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Full Length Research Paper

Characterization of *stx2* and its variants in *Escherichia coli* O157:H7 isolated from patients and animals

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In this study, we investigated 72 *Escherichia coli* O157:H7 strains from humans and animals to determine stx_2 and its variants by polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism. Most isolates were found to carry stx_2 or its variants and the stx_{2c} was the dominant subtype; for the prevalence of stx_{2c} in stx_2-positive isolates was 89.6% (42/47). All stx_2 and stx_{2c} harboring isolates obtained from humans had caused diarrhea or hemolytic-uremic syndrome. Three strains isolated from a piglet and two cattle carried the stx_{2c} gene with an IS*1203*v inserted sequence. Sequencing of this region revealed that, this 1.3 kb insertion was very similar to a previously identified IS *1203*v sequence and the insertion interrupted the carboxyl end of the B subunit coding region of stx_{2c} gene. The corresponding positions in the stx_{2c} gene sequence in which the IS *1203*v sequence was inserted was varied. The isolates possessing IS*1203*v were inactive in the Vero cell toxicity assay.

Key words: Shiga toxin-producing Escherichia coli; stx2; PCR-RFLP; insertion sequence; cytotoxicity.

INTRODUCTION

Shiga toxin (stx)-producing *Escherichia coli* (STEC) has been recognized as an emerging food-borne pathogen of clinical and public health concern and has caused various severe clinical symptoms ranging from diarrhea, hemorrhagic colitis (HC) to hemolytic-uremic syndrome (HUS) in humans (Friedrich et al., 2002; Beutin et al., 2004; Zheng et al., 2005; Bielaszewska et al., 2006; Prager et al., 2009). Shiga toxin is considered to play a prominent role in the pathogenesis of STEC and is presently classified into two broad types, *stx1* and *stx2,* which have similar structure of the molecule and enzymatic activity but are antigenically distinct. Stx1 is highly homologous to stx of *Shigella dysenteriae* type 1 and highly conserved, although, two sequence variants have been reported (Zhang et al., 2002; Burk et al., 2003). *Stx2* shares approximately 56% amino acid homology with stx1 but is relatively more heterogeneous (Jackson et al., 1987). Several related variants of *stx2* have been described that include *stx2c*, *stx2d*, *stx2e*, *stx2f* and *stx2g*, moreover, *stx2c* can be classified into two groups: stx_{2vha}, and stx_{2vhb} (Weinstein et al., 1988; Gannon et al., 1990; Ito et al., 1990; Schmitt et al., 1991; Tyler et al. 1991; Piérard et al., 1998; Schmidt et al., 2000; Teel et al., 2002; Leung et al., 2003; Beutin et al., 2008; Vu-Khac and Cornick, 2008; Prager et al., 2009).

There are many serotypes of STEC strains, but the one most associated with the epidemic cases is O157:H7. The first major outbreak of STEC O157:H7 infection was reported in the United States in 1982 and was linked to eating undercooked ground beef from a fast-food restaurant chain (Riley et al., 1983). Since then, several outbreaks have been reported worldwide, including a large outbreak in Jiangsu and Anhui of Chinese Mainland during 1999 to 2000, which caused more than 200 deaths (Zheng et al., 2005). The pathogenesis of STEC O157:H7 infection in humans depends on many bacterial

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Abbreviations: STEC, Shiga toxin (stx)-producing *Escherichia coli*; **HC,** hemorrhagic colitis; **HUS,** hemolytic-uremic syndrome; **EHEC,** enterohemorrhagic *Escherichia coli coli*; **SAT,** slide agglutination test; **PCR-RFLP,** polymerase chain reaction-restriction fragment length polymorphism; **ORF,** open reading frames.

Table 1. The *stx2* genotype of *E. coli* O157:H7 isolates from Jiangsu and Chongqing, People's Republic of China.

