

Genome Analysis of a Novel Shiga Toxin 1 (Stx1)-Converting Phage Which Is Closely Related to Stx2-Converting Phages but Not to Other Stx1-Converting Phages

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Two Stx-converting phages, designated Stx1 ϕ and Stx2 ϕ -II, were isolated from an *Escherichia coli* O157:H7 strain, Morioka V526, and their entire nucleotide sequences were determined. The genomes of both phages were similar except for the *stx* gene-flanking regions. Comparing these phages to other known Stx-converting phages, we concluded that Stx1 ϕ is a novel Stx1-converting phage closely related to Stx2-converting phages so far reported.

Infection with enterohemorrhagic *Escherichia coli* (EHEC) causes severe illnesses including hemorrhagic colitis, hemolytic-uremic syndrome, and encephalosis (13). Such critical illnesses are due to Shiga toxin (Stx) produced by EHEC. EHEC produces two types of Stx, namely Stx1, which is identical to Shiga toxin produced by *Shigella dysenteriae* type 1 (17), and Stx2, which has immunological properties that are different from those of Stx1 but biological properties that are similar to those of Stx1 (22). Both of these Stxs are encoded by *stx* genes in the genome of the lysogenic bacteriophage (Stx phage) of EHEC (12, 16).

The fact that the expression of *stx* genes is linked to Stx phage induction (1, 11) is clinically quite important because DNA-damaging drugs such as quinolones, which induce an SOS response in bacteria, are supposed to enhance Stx production as well as Stx phage release from EHEC (4, 23). In fact, several studies on the effects of antibiotics on EHEC infection have been published (2, 19, 20). Thus, a need to analyze the nature or structure of Stx-converting phages has led to several studies on genome analysis of some Stx-converting phages (7, 9, 10, 14, 21). We also isolated three Stx-converting phages from EHEC strains collected in Japan, i.e., Stx1 ϕ , Stx2 ϕ -I, and Stx2 ϕ -II (18), and we determined their complete DNA sequences. In this paper, we report the genomic analysis of Stx1 ϕ and Stx2 ϕ -II, both derived from a single EHEC strain, Morioka V526.

Phage isolation and DNA sequence determination. Isolation of Stx-converting phages from the EHEC Morioka V526

strain, preparation of the restriction map, and subcloning were performed as described previously (18). DNA sequencing was done by using the Dye Terminator kit (Applied Biosystems, Norwalk, Conn.) and 377PRISM autosequencer (Applied Biosystems) with synthetic oligonucleotides as primers. It was found that the genome size of Stx1 ϕ was 59,866 bp, while that of Stx2 ϕ -II was 62,706 bp. As shown in Fig. 1, although these two phages carry different *stx* genes, their genomic structures were quite homologous. The 2.8-kb size difference was attributed mainly to the *Bam*HI-*Xho*I fragment-containing *stx* gene (Fig. 1). Also, insertion sequence *IS1203* v (6) was found in this region in Stx2 ϕ -II (Fig. 1).

Comparison to other reported Stx-converting phages. The genomic structures of Stx1 ϕ and Stx2 ϕ -II were compared to those of other Stx-converting phages so far reported. It was found that they were quite similar to those of other known Stx2-converting phages, except for the *stx*-flanking regions (Fig. 1 and Table 1), but not to those of other Stx1-converting phages such as VT1-Sakai and H19B (Fig. 2).

ORF analysis. Open reading frames (ORFs) that showed significant homologies to the genes registered in DDBJ or that consisted of more than 80 amino acid residues were picked up. This definition enabled us to identify 167 putative ORFs in Stx1 ϕ and 170 putative ORFs in Stx2 ϕ -II (for detailed ORF information, please refer to DDBJ). The ORFs that show homology to any genes in other Stx-converting phages or bacterium-associated genes were picked up and are listed in Table 1. ORFs of Stx1 ϕ and Stx2 ϕ -II were also almost completely identical, reflecting the high DNA sequence homology between these two phages. The exception was the *stx*-flanking regions including four ORFs, B69, B73, B74, and b70 in Stx1, which are identical to or almost the same as the corresponding ORFs of VT1-Sakai (Table 1). This region might be characteristic of Stx1-converting phages, since H19B (10) also has a homology in the corresponding region at the DNA level (data not shown). ORFs B4, B5, and B30 of Stx1 ϕ are not identical to the corresponding ORFs of Stx2 ϕ -II due to frameshift mu-

