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## Genotypic variations of Shiga toxin-converting phages from enterohaemorrhagic *Escherichia coli* O157: H7 isolates

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**Pulsed-field gel electrophoresis (PFGE) analysis revealed that enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 strains had considerable variations in their genomes. This study investigated whether or not the molecular profile of Shiga toxin (Stx) 1- and Stx2-converting phages isolated from EHEC O157:H7 strains, derived from various sources in the USA and Japan, corresponded to the variations of host strains' genotypes as determined by PFGE. A total of 51 Stx-converting phages including 12 Stx1-converting phages and 37 Stx2-converting phages was isolated from seven USA isolates and 20 Japanese isolates. The average Dice coefficient values showed 44% similarity between phage DNAs in Stx2-converting phages digested with *Sma*I and 55% in Stx1-converting phages digested with *Hind*III, indicating considerable variation among phage DNA. In particular, restriction fragment length polymorphism (RFLP) patterns of Stx2-converting phage DNA varied according to the PFGE type of their host strain, which suggests that the phage genomes have altered their genotypic characteristics with those of host genomes. However, there are several exceptions: the RFLP patterns of some Stx2-converting phages were quite similar irrespective of the different genotypes of the host strains, indicating that horizontal transfer of Stx2-converting phage may also occur under some circumstances.**

### Introduction

Enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 has been increasingly recognised as a major food-borne pathogen that causes abdominal cramps, diarrhoea, haemorrhagic colitis and haemolytic-uraemic syndrome [1, 2]. Shiga toxin (Stx) produced by EHEC O157:H7 is considered to be a major virulence factor causing these clinical symptoms [3, 4]. Stx is presently classified into two broad types, Stx1 and Stx2 [3]. It has been well described that the structural genes encoding Stx1 and Stx2 (*stx*<sub>1</sub> and *stx*<sub>2</sub>, respectively) are carried on bacteriophages, and are apparently transferred to the chromosomal DNA of host *E. coli*

by lysogenisation of the phages [5–7] (referred to as Stx-converting phages hereafter). Rietra *et al.* [8] reported that Stx-converting phages derived from EHEC O157:H7 of human origin were all similar to each other in their DNA profiles (i.e., genome size, restriction endonuclease digestion and DNA–DNA hybridisation patterns), showing that the phage DNAs were very closely related. However, others [9, 10] have reported appreciable differences in size of fragments in EHEC O157 strains that hybridised with Stx gene probes, suggesting a possible heterogeneity among Stx-converting phages lysogenised in the host strains.

The first major outbreak of EHEC O157:H7 infection was reported in the USA in 1982, and was linked to eating undercooked ground beef from a fast-food restaurant chain [2, 11]. Since then, several outbreaks have been reported world-wide. In 1996, a series of multiple outbreaks and sporadic infections occurred in Japan [12]. To investigate possible epidemiological links between the outbreaks, an extensive molecular analysis was conducted on the outbreak-derived EHEC O157:H7

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isolates by pulsed-field gel electrophoresis (PFGE). This grouped the isolates into various PFGE types (i.e., types I, II, III, IV and V) [13]. Therefore, it was of interest to determine whether or not the above outbreaks in the USA and Japan involved particular types of Stx-converting phages despite the fact that their host strains were genotypically different. The present study reports here on the molecular profiles of 51 Stx-converting phages that have been isolated so far from a total of 27 host bacterial strains belonging to various PFGE types, and relates the data to the hosts' genotypes.

## Materials and methods

### *Bacterial strains and PFGE typing*

A total of 27 EHEC O157:H7 isolates were investigated (Table 1), including seven US isolates, two of which were from two hamburger meat-associated outbreaks in 1982 [2] and 1992–1993 [16], and the remaining 20 isolates were derived from the multiple outbreaks and sporadic infections that occurred in Japan in 1996 [12]. PFGE analysis with restriction endonuclease digestion by *Xba*I was performed as described previously [10].

### *Isolation of phage and preparation of phage DNA*

The methods for isolation of the Stx-converting phages and preparation of phage DNA are described elsewhere [6], and either mitomycin C (Sigma) (2 µg/ml final concentration) or UV irradiation was used for phage induction. *E. coli* MC1061 [17], a strain of *E. coli* K12, was used as an indicator strain for plaque assay [6]. Phage isolation was repeated up to four times in some host strains so that multiple numbers of phages were obtained from each of the host strains.

### *stx profile*

The presence of the *stx* gene in the host strains and in the isolated phages was confirmed by a PCR assay as described by Lin *et al.* [18], with common primers to detect genes for Stx1 (or VT1 [19]), Stx2 (or VT2 [5]), VT2vha [20], VT2vhb [20], VT2vpl [18] (or SLT-IIv [21]) and VT2vp2 [18] (or SLT-IIva [22]). Subsequently, more specific sets of primers were designed to differentiate the *stx*<sub>1</sub> gene from the *stx*<sub>2</sub> gene as described by Cebula *et al.* [14]. Furthermore, variant types of *stx*<sub>2</sub> were determined by the method described by Tyler *et al.* [15]. Briefly, a set of primers to amplify a 285 bp fragment common to the B subunits of *stx*<sub>2</sub> and its variants, genes *stx*<sub>2</sub>vha and *stx*<sub>2</sub>vhb, were used, and the amplified fragment was subsequently digested with *Hae*III *Rsa*I or *Nci*I (Takara Shuzo, Kyoto, Japan) to identify fragment patterns specific to each variant gene.