Province	Source	Genotype	No. of isolates
Jiangsu	Patients	*stx2*	3
		stx2c	3
		ND[a]	2
	Cattle	*stx2*	1
		stx2c	12
		*stx*2 (IS1203v)[b]	2
		ND	6
	Goat	*stx2c*	15
		ND	8
	Swine	*stx2c*	3
	Chicken	*stx2*	1
		stx2c	7
		ND	1
	Fresh mutton	ND	1
Chongqing	Cattle	ND	6
	Swine	*stx2* (IS1203v)[b]	1

[a] *Stx2* or its variants was not detected; [b] containing IS *1203*v.

virulence factors including *stx1, stx2,* enterohemorrhagic *E. coli* (EHEC)-hemolysin, intimin and so on (Wang et al., 2008). But the most important factor is stx2 (Osawa et al., 2000; Kawano et al., 2008). The STEC O157:H7 strains have been reported to produce stx_1, stx_2, and stx_{2c} (Schmitt et al., 1991; Tyler et al., 1991; Osawa et al., 2000; Zheng et al., 2005; Kawano et al., 2008), while other stx_2 variants were often observed from non-O157:H7 STEC. However, the stx_2 gene can be interrupted by a 1310 bp insertion sequence IS*1203* variant (IS*1203*v) which is inserted in the region encoding the amino-terminus of the B subunit with a duplication of 3 bp at the target site and is not cytotoxic to Vero cells (Kusumoto et al., 1999; Jinneman et al., 2000).

Cattle are considered to be the major reservoir of *E. coli* O157:H7 (Baker et al., 2007; Wang et al., 2008; Williams et al., 2008). Recent studies have also demonstrated that, conventional pigs were permissive host for *E. coli* O157:H7 (Booher et al., 2002; Cornick and Helgerson, 2004), though the prevalence of the organism in these studies was generally low, except for the results from Chile and Chinese Mainland and both suggested that, pig might be an important source of this organism in some countries (Borie et al., 1997; Zheng et al., 2005). Also, one family outbreak had been specifically traced back to pork salami and the *E. coli* O157 isolates from the couple and the salami carried stx_1, stx_2 and *eae* genes and shared the same PFGE pattern (Conedera et al., 2007).

In this paper, we analyzed the stx_2 produced by STEC O157:H7 strains isolated from both patients and animals, including cattle, pigs, goats, chicken. While polymerase chain reaction (PCR) analyzing of isolates produced bands of the predicted size for stx_2, three isolates produced abnormally stx_2 gene amplification product containing a 1.3-kb insertion sequence IS*1203*v. This study describes PCR and Vero cell toxicity tests and sequencing of the insertion to fully understand this anomaly.

MATERIALS AND METHODS

Bacterial strains

A total of 72 *E. coli* O157:H7 strains were isolated during several studies performed in Jiangsu Province from 1999 to 2002 and Chongqing Municipality in 2005 of the People's Republic of China (Table 1). The strains were isolated from patients' (two patients with HUS and 6 with diarrhea from Jiangsu), cattle (twenty-one from Jiangsu and 6 from Chongqing.), goats (twenty-three from Jiangsu); pig (Three from Jiangsu and 1 from Chongqing) and chicken feces (Nine from Jiangsu) as well as fresh mutton (One from Jiangsu). Their O and H antigens were further tested with anti-*E. coli* O157 and anti-*E. coli* H7 sera by slide agglutination test (SAT). Molecular identification of the O157 lipopolysaccharide antigen (*rfbE*) and H7 flagella antigen (*fliC*) genes were performed as described previously (Gannon et al., 1997; Desmarchelier et al., 1998).

The *E. coli* O157:H7 reference strain EDL933 was used as positive control for PCR detection of virulent marker and possessed stx_1, stx_2, *eaeA*, *ehxA*, *EspA* and *Tccp* as we have described previously (Wang et al., 2008). *E. coli* DH5α was stored in our laboratory.

Detection of stx_2 and its variants by PCR and PCR-RFLP

The template DNA was prepared from a pure culture of isolates, grown in LB broth for 16 h at 37°C. Three ml of the culture were centrifuged at 8 000 rpm for 5 min and the pellet was re-suspended in 0.2 ml of ddH_2O. The suspension was heated at 100°C for 10 min and then, centrifuged at 12 000 rpm for 5 min. The supernatant was

Table 2. Primers used for detection and typing *stx*$_2$ variants.

