步以Polymerase chain reaction（PCR）方法分析屬Enterohemorrhagic *E. coli*（EHEC）之致病基因，僅該H7菌株具有*slt2*、*eaeA*及*hlyA*等致病基因，同時該菌不能發酵山梨糖醇及缺乏β-D-葡萄糖啟酵素活性。五株non-H7之O157型菌株，再以PCR方法測試其他大腸桿菌致病基因，包括Enterotoxigenic *E. coli*（ETEC；heat-labile enterotoxin, LT；heat-stable enterotoxin, ST），Enteroinvasive *E. coli*（EIEC；invasive plasmid）及Enteropathogenic *E. coli*（EPEC；adherence factor, EAF），發現均不帶有前述之基因。為確定菌株之遺傳關係，選用十種酵素，進行多位點酵素分析（multilocus enzyme analysis, MLEA）。六株菌共區分成五種電泳圖譜型別（electrophoretic type, ET）。同時，以群集法分析菌株之純系關係，發現五株non-EHEC O157與EHEC O157:H7菌株明顯分屬於二個純系（clone）。根據結果推測，台灣本土分離之non-EHEC O157與EHEC O157:H7菌株可能衍生自二個純系，經由多位點酵素分析，可瞭解本土O157型大腸桿菌之遺傳關係。

關鍵詞：多位點酵素分析，大腸桿菌，純系