### *Restriction fragment length polymorphism (RFLP) analysis of phage DNA*

Prepared phage DNA was digested with restriction endonucleases, *Eco*RI *Hind*III and *Sma*I (Takara

Shuzo), according to the manufacturer's instructions, and the digested DNA was analysed by agarose gel electrophoresis. The similarity between DNA from individual phages was calculated from the number of bands showing identical mobility in the agarose gel by the Dice coefficient ( $S_d$ ) [23]:  $S_d = [2A/(2A + B + C)] \times 100$ ; where A indicates the number of identical bands, and B and C show the number of bands present in one phage DNA but not in the other, respectively.

### *Preparation of chromosomal DNA and Southern blot hybridisation*

For Southern blot analysis, the total bacterial DNA of the isolates was prepared by the method described previously [24]. Southern blot hybridisation of the chromosomal DNA preparations was performed to detect *stx*-containing fragments of phage DNA inserted in the host's DNA. The blotting was performed as described previously [25] and subsequent hybridisation was performed by the method described by Yoh *et al.* [26]. Briefly, the total DNA preparations were digested by several endonucleases, including *Sma*I, *Hind*III and *Eco*RI (Takara). The digested DNAs were separated by conventional agarose 0.8% gel electrophoresis, transferred by Hybond-N<sup>+</sup> membrane (Amersham, Buckinghamshire) and probed with alkaline phosphatase-labelled probes (Toyobo, Tokyo, Japan), following the manufacturer's instructions. Probes for the A subunits of *stx*<sub>1</sub> and *stx*<sub>2</sub> comprised DNA corresponding to the nucleotide base sequences from 1106 to 1133 and from 1099 to 1127 from the initiation codon of the *stx*<sub>1</sub> and *stx*<sub>2</sub> genes, respectively [26]. In the *stx*<sub>2</sub> gene, there is only one *Sma*I restriction site, located at position 302–307 from the initiation codon of the gene [5, 20]. As the labelled *stx*<sub>2</sub> probe was designed to hybridise to the 30-bp fragment, 1099–1127, the hybridised fragment included the sequence downstream from the *Sma*I restriction site in the *stx*<sub>2</sub> operon to another *Sma*I restriction site somewhere outside the operon. Likewise, there is only one *Hind*III restriction site in the *stx*<sub>1</sub> operon, located at 803–809 nucleotides from the initiation codon [5], and the labelled *stx*<sub>1</sub> probe recognises the sequence from 1106–1133. Southern blot analysis was also performed on the phage DNA preparations in the same manner as described above.

## Results

### *PFGE types of the host strains*

Eight isolates belonged to PFGE type I, 10 to PFGE type II, two to PFGE type III, four PFGE type IV and one to PFGE type V. Two isolates showed PFGE patterns that were different from any other types (Table 1).

### *stx profile of chromosomal DNA preparations*

All isolates were found to have *stx* genes by PCR assays carried out according to Lin *et al.* [18].