Primer pair	Sequence	Gene specificity	Product size (bp)	Reference or source
stx2-F	GGTCACTGGTTCGAATCCAGTAC	*stx*$_2$, *stx*$_{2vha}$, *stx* $_{2vhb}$	1400	De Baets et al. (2004)
stx2-R	GGGATCCTGAATTGTGACACAGATTACACTTGTTAC			
stx2D-1	ATGAAGAAGATGTTTATGGCGGTTT	*stx*$_{2c}$ with IS *1203*v	210, 254	This study
stx2D-2	TCACAGATAAAACACTCTCCAGGAAAC			
stx2D-3	ATGAAGTGTATATTATTTAAATGGGTAC	*stx*$_{2c}$ with IS *1203*v	1225, 1181	This study
stx2D-4	TCACAGATAAAACACTCTCCAGG			
VT2-c	AAGAAGATGTTTATGGCGGT	*stx*$_2$, *stx*$_{2vha}$, *stx* $_{2vhb}$	285	Tyler et al. (1991)
VT2-d	CACGAATCAGGTTATGCCTC			
VT2v-1	CATTCAGAGTAAAAGTGGCC	*stx* $_{2vha}$, *stx* $_{2vhb}$	385	Tyler et al. (1991)
VT2v-2	GGGTGCCTCCCGGTGAGTTC			

Table 3. Characteristics of *E. coli* O157:H7 isolates and DH5α.

Isolate	Serotype	Genotype				Source
		stx$_1$	*stx*$_2$	*stx*$_{2c}$	*stx*$_{2c}$ (IS1203v)	
EDL933	O157:H7	+	+	-	-	Human
00B015	O157:H7	-	-	+	-	Goat
BRY24	O157:H7	-	-	-	+	Swine
00F077	O157:H7	-	-	-	+	Cattle
00F078	O157:H7	-	-	-	+	Cattle
CYB24	O157:H7	-	-	-	-	Cattle
DH5α		-	-	-	-	

used for the PCR template. *E. coli* O157:H7 isolates were examined by PCR assay to determine the presence of *stx*$_2$ and its variants. PCR was performed in a total volume of 20 µl containing 0.5 unit of PrimeSTAR® HS DNA polymerase (Takara, Japan), 25 pmol of appropriate primers, 1.6 µl of dNTPs（2.5 mmol/L each）, 4 µl 5×buffer（Mg^{2+} plus）and 0.4 µl of the DNA template. The reaction was carried out in a Bio-Rad PCR system PTC-100 Peltier thermal cycler.

Primers used for the detection of *stx*$_2$ gene and its variants are shown in Table 2. The *stx*$_2$ genotypes harbored by the *E. coli* O157:H7 isolates were examined by PCR assay as described previously (Tyler et al., 1991; De Baets et al., 2004). The B subunit genes of strains positive with primers VT2-c and VT2-d-specific for *stx*$_2$ and its *stx*$_{2c}$ variants were sub-typed by Tyler's PCR- restriction fragment length polymorphism (RFLP) method, which identifies *stx*$_2$, *stx*$_{2vha}$, and *stx*$_{2vhb}$ (Tyler et al., 1991). Amplicons were digested by enzymes *Hae*III to discriminate *stx*$_2$ from *stx*$_{2c}$ subtype genes. Use of restriction enzymes *Nci*I and *Rsa*I for PCR-RFLP analysis enabled classification of *stx*$_{2c}$ genes as *stx*$_{2vha}$ and *stx*$_{2vhb}$. Primers VT2v-1 and VT2v-2 were also used to discriminate *stx*$_{2c}$ from *stx*$_2$ subtype genes (Tyler et al., 1991).

Primers stx2D-1 and stx2D-2 (Table 2) were used for amplification of the initial 210 bp region of the *stx*$_2$ B subunit of the BRY24 strain. The 210 bp region cycling conditions were as follows: Initial denaturation at 98°C for 5 min; 30 cycles of denaturation at 98°C for 10 s, annealing at 55°C for 10 s and extension for 20 s at 72°C; and final extension at 72°C for 6 min. Primers stx2D-3 and stx2D-4 (Table 2) set for the *stx*$_2$ gene of 00F077 and 00F078 strains amplified a 1225 bp fragment from a region which spanned B subunit. The 1225 bp fragment cycling conditions were as follows: Initial denaturation at 98°C for 5 min; 30 cycles of denaturation at 98°C for 20 s, annealing at 48°C for 20 s and extension for 1 min 15 s at 72°C and final extension at 72°C for 6 min. PCR amplicons were run on a 2.5% agarose gel, stained with ethidium bromide and visualized under UV illumination.

