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A Genomic Island, Termed High-Pathogenicity Island, Is Present in Certain Non-O157 Shiga Toxin-Producing *Escherichia coli* Clonal Lineages

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Shiga toxin-producing *Escherichia coli* (STEC) strains cause a wide spectrum of diseases in humans. In this study, we tested 206 STEC strains isolated from patients for potential virulence genes including *stx*, *eae*, and enterohemorrhagic *E. coli hly*. In addition, all strains were examined for the presence of another genetic element, the high-pathogenicity island (HPI). The HPI was first described in pathogenic *Yersinia* species and encodes the pesticin receptor FyuA and the siderophore yersiniabactin. The HPI was found in the genome of distinct clonal lineages of STEC, including all 31 *eae*-positive O26:H11/H$^-$ strains and 7 of 12 *eae*-negative O128:H2/H$^-$ strains. In total, the HPI was found in 56 (27.2%) of 206 STEC strains. However, it was absent from the genome of all 37 O157:H7/H$^-$, 14 O111:H$^-$, 13 O103:H2, and 13 O145:H$^-$ STEC isolates, all of which were positive for *eae*. Polypeptides encoded by the *fyuA* gene located on the HPI could be detected by using immunoblot analysis in most of the HPI-positive STEC strains, suggesting the presence of a functional yersiniabactin system. The HPI in STEC was located next to the tRNA gene *asnT*. In contrast to the HPI of other pathogenic enterobacteria, the HPI of O26 STEC strains shows a deletion at its left junction, leading to a truncated integrase gene *int*. We conclude from this study that the *Yersinia* HPI is disseminated among certain clonal subgroups of STEC strains. The hypothesis that the HPI in STEC contributes to the fitness of the strains in certain ecological niches rather than to their pathogenic potential is discussed.

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) strains are a worldwide cause of human disease, the spectrum of which ranges from mild diarrhea to life-threatening hemolytic-uremic syndrome (HUS). In addition to expressing Stx, most of these strains possess other virulence characteristics such as the ability to cause attaching-and-effacing (A-E) lesions on mucosal epithelial cells of the large intestine (52), and they contain an approximately 90-kb plasmid (54). STEC strains which, in addition to Stx production, display the A-E activity may also be referred to as enterohemorrhagic *E. coli* (EHEC). Although most STEC strains belong to the serotype O157:H7, non-O157 STEC, mostly those of the serogroups O26, O111, O103, O145, and O128, are a significant cause of human disease in Europe (5, 8).

STEC produce one or more Stx. The *E. coli* Stx family consists of two major toxin types, Stx1 and Stx2, that display only 58% overall nucleotide sequence homology (31). The genes encoding Stx1 and Stx2 are located in the genomes of temperate lambdoid bacteriophages (30, 33, 46, 50), and this may facilitate the spread of the genes via transduction. The large plasmids of STEC O157 and non-O157 encode determinants that may serve as additional virulence factors (24), such as the EHEC hemolysin, which has the function of a pore-forming cytolysin (45). In STEC O157:H7, the genes encoding proteins involved in producing the A-E lesions are located on a 42-kb pathogenicity island (PAI) termed the locus of enterocyte effacement (LEE). The LEE consists of three functional domains: the *eae* and *tir* genes in the central region, a type III secretion system, and genes for other secreted proteins (*esp* loci) (for review, see reference 22).

Whereas most PAIs are species- and even pathotype-specific, e.g., PAIs encoding alpha-hemolysin and P fimbriae are found exclusively in extraintestinal *E. coli* (3, 16, 40), one PAI, termed the high-pathogenicity island (HPI) and first described in pathogenic *Yersinia* strains, is widespread among enterobacteria (49). The HPI region carries the gene *fyuA*, which is specific for the pesticin receptor and the *irp* (iron repressible protein) loci encoding the siderophore yersiniabactin. The HPI element is associated with asparagine-specific tRNA loci and carries an integrase gene, *int*, often associated with a phage genome (7, 38). It is of interest that the HPI is not only present in the genomes of the pathogenic *Yersinia* species, including *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, and *Yersinia pestis*, but is also a part of the genomes of other enterobacteria such as *Klebsiella* spp., *Citrobacter* spp., and *E. coli* (16, 17, 49).

In pathogenic *E. coli*, the HPI element is frequently found in the genomes of enteroaggregative *E. coli* and of extraintestinal *E. coli* strains associated with urinary tract infections and sepsis (49). The HPI has also been detected in more than 30% of *E. coli* strains from physiological intestinal microflora (49). In a previous publication, we reported that STEC strains of serotype O157:H7 did not possess the HPI element (49). In this report, we confirm this observation and show that the *Yersinia* HPI is a part of the genome of certain non-O157 STEC clonal lineages.

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MATERIALS AND METHODS

Bacterial strains. In total, 206 STEC strains isolated from patients were investigated. The strains belonged to the serotype O157:H7/H$^-$ and to 57 dif-

TABLE 1. Potential virulence factors of STEC strains used in this study

Serotype	Total no. of strains	No. containing *stx* gene			No. containing *eae*	No. containing EHEC *hly*
		stx$_1$	*stx*$_2$	*stx*$_1$ plus *stx*$_2$		
O157:H7/H$^-$	37	2	25[a]	10[a]	37	37
O26:H11/H$^-$	31	13	15	3	31	31
O103:H2	13	12	0	1	13	13
O111:H$^-$	14	9	0	5	14	13
O145:H$^-$	13	3	9[a]	1[a]	13	13
O128:H2/H$^-$	12	2	1[b]	9[b]	0	7
Other[c]	86	37	28[d]	21[d]	21	45
Total	206	78	78	50	129	159