**Table 1.** Stx-converting phages isolated from EHEC O157:H7 strains in the USA and Japan

EHEC O157:H7 host strains					Stx-converting phages derived		
ID no.	Origin/source	Date of isolation	stx profile	PFGE type	Phage ID no.	stx profile [14]	[15]
EDL933	USA/food	1982	stx <sub>1</sub> , stx <sub>2</sub>	ND	φ933SI	stx <sub>2</sub>	stx <sub>2</sub>
					φEDL933V2	stx <sub>2</sub>	stx <sub>2</sub>
					φEDL933V2'	stx <sub>2</sub>	stx <sub>2</sub>
CDC-1	USA/patient	1993	stx <sub>1</sub> , stx <sub>2</sub>	ND	φCDC1-a	stx <sub>1</sub>	NT
					φCDC1V1	stx <sub>1</sub>	NT
					φCDC1-b	stx <sub>2</sub>	stx <sub>2</sub>
					φCDC1V2	stx <sub>2</sub>	stx <sub>2</sub>
YO-1	Hiroshima/patient	June 1996	stx <sub>1</sub> , stx <sub>2</sub>	Ia	φYO-1	stx <sub>2</sub>	stx <sub>2</sub>
					φYo-1V2	stx <sub>2</sub>	stx <sub>2</sub>
YO-3	Fukuoka/patient	June 1996	stx <sub>1</sub> , stx <sub>2</sub>	Ia	φYO-3	stx <sub>2</sub>	stx <sub>2</sub>
YO-5	Gifu/patient	June 1996	stx <sub>1</sub> , stx <sub>2</sub>	Ib	φYO-5	stx <sub>2</sub>	stx <sub>2</sub>
YO-10	Aichi/patient	June 1996	stx <sub>1</sub> , stx <sub>2</sub>	Ib	φYO-10	stx <sub>2</sub>	stx <sub>2</sub>
YO-12	Okayama (Niimi)/patient	June 1996	stx <sub>1</sub> , stx <sub>2</sub>	Ib	φYO-12	stx <sub>2</sub>	stx <sub>2</sub>
					φYo-12V2	stx <sub>2</sub>	stx <sub>2</sub>
YO-15	Osaka/patient	June 1996	stx <sub>1</sub> , stx <sub>2</sub>	Ib	φYO-15	stx <sub>2</sub>	stx <sub>2</sub>
YO-8	Okayama (Oku)/patient	June 1996	stx <sub>1</sub> , stx <sub>2</sub>	Ic	φYO-8	stx <sub>2</sub>	stx <sub>2</sub>
					φYo-8V2	stx <sub>2</sub>	stx <sub>2</sub>
YO-16	Shiga/patient	August 1996	stx <sub>1</sub> , stx <sub>2</sub>	Ic	φYO-16	stx <sub>2</sub>	stx <sub>2</sub>
YO-19	Osaka (Sakai)/patient	July 1996	stx <sub>1</sub> , stx <sub>2</sub>	IIa	φ19V2	stx <sub>2</sub>	stx <sub>2</sub>
YO-20	Osaka (Sakai)/patient	July 1996	stx <sub>1</sub> , stx <sub>2</sub>	IIa	φYO20-2	stx <sub>1</sub>	NT
					φYO20V1	stx <sub>1</sub>	NT
					φYo20bV1	stx <sub>1</sub>	NT
					φYO20-1	stx <sub>2</sub>	stx <sub>2</sub>
					φ20V2	stx <sub>2</sub>	stx <sub>2</sub>
					φYo20aV2	stx <sub>2</sub>	stx <sub>2</sub>
					φYo20eV2	stx <sub>2</sub>	stx <sub>2</sub>
YO-22	Osaka (Sakai)/patient	July 1996	stx <sub>1</sub> , stx <sub>2</sub>	IIa	φ22V1	stx <sub>1</sub>	NT
					φYo22V2	stx <sub>2</sub>	stx <sub>2</sub>
YO-23	Kyoto/patient	July 1996	stx <sub>1</sub> , stx <sub>2</sub>	IIa	φ23V1	stx <sub>1</sub>	NT
					φ23V2	stx <sub>2</sub>	stx <sub>2</sub>
YO-27	Wakayama/patient	August	stx <sub>1</sub> , stx <sub>2</sub>	IIa	φ27V1	stx <sub>1</sub>	NT
					φYO27V2	stx <sub>2</sub>	stx <sub>2</sub>
CDC-8	USA/food	1993	stx <sub>1</sub> , stx <sub>2</sub>	IIa	φCDC-8	stx <sub>2</sub>	stx <sub>2</sub>
CDC-10	USA/cattle	1993	stx <sub>1</sub> , stx <sub>2</sub>	IIa	φCDC-10	stx <sub>2</sub>	stx <sub>2</sub>
CDC-11	USA/cattle	1993	stx <sub>1</sub> , stx <sub>2</sub>	IIc	φCDC-11	stx <sub>1</sub>	NT
YO-25	Tokyo/patient	July 1996	stx <sub>1</sub> , stx <sub>2</sub>	IId'	φYO-25	stx <sub>1</sub>	NT
					φ25V1	stx <sub>1</sub>	NT
					φYo25aV1	stx <sub>1</sub>	NT
					φYo25cV1	stx <sub>1</sub>	NT
					φ25V2	stx <sub>2</sub>	stx <sub>2</sub>
					φYo25eV2	stx <sub>2</sub>	stx <sub>2</sub>
YO-31	Iwate (Morioka)/patient	Sept. 1996	stx <sub>1</sub> , stx <sub>2</sub>	IIj	φ31V1	stx <sub>1</sub>	NT
					φYo31cV2	stx <sub>2</sub>	stx <sub>2</sub>
CDC-2	USA/patient	1993	stx <sub>1</sub> , stx <sub>2</sub>	III	φCDC-2	stx <sub>2</sub>	stx <sub>2</sub>
					φCDC2V2	stx <sub>2</sub>	stx <sub>2</sub>
CDC-3	USA/patient	1993	stx <sub>1</sub> , stx <sub>2</sub>	IIIa	φCDC3V2	stx <sub>2</sub>	stx <sub>2</sub>
YO-36	Chiba/patient	June 1996	stx <sub>2</sub>	IV	φYO-36	stx <sub>2</sub>	stx <sub>2</sub>
YO-38	Gunma/patient	July 1996	stx <sub>2</sub>	IV	φYO-38	stx <sub>2</sub>	stx <sub>2</sub>
YO-40	Saga/patient	Sept. 1996	stx <sub>2</sub>	IV	φ40V2	stx <sub>2</sub>	stx <sub>2</sub>
YO-42	Saga/fly	Oct. 1996	stx <sub>2</sub>	IV	φ42V2	stx <sub>2</sub>	stx <sub>2</sub>
YO-43	Kanagawa/patient	June 1996	stx <sub>2</sub> , stx <sub>2</sub> vha	Va	φKE-1	stx <sub>2</sub>	stx <sub>2</sub>

ND, not determined, NT, not tested.

Subsequent PCR assay as described by Cebula *et al.* [14] indicated that 21 isolates had both the stx<sub>1</sub> and stx<sub>2</sub> genes, and that six isolates had the stx<sub>2</sub> gene only (Table 1). PCR-RFLP analysis [15] revealed that one isolate, YO-43 had both stx<sub>2</sub> gene and its variant gene, stx<sub>2</sub>vha.

#### stx profile of phage DNA

A total of 51 Stx-converting phages was isolated from the 27 host strains. The phages, their host strains and the stx profiles obtained by three different PCR analysis are summarised in Table 1. All phages produced a 900-bp PCR-amplified product common to both stx<sub>1</sub> and stx<sub>2</sub> by primers described by Lin *et al.* [18]. The PCR

analysis of Cebula *et al.* [14], which differentiates stx<sub>1</sub> from stx<sub>2</sub>, showed that 14 of the phages had stx<sub>1</sub> and that 37 phages had stx<sub>2</sub> genes. Further PCR analysis based on the method of Tyler *et al.* [15] indicated that none of the stx<sub>2</sub> genes belonged to its variant genes (i.e., stx<sub>2</sub>vha and stx<sub>2</sub>vhb). It should also be noted that phages carrying stx<sub>1</sub> were seldom isolated from either strain EDL933 of PFGE nondescript type or strains belonging to PFGE type I in spite of repeated tests.