DNA sequencing

The resulting PCR products of BRY24 and 00F078 with stx2-F and stx2-R primers were gel purified and ligated into pMD19-T simple vector, designating pMD19-BRY24 and pMD19-00F078, respectively. Their constructed plasmids were sent to Takara Company for sequencing. DNA sequence information was analyzed using DNAssist software.

Vero cell cytotoxicity assay

Vero cell cytotoxicity assay was performed as described previously with minor modification (Teel et al., 2002). Isolates used were listed in Table 3. *E. coli* DH5α, a nontoxic laboratory strain, was used as the negative control. Briefly, Vero cells suspended in tissue culture medium (DMEM containing 10% fetal bovine serum) were seeded into wells (approximately10^4 cells/well) of a 96-well microtiter plate, leaving the exterior rows empty and incubated for 24 h at 37°C in the presence of 5% CO_2. The tissue culture medium was removed by aspiration and replaced with 100 µl fresh medium. On the other hand, a single colony of each bacterial strain was removed, inoculated into 5 ml LB broth and shaken overnight at 37°C. After

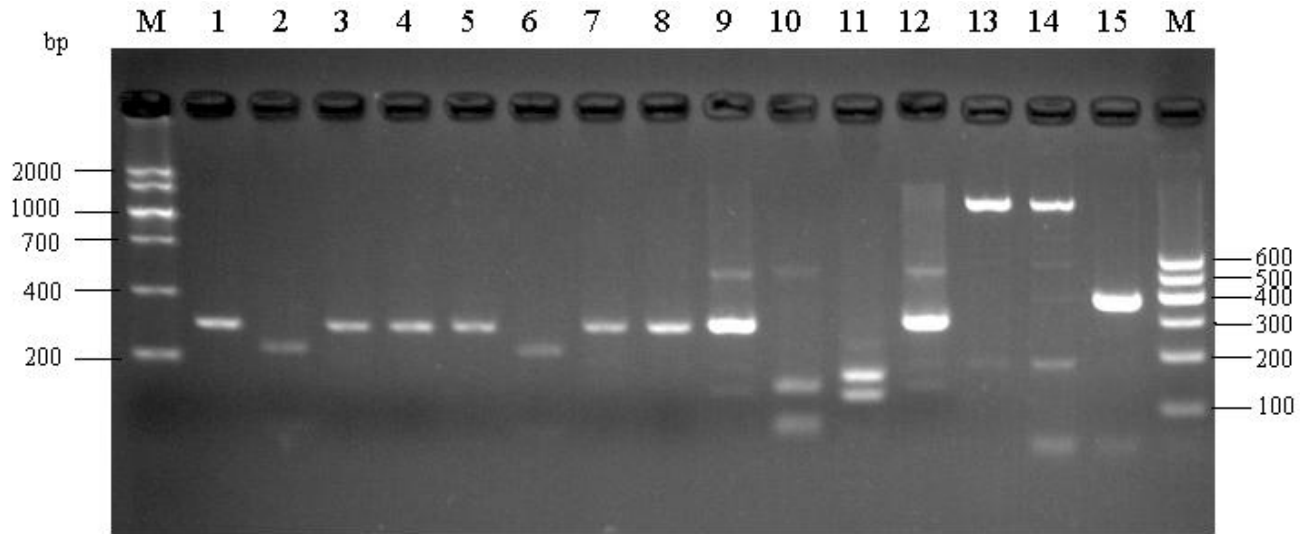

Figure 1. Products on gel after PCR with different pairs of primers and RFLP analysis of VT2 genotypes by digestion of the amplified products. Lane 1 to 12, Amplified with VT2 primers; Lane 13 to 15, amplified with VT2v primers. Lane 1, stx_2 (EDL933); Lane 2, stx_2 (EDL933) digested with *Rsa*I; Lane 3, stx_2 (EDL933) digested with *Hae*III; Lane 4, stx_2 (EDL933) digested with *Nci*I; Lane 5, stx_2 (99A038); Lane 6, stx_2 (99A038) digested with *Rsa*I; Lane 7, stx_2 (99A038) digested with *Hae*III; Lane 8, stx_2 (99A038) digested with *Nci*I; Lane 9, stx_{2c} (99A021); Lane 10, stx_{2c} (99A021) digested with *Rsa*I; Lane 11, stx_{2c} (99A021) digested with *Hae*III; Lane 12, stx_{2c} (99A021) digested with *Nci*I; Lane 13, EDL933 product; Lane 14, 99A038 product; Lane 15, 99A021 product. Molecular weight standards are given to the left and right.