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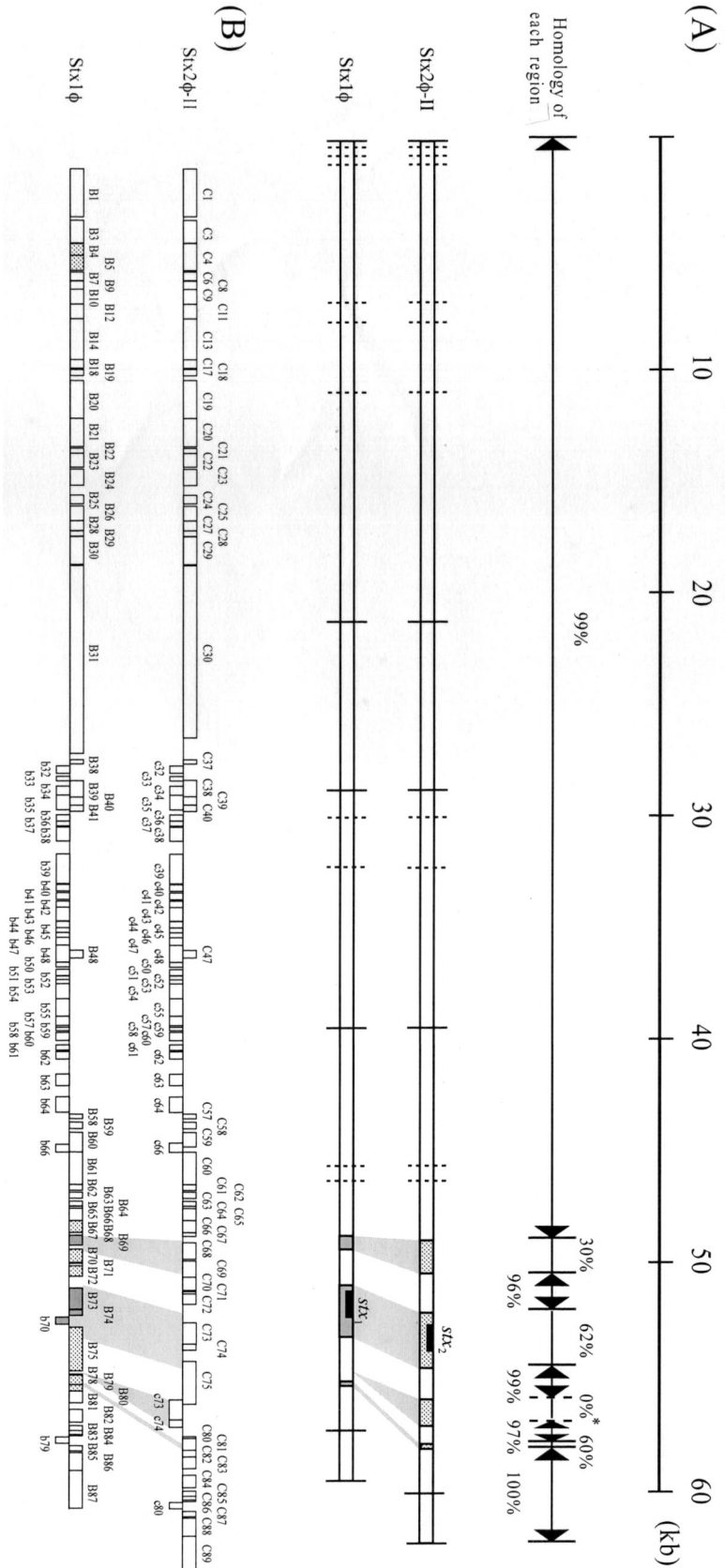


FIG. 1. Schematic representation of DNAs of Stx2φ-II and Stx1φ. (A) Comparison of DNAs of Stx2φ-II and Stx1φ. The linear sequences of *Xho*I fragments are shown. The linear sizes are shown on the top of the figure in kilobases (kb). Homology percentages are given above and below the second line of the figure. The asterisk indicates 0% homology, which is due to IS1203 v. The boxes above and below the horizontal lines are ORFs with rightward and leftward transcription directions, respectively. Open boxes are ORFs identical to the corresponding ORFs in any Stx2-converting phage(s) described in Table 1. Shaded boxes are ORFs characteristic of Stx1-converting phages, and dotted boxes are ORFs nearly identical to those of any Stx2-converting phage(s).