#### RFLP of phage DNA

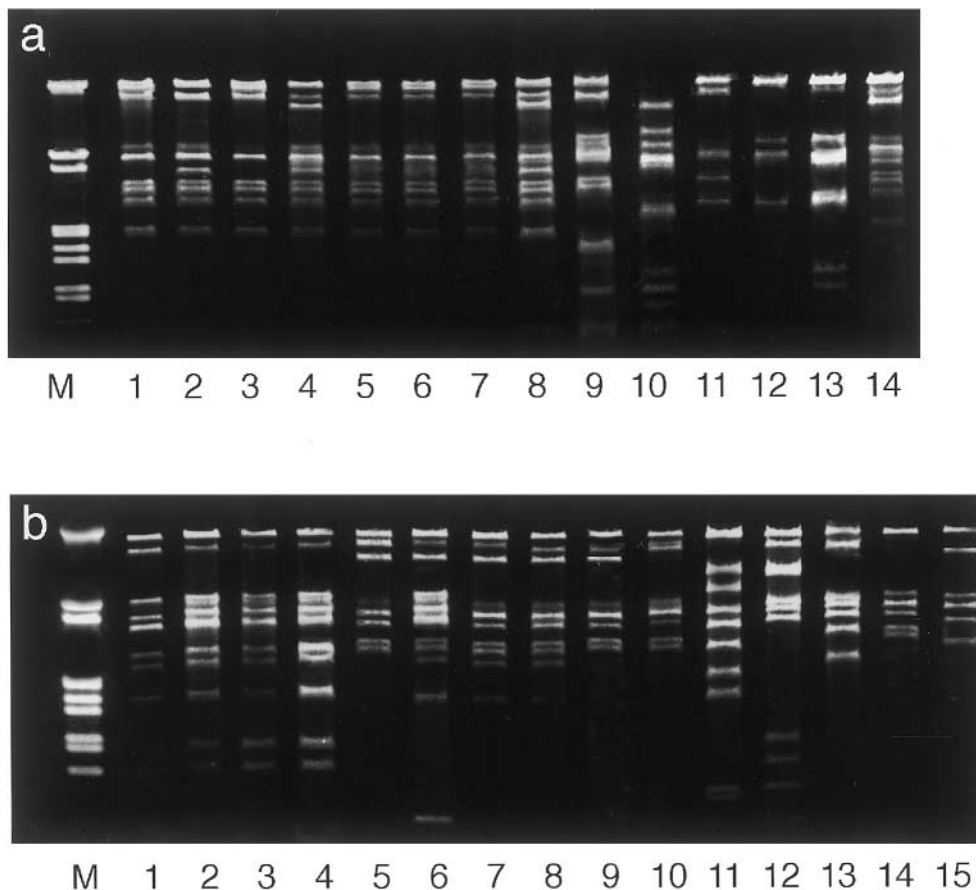
On the basis of the sum of the sizes of the individual restriction fragments, the whole genome size of each intact phage DNA ranged from 42 kb to 54 kb. The

agarose gel electrophoresis of restriction endonuclease (*EcoRI* and *HindIII* or *SmaI*) digests of the phage DNA preparations showed appreciable variations in their RFLP (Fig. 1a and b show *EcoRI* digests; data for *HindIII* and *SmaI* digests not shown). RFLP patterns revealed that the average  $S_d$  of 37 Stx2-converting phages by their *EcoRI* and *SmaI* digests was 55% and 44% respectively. The phages isolated from the host strains belonging to PFGE types I, III, and IV were identical ( $S_d$  values of 100%) within the same PFGE type. The phages from the host strains belonging to PFGE type II showed an appreciable variation in their patterns, but they were clearly distinguishable from the phages of other PFGE types (Fig. 2a), and generally the results showed that there were differences between the phages from the host strains of different PFGE types. However, there were some exceptions; those from a US isolate (EDL933) of the PFGE nondescript type were almost identical to those from the Japanese isolates belonging to PFGE type I with  $S_d$  value of >95% (Fig. 2a and Fig. 3a, b and c). Likewise, the RFLP patterns observed in the 14 Stx1-converting phages showed appreciable variations with an average  $S_d$  value of 67% on the *EcoRI*

digests and 55% on the *HindIII* digests. The exceptions were the phages ( $\phi$ YO20V1,  $\phi$ YO27V1 and  $\phi$ 31V1) from the host strains YO-20, YO-27 and YO-31, which were identical (Fig. 2b). These results indicate that some phages conserve their genomic structure in spite of the variation of host genome (i.e., the difference of PFGE types). It should be also noted that several Stx1- and Stx2-converting phages were isolated from a strain (i.e., CDC-1, YO-20 and YO25), which showed some variations from each other in RFLP patterns (Fig. 4).