the cell concentration was adjusted to 1 x 10^9 CFU/ml, bacterial cultures were centrifuged and filtered with a 0.22 µm pore size filter. Filter-sterilized bacterial culture supernatants (100 µl) were then added to the first row of wells. Serial dilutions (1:5) of each supernatant were prepared in 96-well microtiter plates using tissue culture medium. The last row was not inoculated and served as a control for unintoxicated cell background. After incubation for 48 h, the cells were fixed in buffered formalin and stained with crystal violet. The intensity of the color of the fixed and stained cells was measured with a Bio-Rad microplate reader at 570 nm (A_{570}). The staining intensity was proportional to the number of viable, attached tissue culture cells present before they were fixed to the well. The 50% cytotoxic dose (CD_{50}) was the amount of toxin required to kill 50% of the cells in a well. The CD_{50} for each bacterial strain was determined by plotting the optical density at 570 nm of each dilution well after subtraction of the optical density at 570 nm for the blank against the log-transformed toxin dilution using Originpro7.5 software (OriginLab, Northampton, MA).

Nucleotide sequence accession number

The nucleotide sequences of the stx_2 variant genes of STEC O157:H7 strain BRY24 and 00F078 have been submitted to the Genbank database under accession numbers GU983682 and GU983683.

RESULTS

Stx_2 genotypes among *E. coli* O157:H7 isolates from human and livestock sources

E. coli O157:H7 isolates (n =72) were isolated from a variety of human and livestock sources. Forty eight isolates possessed stx_2 or its variants (Table 1). One isolate from cattle in Chongqing can amplify a 2740 bp fragment by using stx2-F and stx2-R primer pairs and a total of 47 (72.3%) isolates from Jiangsu Province had stx_2 or its variants. By using primers VT2-c and VT2-d, stx_2 can be detected in 45 isolates. Negative PCRs were repeated for confirmation, along with positive control. The PCR-RFLP and PCR with primers VT2v-1 and VT2v-2 of subtypes was used to distinguish stx_2, stx_{2vha}, and stx_{2vhb}. The stx_2 was dominantly present in patients (3 of 5, 60%), only two stx_2-positive isolates can be detected in animal. Stx_{2vha} were the dominant subtype in *E. coli* O157:H7 strains from animals; the prevalence of stx_{2vha} in stx_2-positive isolates was 83.3% (40/48). Stx_{2vhb} variant was not found in any isolate (Figure 1).

Using stx_2 gene primers, stx2-F and stx2-R, a sequence of about 2 740 bp fragment was amplified in strains BRY24 and 00F078, which was larger than the normal amplified nucleotide sequence of stx_2 (1 400 bp). Then, the nucleotides fragments were sequenced by ligating into pMD19-T simple vector and the DNA sequence information was analyzed by using several bioinformatics software. The nucleotides sequences of subunit A of $stx_{2\ BRY24}$ and $stx_{2\ 00F078}$ were identical and 99.1% homologous to $stx_{2\ EDL933}$. According to the nucleotide sequences of the B subunit of $stx_{2\ BRY24}$ and $stx_{2\ 00F078}$, two pairs of primers were designed. With primers stx2D-1 and stx2D-2, 210 bp fragment was obtained from strain BRY24, 254 bp fragment was obtained from strains 00F077 and 00F078. As the same, with primers stx2D-3 and stx2D-4, 1181 bp fragment was obtained from strain BRY24, and