TABLE 1. ORFs of Stx1 ϕ and Stx2 ϕ -II

ORF	Stx1 ϕ		% Identity between Stx1 ϕ and Stx2 ϕ -II ^a		Gene	Orientation ^b	Stx2 ϕ -II			Homologous ORFs in ^c :			Description	% Identity (aa)
	Start	Stop	aa ^c	aa			ORF	Start	Stop	aa	933W	VT2-Sakai		
B0	-452	1255	568	100 (568)	C0	>	-452	1255	568	L0114	100 (714)	H0130		
B1	1255	3399	714	100 (714)	C1	>	1255	3399	714	L0115	100 (335)	H0131		
B3	3557	4564	335	100 (335)	C3	>	3557	4564	335	L0116	100 (162)	H0132		
B4	4588	5121	177	100 (162)	C4	>	4588	5802	404	L0117	100 (227)	H0133		
B5	5118	5801	227	100 (227)	C4	>	4588	5802	404	L0118	100 (129)	H0133		
B7	5857	6246	129	100 (129)	C6	>	5858	6247	129	L0119	100 (162)	H0134		
B9	6269	6757	162	100 (162)	C8	>	6270	6758	162	L0120	100 (223)	H0135		
B10	6633	7304	223	100 (223)	C9	>	6634	7305	223	L0121	100 (216)	H0136		
B12	7304	7954	216	100 (216)	C11	>	7305	7955	216	L0122	99 (645)	H0137	Collagen alpha 1 (I) chain	40 (422)
B14	7951	9888	645	100 (645)	C13	>	7952	9889	645	L0123	100 (93)	H0138		
B18	9770	10159	129	100 (129)	C17	>	9771	10160	129	L0124	100 (567)	H0139		
B19	10206	10487	93	100 (93)	C18	>	10207	10488	93	L0125	100 (422)	H0140		
B20	10704	12407	567	100 (567)	C19	>	10705	12408	567	L0126	100 (75)	H0141		
B21	12404	13672	422	100 (422)	C20	>	12405	13673	422	L0127	100 (205)	H0142		
B22	13738	13965	75	100 (75)	C21	>	13739	13966	75	L0128	100 (244)	H0143		
B23	13971	14588	205	100 (205)	C22	>	13972	14589	205	L0129	100 (133)	H0144		
B24	14679	15413	244	100 (244)	C23	>	14680	15414	244	L0130	100 (148)	H0145		
B25	15843	16244	133	100 (133)	C24	>	15844	16245	133	L0131	100 (83)	H0146		
B26	16338	16994	218	100 (218)	C25	>	16339	16995	218	L0132	100 (421)	H0147		
B28	16997	17443	148	100 (148)	C27	>	16998	17444	148	L0133	100 (2,806)	H0148		
B29	17453	17704	83	100 (83)	C28	>	17454	17705	83	L0134	100 (62)	H0149		
B30	17715	18980	421	100 (421)	C29	>	17716	18981	421	L0135	100 (114)	H0150		
B31	19011	27431	2,806	99 (2,565)	C30	>	19012	26742	2,576	L0136	100 (249)	H0151		
B38	27714	27902	62	100 (62)	C37	>	27714	27902	62	L0137	100 (70)	H0152		
B32	28326	27982	114	100 (114)	C32	>	28326	27982	114	L0139	92 (130)	H0153		
B39	28646	29395	249	100 (249)	C38	>	28646	29395	249	L0140	100 (94)		<i>E. coli</i> K-12 Gef	73 (69)
B33	28658	28446	70	100 (70)	C33	>	28658	28446	70	L0141	98 (73)			
B34	29362	28892	156	100 (156)	C34	>	29362	28892	156	L0142	100 (209)		Bacteriophage C3208 enterohemolysin 2	89 (74)
B40	29380	29748	122	100 (122)	C39	>	29380	29748	122	L0061	100 (444)		Enterohemolysin-associated protein	88 (77)
B41	29735	30019	94	100 (94)	C40	>	29735	30019	94	L0062	100 (99)			
B35	29946	29287	219	100 (219)	C35	>	29946	29287	219	L0063	100 (103)		<i>E. coli</i> AntB	84 (209)
B36	30456	30172	94	100 (94)	C36	>	30456	30172	94	L0064	100 (121)		<i>E. coli</i> K-12 putative transposase	55 (385)
B37	30674	30453	73	100 (73)	C37	>	30674	30453	73	L0065	100 (207)		<i>E. coli</i> YdaQ	35 (62)
B38	31351	30722	209	100 (209)	C38	>	31351	30722	209	L0066	100 (95)			
B39	33251	31917	444	100 (444)	C39	>	33251	31917	444	L0067	100 (72)			
B40	33579	33280	99	100 (99)	C40	>	33579	33280	99	L0068	100 (95)			
B41	33961	33650	103	100 (103)	C41	>	33961	33650	103	L0069	100 (115)			
B42	34386	34021	121	100 (121)	C42	>	34386	34021	121	L0070	100 (257)		Bacteriophage C3208 enterohemolysin 2	95 (102)
B43	34921	34298	207	100 (207)	C43	>	34921	34298	207	L0071	100 (93)		Enterohemolysin-associated protein	97 (177)
B44	35212	34925	95	100 (95)	C44	>	35212	34925	95	L0072	100 (116)			
B45	35432	35214	72	100 (72)	C45	>	35432	35214	72	L0073	100 (115)			
B46	35721	35434	95	100 (95)	C46	>	35721	35434	95	L0074	100 (115)			
B47	36118	35651	155	100 (155)	C47	>	36118	35651	155	L0075	100 (116)			
B48	36217	36567	116	100 (116)	C47	>	36217	36567	116	L0076	100 (115)			
B48	36764	35991	257	100 (257)	C48	>	36764	35991	257	L0077	100 (115)			
B50	36982	36761	73	100 (73)	C50	>	36982	36761	73	L0078	100 (115)			
B51	37362	37081	93	100 (93)	C51	>	37362	37081	93	L0079	100 (257)			
B52	37564	37373	63	100 (63)	C52	>	37564	37373	63	L0080	100 (93)		Phage lambda orf61	93 (46)
B53	37725	37537	62	100 (62)	C53	>	37725	37537	62	L0081	100 (93)		Phage lambda orf63	90 (61)
B54	38396	37716	226	100 (226)	C54	>	38396	37716	226	L0082	100 (63)		Phage lambda orf60	96 (60)
B55	39178	38393	261	100 (261)	C55	>	39178	38393	261	L0083	100 (62)		Phage lambda orf60	98 (226)
B57	39600	39184	138	100 (138)	C57	>	39600	39184	138	L0084	100 (226)		Phage lambda Bet	99 (261)
B58	39824	39555	89	100 (89)	C58	>	39824	39555	89	L0085	100 (261)		Phage lambda Gam	97 (138)
										L0076	100 (89)		Phage lambda Kil	98 (89)