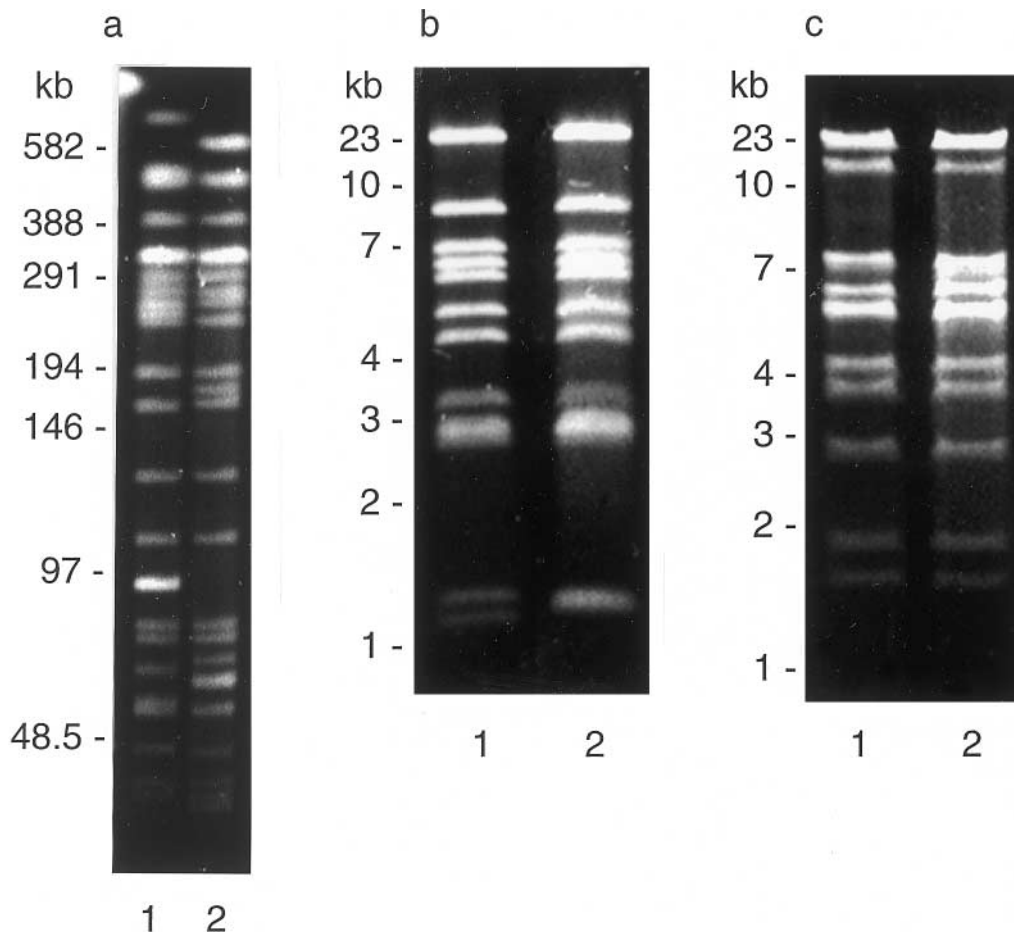
#### *Stx genes-hybridised RFLP of chromosomal and phage DNA*

The *stx*<sub>2</sub> gene probe reacted with a common 4.5-kb or 2.5 kb-*SmaI* fragment of DNA from the various phage (Table 2). There were less variations around the *stx*<sub>2</sub> gene compared with the variations in the whole phage DNA described above. The majority of the host chromosomes contained the *stx*<sub>2</sub> genes in the same sized *SmaI* fragment as the phage DNA while some (YO-19, YO-20, YO-22, YO-23 and YO-27) of the PFGE type IIa strains hybridised with a 7-kb *SmaI*



**Fig. 1.** Conventional agarose gel electrophoresis of *EcoRI*-digested DNA from: (a) Stx1-converting phages; M, DNA mol.-wt marker (*HindIII-EcoRI* double digest of  $\lambda$  phage DNA (0.83 ~ 21.23 kb)); lane 1,  $\phi$ CDC1-a; 2,  $\phi$ CDC1V1; 3,  $\phi$ YO20-2; 4,  $\phi$ YO20V1; 5,  $\phi$ YO20bV1; 6,  $\phi$ 22V1; 7,  $\phi$ 23V1; 8,  $\phi$ 27V1; 9,  $\phi$ CDC11; 10,  $\phi$ YO25; 11,  $\phi$ 25V1; 12,  $\phi$ YO25aV1; 13  $\phi$ YO25cV1; 14,  $\phi$ 31V1; (b) Stx2-converting phages (data from 15 of the 37 phages only shown); M, DNA mol.-wt marker (*HindIII-EcoRI* double digest of  $\lambda$  phage DNA (0.83 ~ 21.23 kb)); 1,  $\phi$ 933SI; 2,  $\phi$ YO-1; 3,  $\phi$ YO-10; 4,  $\phi$ Yo-8V2; 5,  $\phi$ CDC-1b; 6,  $\phi$ CDC-2; 7,  $\phi$ CDC3V2; 8,  $\phi$ 19V2; 9,  $\phi$ YO20-1; 10,  $\phi$ CDC1V2; 11,  $\phi$ YO-36; 12,  $\phi$ KE-1; 13,  $\phi$ 25V2; 14,  $\phi$ YO25eV2; 15,  $\phi$ Yo31cV2.





**Fig. 3.** (a) PFGE of EHEC O157:H7 strains showing different fragment patterns: 1, EDL933 (USA isolate); 2, YO-1 (Japanese isolate). Conventional agarose gel electrophoresis of (b) *Sma*I digests and (c) *Eco*RI digests of the Stx2-converting phages that had been isolated from the above host strains: 1,  $\phi$ 933SI; 2,  $\phi$ YO-1.

fragment (Table 2) despite the fact that the probe-reacted *Sma*I fragment in the corresponding phage DNA was 5 kb in size.