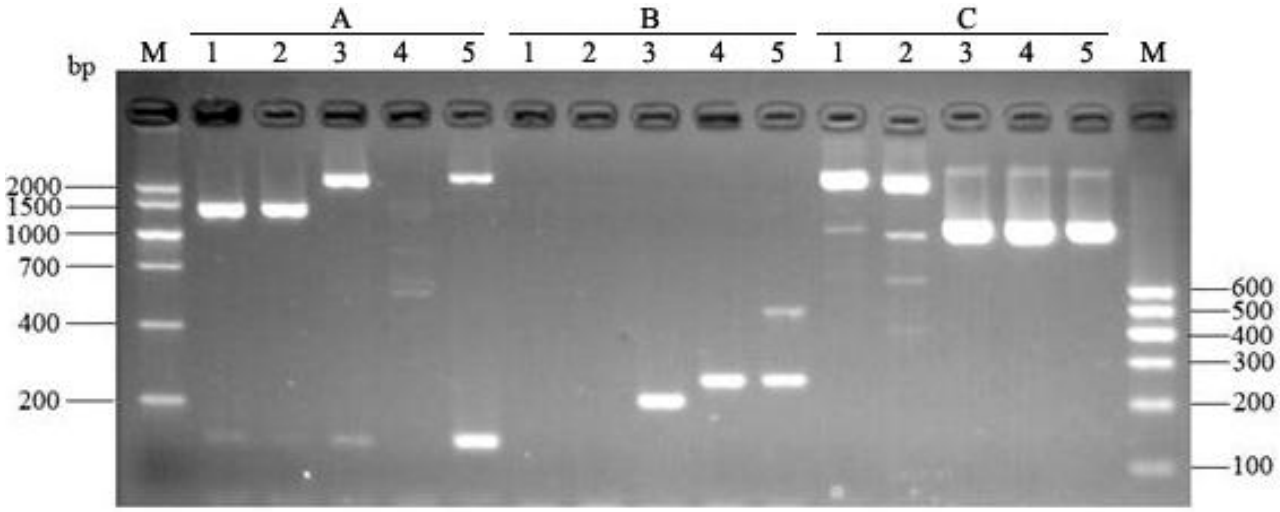

Figure 2. Products on gel after PCR with stx2 (A), stx2D-1 and stx2D-2(B) and stx2D-3 and stx2D-4(C) primers. Lane M, Marker; Lane 1, EDL933; Lane 2, 99A021; Lane 3, BRY24; Lane 4, 00F077; Lane 5, Molecular weight standards are given to the left and right.

1225 bp fragment was obtained from strains 00F077 and 00F078 (Figure 2). However, no amplicon was amplified in other isolates with these two pairs of primers. The amplicon of 00F077, amplified with primers stx2D-3 and stx2D-4, was sequenced by ligating into pMD19-T simple vector and the sequenced nucleotide was identical to the sequence of $stx_{2\,00F078}$.

Analysis of insertion sequence in BRY24, 00F077 and 00F078

The nucleotide sequences of the stx_2 genes of three *E. coli* O157:H7 were identical to the stx_{2c} gene sequence described by some researchers (Kusumoto et al., 1999; Lin et al., 1993; Schmitt et al., 1991; Teel et al., 2002), although, they were interrupted by a 1310 bp insertion sequence. In all three isolates, the IS were in the reverse orientation within the stx_{2c} gene. Both 1310 bp insertion sequences in 00F077 and 00F078 were identical to IS *1203*v (Kusumoto et al., 1999). However, the 1310 bp sequence was most similar to IS *1203*v with only a 2 bp substitution in the insertion sequence region of RBY24 when compared with the same region from the AB017524 sequence ("A" residue at position 581 in the RBY24 sequence and a "G" at the corresponding position 642 in sequence AB017524; "G" residue at position 587 in the RBY24 sequence and a "A" at the corresponding position 648 in sequence AB017524). However, the start codon of IS *1203*v ORFb in 00F078 was mutated to "ATA" in BRY24 and the length of ORFb in BRY24 was 3 bp shorter than AB017524's (Figure 3). The corresponding positions in the stx_{2c} gene sequence at which the IS *1203*v sequence were located were 212 in the B subunit for the isolates 00F077 and 00F078, and 168 in the B subunit for RBY24.

For 00F077, 00F078 and BRY24 IS*1203*v sequences, the three nucleotides ("ATT", "ATT" and "AAC", respectively) preceding the insertion site were repeated after IS *1203*v followed by the remainder of the stx_{2c} gene sequence for the regions sequenced. IS *1203*v in all three isolates were inserted in the region encoding the amino-terminus of the B subunit.