TABLE 1—Continued

b59	39831	39667	54	100 (54)	c59	39831	39667	54	<i>cIII</i>	<	98 (54)	L0077	100 (54)	H0088	100 (54)	H0057	Phage lambda CIII	100 (54)
b60	40272	39904	122	100 (122)	c60	40272	39904	122	<i>ea10</i>	<	98 (122)	L0078	100 (122)	H0089	98 (122)	H0056	Phage lambda Eal0	99 (122)
b61	40706	40455	83	100 (83)	c61	40706	40455	83		<	41 (127)	L0080	100 (83)	H0090				42 (127)
b62	41109	40765	114	100 (144)	c62	41109	40765	114	<i>N</i>	<		L0085	100 (90)	H0091	47 (90)	H0054	Phage HK97 N	
b63	42287	41766	173	100 (173)	c63	42287	41766	173		<		L0085	100 (173)	H0093				71 (212)
b64	43484	42789	231	100 (231)	c64	43484	42789	231	<i>cl</i>	<		L0086	99 (217)	H0094				69 (71)
B58	43560	43775	71	100 (71)	C57	43560	43775	71	<i>cro</i>	>		L0087	100 (71)	H0095				
B59	43917	44213	98	100 (98)	C58	43917	44213	98	<i>cII</i>	>	98 (98)	L0087	100 (98)	H0096	90 (98)	H0050	Phage HK022 CII	96 (98)
B60	44385	45284	299	92 (194)	C59	44385	45029	214	<i>O</i>	>		L0088	100 (299)	H0098				98 (299)
B61	45259	46710	483	100 (483)	C60	45258	46709	483	<i>P</i>	>		L0089	100 (478)	H0099				99 (413)
b66	45272	44913	119	100 (117)	c66	45271	44855	138		<		L0089	100 (119)	H0099				
B62	46710	46979	89	100 (89)	C61	46709	46978	89		>		L0091	100 (89)	H0100				
B63	47050	47328	92	100 (92)	C62	47049	47327	92		>	100 (92)	L0091	100 (92)	H0101	98 (92)	H0045		
B64	47461	47676	71	100 (71)	C63	47460	47675	71		>	97 (71)	orf 6 ^c	100 (71)	H0102	98 (71)	H0044		
B65	47681	47923	80	100 (80)	C64	47680	47922	80		>	100 (48)	L0092	100 (78)	H0103	100 (78)	H0043		
B66	47781	48326	181	100 (181)	C65	47780	48325	181	<i>ninB</i>	>	99 (181)	L0093	100 (148)	H0104	100 (148)	H0042	Bacteriophage 21 NinB	43 (148)
B67	48323	48850	175	98 (175)	C66	48322	48849	175	<i>dam</i>	>	98 (175)	L0094	98 (175)	H0105	100 (175)	H0041	DNA adenine methyltransferase	32 (158)
B68	48847	49023	58	100 (58)	C67	48846	49028	60	<i>ninE</i>	>	85 (27)	orf 11 ^c	100 (58)	H0106	100 (58)	H0040	Bacteriophage P22 NinE	98 (58)
B69	48984	49427	147		C68	49303	50037	244	<i>ant</i>	>		L0096	100 (242) ^d	H0108				82 (104)
B70	49589	50194	201	96 (201)	C70	50834	51439	201	<i>roi</i>	>	88 (242) ^d	L0096	100 (242) ^d	H0108				82 (241)
B71	50221	50385	54	100 (54)	C71	51466	51630	54	<i>ninG</i>	>	96 (201)	L0097	96 (201)	H0109	100 (135)	H0039	Phage lambda Nin G	90 (203)