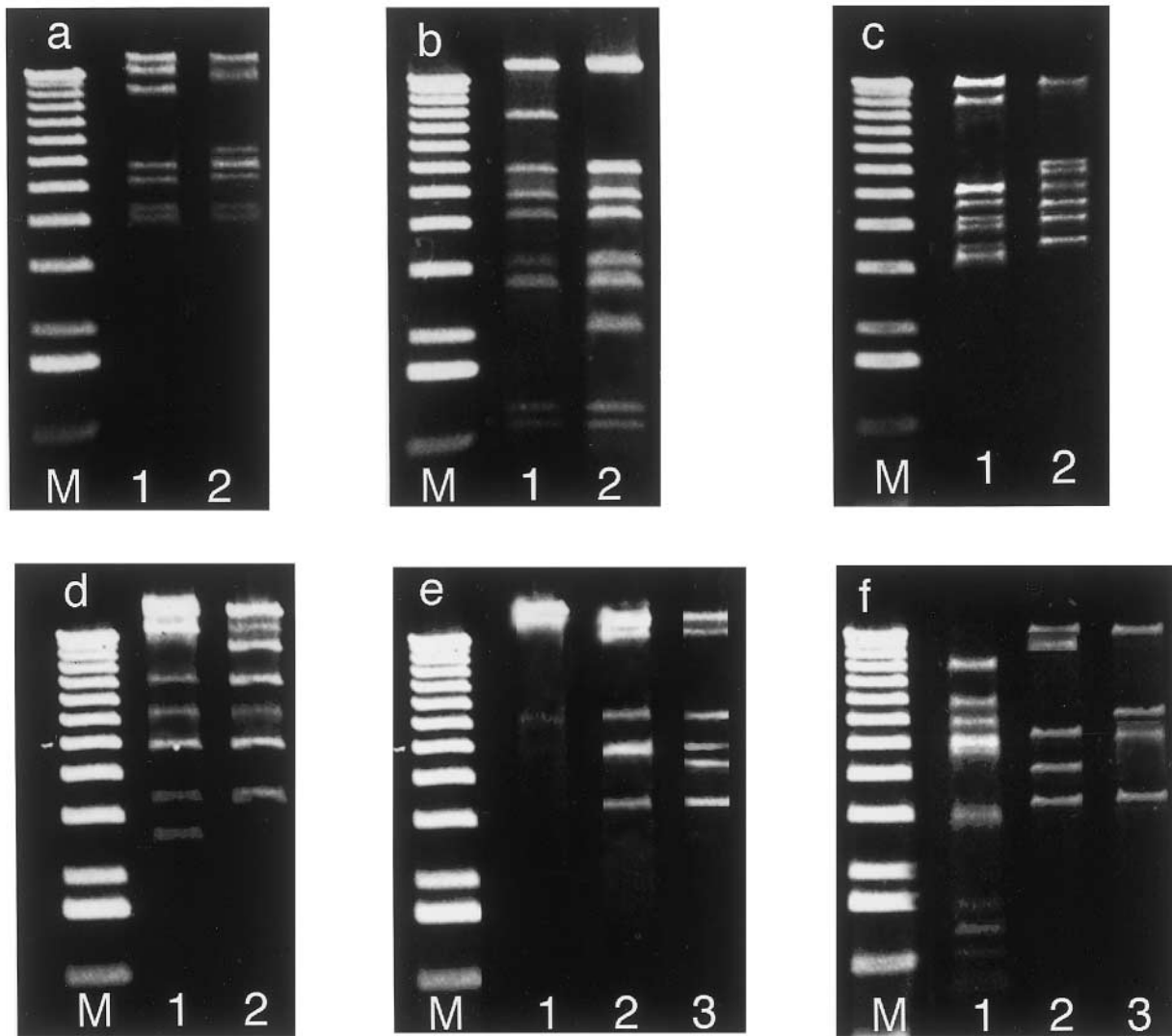
*Hind*III-digested RFLP patterns of the 14 Stx1-converting phages and their host strains with *stx*<sub>1</sub> DNA probe are also shown in Table 2. The size of the *stx*<sub>1</sub> probe-reacted fragments of the phage DNA corresponded to that of the host genome DNA in most cases. However, four Stx1-converting phages which had been isolated independently from YO-25 had reactive fragments (5 or 7 kb) markedly smaller than the host DNA fragment (19 kb).

## Discussion

Previous PFGE analyses have shown that various genotypes of EHEC O157:H7 have spread in Germany [27], the USA [28] and Japan [13, 29]. The PFGE types of the Japanese isolates derived from the major outbreaks occurring in 1996 were classified into at least five groups [13]. However, the PFGE patterns exceeded >200 when those from other sporadic infections occurring between 1996 and 1997 were included [30]. As the *stx* genes are encoded on the lambdoid phage DNA lysogenised in the genome of

EHEC O157:H7 [6], the present study investigated whether the host's genotypic diversity reflects any genotypic variations of the phages. The results showed that, generally, the Stx2-converting phages were genotypically homogeneous within the same PFGE types of the host strains, but they were different from each other when the host strains belonged to different PFGE types. This would indicate that the host genome has co-evolved with the phage genome. However, it was not possible to make such a comparison with Stx1-converting phages, because only two Stx1-converting phages ( $\phi$ CDC1-a and  $\phi$ CDC1V1) could be isolated from a host strain (CDC-1; PFGE nondescript type) other than PFGE type II.

Although RFLP patterns of phages derived from different PFGE types were quite different, the Stx-2 converting phages from one of the US isolates (EDL933) that had been derived from the outbreak in 1982 shared remarkably similar molecular profiles with those from the Japanese host strains of PFGE type I. This result seems to be consistent with the recent finding [31] that an Stx2-converting phage from EHEC O157:H7 derived from the Okayama outbreak in 1996 resembled the Stx2-converting phage, 933W, isolated from EDL933, in its infective properties. The PFGE



**Fig. 4.** Conventional agarose gel electrophoresis of the DNA from Stx-converting phages showing variations (<90%  $S_d$  values) in their RFLP patterns despite the fact that they were isolated from the same host strain. (a) *EcoRI* digests of lane 1,  $\phi$ CDC1-b; 2,  $\phi$ CDC1V2. (b) *SmaI* digests of: lane 1,  $\phi$ YO20-1; 2,  $\phi$ 20V2. (c) *EcoRI* digests of: lane 1,  $\phi$ 25V2; 2,  $\phi$ Yo25eV2. (d) *HindIII* digests of: lane 1,  $\phi$ CDC1-a, 2,  $\phi$ CDC1V1. (e) *HindIII* digests of: lane 1,  $\phi$ YO20-2, 2  $\phi$ YO20V1; 3,  $\phi$ Yo20bV1. (f) *EcoRI* digests of: lane 1,  $\phi$ YO-25; 2,  $\phi$ 25V1; 3,  $\phi$ Yo25aV1. M, DNA mot.-wt marker (1-kb DNA ladder, 1 ~ 12 kb).

analysis of these USA and Japanese host strains indicated that they were genotypically different from each other. On this basis, two hypotheses may be put forward to explain why homogeneous phages are found in the heterogeneous host strains. One is that the host strain carrying an Stx-converting phage altered its genotypic characteristics with time, and ramified into multiple PFGE types with the same phage being 'vertically' transferred. Alternatively, an Stx-converting phage might be released from a host strain and lysogenised into appropriate recipient host strains of different genotypes. The occurrence of such 'horizontal transfer' of Stx-converting phages has been demonstrated both *in vivo* and *in vitro* by recent experimental work conducted elsewhere [32, 33]. If this is the case the USA and Japanese isolates described above have originated from the same source.