Cytotoxicity

The Vero cell cytotoxicity of selected isolates was analyzed by using a microtiter plate cytotoxicity assay. The supernatants of CYB42 and DH5α were not cytotoxic to Vero cells as expected. On the contrary, the supernatants of EDL933 (Containing stx1 and stx2) and 00B015 (Containing stx2c but not containing stx1) isolates were cytotoxic to Vero cells as expected. Nevertheless, BRY24, 00F077 and 00F078 were not cytotoxic to Vero cells as expected (Jinneman et al., 2000).

DISCUSSION

In human, Shiga toxins are the major virulence factors of STEC responsible for HC and HUS. The association of STEC with HC and HUS implies that stx_2 is more closely to these diseases than stx_1 (Miceli et al., 1999; Siegler et al., 2003). Recent studies showed that, the strains carrying stx_{2vha} might be less virulent and less frequently cause bloody diarrhea (Zheng et al., 2005; Kawano et al., 2008). Therefore, stx_2 sub-typing is suggested to be helpful in understanding the role of the different subtypes in clinical medicine and epidemiology. In this study, we

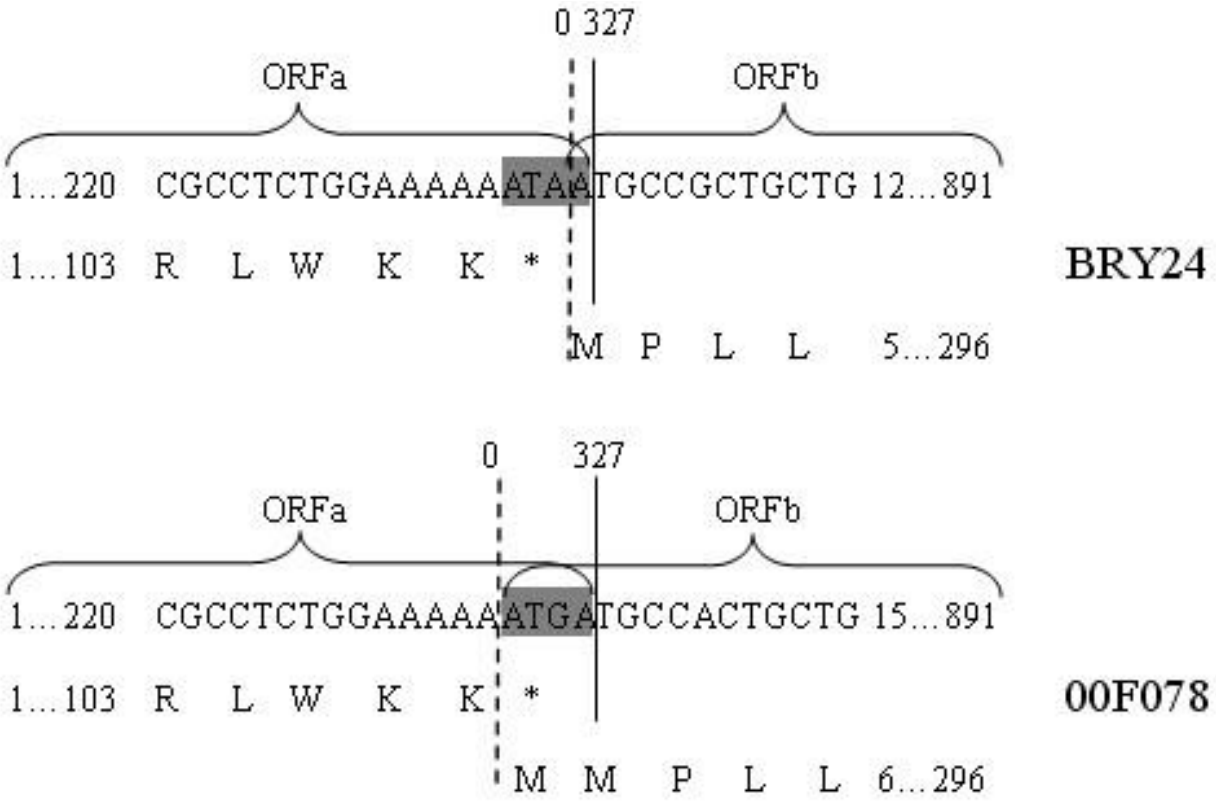

Figure 3. Nucleotide sequences comparison of IS*1203*v ORFb in the stx_{2c} for both BRY24 and 00F078 strains. The 1310 bp insertion sequence in 00F078 was identical to IS 1203v (Kusumoto et al., 1999). However, the start codon of IS *1203*v ORFb in 00F078 was mutated to "ATA" in BRY24, and the length of IS *1203*v ORFb in BRY24 was 3 bp shorter than AB017524's (Kusumoto et al., 1999). The solid lines represent the stop of IS *1203*v ORFa and the dash lines represent the start of IS *1203*v ORFb.