B72	50339	50812	157	97 (157)	C72	51584	52057	157	<i>Q</i>	>	100 (54)	L0098	100 (54)	H0110	100 (54)	H0036	Phage lambda Nin H	78 (50)
B73	51319	52266	315		C73	52841	53800	319	<i>stxB₁</i>	>	97 (157)	L0099	97 (157)	H0111	100 (157)	H0035	Q	
B74	52276	52545	89		C74	53812	54081	89	<i>stxB₂</i>	>	100 (319) ^d	L0103	100 (319) ^d	H0112	100 (89)	H0033	StxA1 subunit	100 (315)
b70	52929	52606	107		C75	54568	56472	634	<i>yjH₅</i>	>	100 (89) ^d	L0104	100 (89) ^d	H0113	100 (89)	H0033	StxB1 subunit	100 (89)
B75	53056	55002	648	89 (570)	C75	54568	56472	634		>	91 (648)	L0105	98 (415)	H0115	100 (107)	H0032	<i>S. dysenteriae</i> hypothetical protein	100 (319)
B78	55140	55319	59	100 (59)	c73	57169	56279	296		<		L0106	81 (136)	H0116	100 (296) ^d	H0021	<i>S. dysenteriae</i> hypothetical protein	59 (61)
B79	55186	55605	139	82 (136)	c74	57492	57166	108		<	100 (59)	orf 25 ^c	100 (59)	H0117	99 (108) ^d	H0020	<i>S. dysenteriae</i> YjH5	67 (656)
B80	55608	55898	96	100 (71)	C81	58000	58446	148		>	79 (148)	L0106	81 (136)	H0119	100 (59)	H0031	<i>S. sonnei</i> bacteriophage 7888	91 (648)
B81	55903	56436	177	100 (177)	C82	58523	58738	71	<i>S</i>	>	100 (71)	L0107	100 (71)	H0121	100 (71)	H0029	<i>S. sonnei</i> bacteriophage 7888	100 (296)
B82	56707	57276	189	100 (189)	C83	58743	59276	177	<i>R</i>	>	100 (177)	L0108	100 (177)	H0122	100 (177)	H0028	<i>S. sonnei</i> bacteriophage 7888 R	100 (108)
B83	57430	57894	154	100 (154)	C84	59547	60116	189	<i>ant</i>	>	100 (189)	L0109	100 (189)	H0123	100 (189)	H0027	<i>S. sonnei</i> bacteriophage Ant	95 (71)
B84	57650	57835	61	100 (61)	C85	60270	60734	154	<i>Rz</i>	>	100 (154)	L0110	100 (154)	H0124	96 (153)	H0026	<i>S. sonnei</i> bacteriophage 7888 Rz	96 (177)
b79	58219	57926	97	100 (97)	C86	60490	60675	61		>	100 (61)	L0110	100 (61)	H0125	98 (61)			84 (133)
B85	58327	58572	81	100 (81)	c80	61059	60766	97	<i>bor</i>	<	100 (97)	L0111	100 (97)	H0126				72 (61)
B86	58628	59434	268	100 (268)	C87	61167	61412	81		>	100 (81)	L0112	100 (81)	H0127				96 (97)
B87	59415	1255	568	100 (568)	C88	61468	62274	268		>	100 (268)	L0113	100 (568)	H0128				
B87	59415	1255	568	100 (568)	C89	62255	1255	568		>	100 (568)	L0113	100 (568)	H0129				