The RFLP patterns seen in both Stx1- and Stx2-

converting phages isolated from the host strains of PFGE type II seemed to be rather problematic, in that the patterns of most phages varied even when they were from the same host strain. A straightforward interpretation of this observation may be the gain or loss of a restriction endonuclease site of the phages. Alternatively, those *E. coli* strains might have been lysogenised by more than one Stx1- or Stx2-converting phage of different genotypes. Recent genome sequencing revealed that EHEC O157:H7 Sakai strain (PFGE type IIa) carried at least 10 lambdoid phages in the genome [34]. It is also likely that phage propagation promotes genetic recombination between those lambdoid phages, which leads to the diversity of Stx-converting phages. Further molecular analysis, including cross-hybridisation of the phages from different PFGE groups, would be necessary to confirm the genotypic diversity or instability of Stx-converting phages.

**Table 2.** Southern blot analysis of phage DNA and host strains total DNA

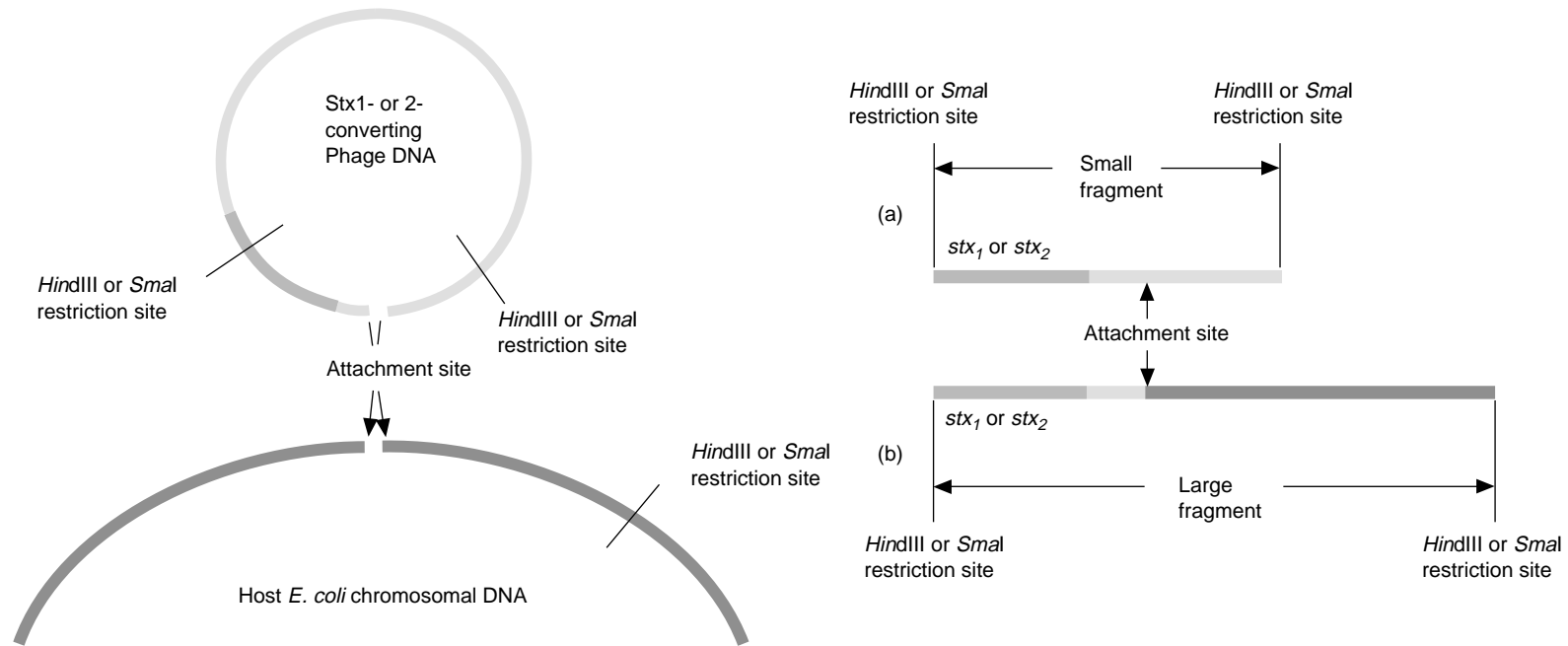
Host strain no.	PFGE type	Size (kb) of fragment that reacted with*		Phage no.	Size (kb) of fragment that reacted with*	
		<i>stx</i> <sub>1</sub> probe	<i>stx</i> <sub>2</sub> probe		<i>stx</i> <sub>1</sub> probe	<i>stx</i> <sub>2</sub> probe
EDL933	ND	–	4.5	<i>φ</i> 933SI	–	4.5
				<i>φ</i> EDL933V2	–	4.5
				<i>φ</i> EDL933V2'	–	4.5
CDC-1	ND	19	2.5	<i>φ</i> CDC1-a	19	–
				<i>φ</i> CDC1V1	19	–
				<i>φ</i> CDC-1b	–	2.5
				<i>φ</i> CDC1V2	–	2.5
YO-1	Ia	–	4.5	<i>φ</i> YO-1	–	4.5
				<i>φ</i> Yo-1V2	–	4.5
YO-3	Ia	–	4.5	<i>φ</i> YO-3	–	4.5
YO-5	Ib	–	4.5	<i>φ</i> YO-5	–	4.5
YO-10	Ib	–	4.5	<i>φ</i> YO-10	–	4.5
YO-12	Ib	–	4.5	<i>φ</i> YO-12	–	4.5
				<i>φ</i> Yo-12V2	–	4.5
YO-15	Ib	–	4.5	<i>φ</i> YO-15	–	4.5
YO-8	Ic	–	4.5	<i>φ</i> YO-8	–	4.5
				<i>φ</i> Yo-8V2	–	4.5
YO-16	Ic	–	4.5	<i>φ</i> YO-16	–	4.5
YO-19	IIa	–	7	<i>φ</i> 19V2	–	<b>4.5</b>
YO-20	IIa	19	7	<i>φ</i> YO20-2	19	–
				<i>φ</i> YO20V1	19	–
				<i>φ</i> Yo20bV1	19	–
				<i>φ</i> YO20-1	–	<b>4.5</b>
				<i>φ</i> 20V2	–	<b>4.5</b>
				<i>φ</i> Yo20aV2	–	<b>4.5</b>
				<i>φ</i> Yo20eV2	–	<b>4.5</b>
YO-22	IIa	19	7	<i>φ</i> 22V1	19	–
				<i>φ</i> Yo22V2	–	<b>4.5</b>
YO-23	IIa	19	7	<i>φ</i> 23V1	19	–
				<i>φ</i> 23V2	–	<b>4.5</b>
YO-27	IIa	19	7	<i>φ</i> 27V1	19	–
				<i>φ</i> YO27V2	–	<b>4.5</b>
CDC-8	IIa	–	2.5	<i>φ</i> CDC-8	–	2.5
CDC-10	IIa	–	4.5	<i>φ</i> CDC-10	–	4.5
CDC-11	IIc	19	–	<i>φ</i> CDC-11	19	–
YO-25	IId'	19	4.5	<i>φ</i> YO-25	7	–
				<i>φ</i> 25V1	7	–
				<i>φ</i> Yo25aV1	5	–
				<i>φ</i> Yo25cV1	5	–
				<i>φ</i> 25V2	–	4.5
				<i>φ</i> Yo25eV2	–	4.5
YO-31	IIj	23	7	<i>φ</i> 31V1	23	–
				<i>φ</i> Yo31cV2	–	<b>4.5</b>
CDC-2	III	–	2.5	<i>φ</i> CDC-2	–	2.5
				<i>φ</i> CDC2V2	–	2.5
CDC-3	IIIa	–	2.5	<i>φ</i> CDC3V2	–	2.5
YO-36	IV	–	4.5	<i>φ</i> YO-36	–	4.5
YO-38	IV	–	4.5	<i>φ</i> YO-38	–	4.5
YO-40	IV	–	4.5	<i>φ</i> 40V2	–	4.5
YO-42	IV	–	4.5	<i>φ</i> 42V2	–	4.5
YO-43	Va	–	4.5	<i>φ</i> KE-1	–	4.5