analyzed a collection of 72 *E. coli* O157:H7 isolates recovered from cattle, goat, pig, chicken and human patients to further characterize the stx_2 that they contained. Two main stx_2 subtypes were detected (stx_2 and stx_{2c}) and no combination of these two subtypes was detected. Two pairs of primers (stx2-F and stx2-R, VT2-c and VT2-d) can amplify stx_2 and stx_{2c}, though the amplicons amplified by primers VT2-c and VT2-d can be further subtyped by using Tyler's PCR-RFLP method (Piérard et al., 1997; Piérard et al., 1998). Stx_2 genotype isolates caused more severe symptoms (two with HUS and 1 with bloody diarrhea) than stx_{2c} harboring isolates (three with watery diarrhea), though the case load was shortage. However, Friedlich et al. (2002) have shown that stx_{2c} is the only stx_2 variant associated with HUS, even the risk of developing HUS was significantly lower after infection with stx_2-bearing. Therefore, *E. coli* O157:H7 harboring stx_{2c} may be a threaten pathogen and become the dominant variant in the outbreak. Nevertheless, two isolates that did not contain stx_2 can also cause clinical symptoms, such as watery diarrhea. It may suggest that accessory virulence factors can be present in some fully pathogenic strains, such as intimin, EHEC hemolysin, and tccp (Wang et al., 2008).

Compared to other STEC O157:H7, the identification of an insertion of approximately 1300 bp in the stx_{2c} gene for BRY24, 00F077 and 00F078 isolates was of particular concern to us. Kusumoto et al. (1999) reported that the stx_{2vhd} gene (In fact the stx_{2c} gene) (Lin et al., 1993), one of the stx_2 variants was interrupted by IS*1203*v, whose target site was 33 bp away at the amino terminal end of the coding region for the B subunit, resulting in the inactivation of the stx_2 gene. Based on the related nucleotide sequences data, the IS *1203*v element in stx_{2c} of 00F077 or 00F078 was inserted nearer the carboxyl terminal end of the B subunit coding region than BRY24's. IS *1203* belongs to the IS 3 family which are between 1200 and 1550 bp with inverted terminal repeats and containing two consecutive and partially overlapping open reading frames (ORF) (Mahillon and Chandler, 1998). IS *1203* is flanked by 26 bp imperfect terminal repeats and two ORFs, with the second ORF being previously identified as encoding a transposase (Paton and Paton, 1994). The *E. coli* O157:H7 plasmid sequences include IS *1203*, the sequence of which has been reported in other STEC strains including preceding the stx_1 gene in a *E. coli* O111:H- strain (Paton et al., 1993). IS *1203* has been reported to interrupt the *esp*P gene of a non-sorbitol

fermenting *E. coli* O157:H7 strain (Brunder et al., 1999). Moreover, other researches indicated that, IS*1203*v was inserted into the stx_2 gene of *E. coli* O157:H7 strains at different sites (Jinneman et al., 2000; Okitsu et al., 2001). Interestingly, two O157:H7 strains isolated from humans carrying the *stx1* gene with an IS *1203* that was inserted into the coding region of the A subunit (Suzuki et al., 2004). In this study, the result was similar to a report from Chinese Mainland, in which *E. coli* O157:H7 possessing IS *1203*v inserted stx_{2c} gene was prevalent (Zheng et al., 2005). Moreover, the 00F077 and 00F078 isolates were obtained from cattle in the same farm and their stx_{2c} gene had the identical sequence and that might suggest a common origin.

In conclusion, based on the analysis of stx_2 genotype and clinical manifestations caused by *E. coli* O157:H7, it might be confirmed that, stx_2 genotype was one of the important risky factors of disease severity and that, stx_{2c} was the dominant genotype of STEC O157:H7 isolated from domestic animals. Three isolates, obtained from a piglet and two cattle, have acquired an IS *1203*v within the stx_{2c} gene and did not produce a functional stx2c protein.

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