^a Homologous ORF(s) of Six2b-II, 933W (14), VT2-Sakai (7) and VT1-Sakai (21) compared with each ORF of Six1φ are shown as % identity in amino acid residues indicated. Identities higher than 95% are shown in bold, and ORFs which are identical to the corresponding ORF in Six1φ are underlined.
^b >, rightward transcription; <, leftward transcription in Fig. 1.
^c This ORF was reported in reference 3.
^d Comparison with Six2b-II.
^e aa, amino acids.

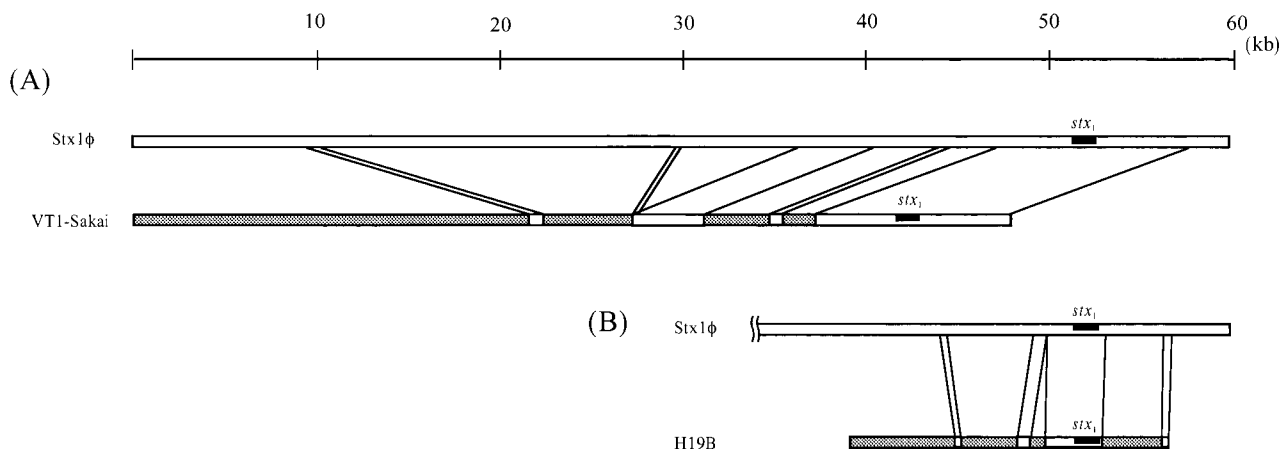


FIG. 2. Comparison of Stx1φ with other related Stx1-converting phages. The open bars represent portions homologous to Stx1φ, while the different portions of each Stx1-converting phage are cross-hatched. (A) Comparison between Stx1φ and VT1-Sakai phages; (B) comparison between Stx1φ and H19B phages. The DNA sequence of VT1-Sakai phage was modified from that reported by Yokoyama et al. (21) for convenience. Note that most regions of Stx1φ were not homologous to Stx1-converting phages, except for the *stx*₁-flanking region.

tations (data not shown). From these data, we conclude that Stx1φ is closely related to other Stx2-converting phages even at the ORF level.

It is noteworthy that there are several ORFs homologous to those of *Shigella sonnei* phage 7888 (15) and *S. dysenteriae* (8) in the *stx*-flanking regions of Stx1φ and Stx2φ-II (Table 1). Recently, an Stx-converting phage was isolated from Stx1-producing *S. sonnei* (L. Beutin, E. Strauch, and I. Fischer, Letter, Lancet 353:1498, 1999). Treatment with mitomycin C increases Stx production and induces Stx phage from some EHEC (5) and *S. sonnei* (Beutin et al., letter) bacteria. It has been a focus of discussion whether Stx-converting phages in EHEC are derived from *Shigella* species. Our data rather support that Stx-converting phages might be derived from, or at least related to, *Shigella* species.

Nucleotide sequence accession numbers. The entire nucleotide sequences of Stx1φ and Stx2φ-II were submitted to DDBJ under accession numbers AP005153 and AP005154, respectively.

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