Fragment sizes in bold letters indicate that the reactive fragments were markedly smaller than the corresponding fragments of the host total DNA. ND, not determined.

\*Southern blotting was performed with *stx*<sub>1</sub> and *stx*<sub>2</sub> probes for *Hind*III and *Sma*I digests of the prepared DNA, respectively.

The *stx*<sub>1</sub> and *stx*<sub>2</sub> genes have only one cleavage site for *Hind*III and *Sma*I, respectively. The DNA hybridisation probes that were used for *stx*<sub>1</sub> and *stx*<sub>2</sub> detected the sequences downstream from each restriction site of the *stx* genes to another restriction site somewhere outside the *stx* operons. The DNA probe hybridised with the same sized DNA fragment between DNA preparations of a phage and its host chromosome belonging to PFGE type I, III, IV, V and other nondescript types. In contrast, the fragments of the phage DNAs hybridised were, in many cases, different in size from those of the host strains' chromosome belonging to PFGE type II

(see Table 2). This may indicate that, for PFGE type II, the phages were often integrated into the recipient host chromosome through the attachment site located downstream of the *stx* operon, as shown in Fig. 5. Huang *et al.* [35] suggested that *stx*<sub>1</sub> was located on the phage DNA at least 12–13 kb apart from its attachment site (estimated from the figure provided by Huang *et al.* [35]); however, sufficient details were not available. As the probe-reacted restriction fragments of the phage DNA were much smaller than those of the total DNA from several host strains, the *stx*<sub>1</sub> and *stx*<sub>2</sub> genes are likely to be located in much closer proximity to the



**Fig. 5.** Schematic drawings of phage attachment site proposed for four Stx1-converting phages,  $\phi$ YO25,  $\phi$ 25V1,  $\phi$ Yo25aV1 and  $\phi$ Yo25cV1, and eight Stx2-converting phages,  $\phi$ 19V2,  $\phi$ YO20-1,  $\phi$ 20V2,  $\phi$ Yo20aV2,  $\phi$ Yo20ev2,  $\phi$ Yo22V2,  $\phi$ 23v2 and  $\phi$ YO27V2 (not to scale). (a) *Hind*III or *Sma*I cut fragment of the total phage DNA preparation; (b) *Hind*III or *Sma*I cut fragment of the total DNA preparation of the host strain after lysogenisation of the phage.

phage attachment site. This in turn suggests that the *stx* genes can be either lost or gained by an aberrant excision of the prophage like other toxin-converting bacteriophages reported for several pathogenic bacteria [36, 37].

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