[a] *stx*$_2$ variant was *stx*$_{2c}$.
[b] *stx*$_2$ variant was *stx*$_{2d}$.
[c] Fifty different serotypes within 39 O serogroups; the serotypes that comprised at least three strains were O8:H$^-$ (six strains), O62:H$^-$ (three strains), O78:H$^-$ (three strains), O113:H$^-$ (three strains), and O118:H$^-$ (three strains).
[d] *stx*$_2$ variant was *stx*$_{2c}$ (nine strains) or *stx*$_{2d}$ (22 strains).

ferent non-O157 serotypes (Table 1). Most of them were isolated from German patients with HUS or diarrhea in our laboratory during routine diagnostic work between 1987 and 1998. Sixteen strains originated from patients with HUS or diarrhea in France, Italy, Canada, and the United States and were described elsewhere (6, 25, 27, 42, 47, 51). Enteroaggregative *E. coli* strain 17-2 (53) was a gift from J. P. Nataro (Center for Vaccine Development, Baltimore, Md.). Strains of *Y. pestis* KIM6$^+$, *Y. enterocolitica* WA-314, *Y. pseudotuberculosis* O1, *E. coli* K-12 MG1655, and *E. coli* DH5α were described previously (1, 13, 34, 37).

Isolation and identification of STEC. Screening for STEC in stool cultures was performed by PCR as described previously (23) using primer pair KS7 and KS8 (42) and either GK3 and GK4 (14) or LP43 and LP44 (10) complementary to the *stx*$_1$ and *stx*$_2$ genes (Table 2). The *stx*$_{2c}$ was demonstrated by restriction analysis of the *stx*$_2$*B* PCR products with *Hae*III and *Fok*I as described (41). The strategy to detect the *stx*$_{2d}$ was that described by Piérard et al. (35). To identify STEC strains in PCR-positive samples, colony hybridization with 100 to 200 well-separated colonies was performed (44) by using digoxigenin-labeled *stx*$_1$ and *stx*$_2$ probes prepared as described (42, 44). The identified STEC strains were serotyped according to Bockemühl et al. (4).

Detection of STEC virulence genes. The presence of the STEC virulence genes, including the *stx*$_1$, *stx*$_2$ and *stx*$_2$ variants (*stx*$_{2c}$, *stx*$_{2d}$), *eae*, and EHEC *hly* was detected by PCRs performed with the GeneAmp PCR System 9600 (Perkin-Elmer, Weiterstadt, Germany). Amplifications were carried out in a total volume of 50 μl containing 15 μl of bacterial suspension (10^6 cells), each deoxynucleoside triphosphate at a concentration of 200 μM, 30 pmol of each primer, 5 μl of 10-fold-concentrated polymerase synthesis buffer, 1.5 mM $MgCl_2$, and 2.0 U of Ampli*Taq* DNA polymerase (Perkin-Elmer). The primer sequences and PCR conditions are shown in Table 2. After 30 cycles had been completed, a 5-μl volume of each PCR sample was analyzed by submarine gel electrophoresis on a 1.5% (wt/vol) agarose gel and was visualized by staining with ethidium bromide. Strains EDL933 (O157:H7; *stx*$_1^+$ *stx*$_2^+$ *eae*$^+$, EHEC *hly*$^+$) (32, 44, 45), E32511 (O157:H$^-$; *stx*$_{2c}^+$ *eae*$^+$, EHEC *hly*$^+$) (48), and 4797/97 (O103:H$^-$; *stx*$_{2d}^+$) from our collection were used as positive controls.

Detection of the *Yersinia* HPI genes in STEC strains. The *irp2* and *fyuA* genes of the *Yersinia* HPI were detected by PCR as described by Schubert et al. (49) with small modifications. For a more detailed analysis of the HPI in STEC strains, several primer pairs targeting further genes described in the *Yersinia* HPI were designed, mostly according to sequences published for *Y. pestis* HPI (13). The target regions and the primers and PCR conditions are shown in Fig. 1 and Table 2, respectively.

Characterization and sequencing of the integrase gene. The presence of the integrase gene in the HPI of STEC strains was demonstrated by PCR amplification using the primers and conditions shown in Table 2. In order to sequence the integrase gene of STEC strains, the amplified DNA PCR products obtained with primers asnT1 and int2 were purified with the QIAquick PCR purification kit (Qiagen, Hilden, Germany). For each sequencing reaction, 12 μl (100 ng) of DNA was subjected to the thermosequenase fluorescent-labeled primer cycle sequencing kit (Amersham, Pharmacia Biotech, Freiburg, Germany). Electrophoresis of the sequencing products was performed on a model 4000 automated sequencer (MWG-Biotech, Ebersberg, Germany).

Detection of FyuA by immunoblotting. For immunoblotting, ultrasonicated bacterial cell pellets were treated with Triton X-100, and the insoluble membrane material was purified and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described previously (18, 21). After electrotransfer to polyvinylidene difluoride membranes (Millipore, Eschborn, Germany), FyuA was detected by antiserum (anti-FyuA) raised against FyuA from *Y. enterocolitica* O8 strain Y1852 in rabbits (21). Goat anti-rabbit antibody conjugated to horseradish peroxidase was employed as a second antibody (ECL Western blotting detection reagents; Amersham Pharmacia Biotech). The membrane was soaked briefly in the detection reagent. This elicited a peroxidase-catalyzed oxidation of luminol and subsequently enhanced chemiluminescence when the horseradish peroxidase-labeled protein was bound to the antigen on the membrane. The chemiluminescence was detected by exposing the membrane to Kodak Bio Max MR film at room temperature (19).

Nucleotide sequence accession numbers. The nucleotide sequences for the HPI integrase genes of *E. coli* O128:H2 (strain 3172/97) and *E. coli* O26:H$^-$ (strain 5720/96) have been entered into the EMBL database under the accession no. AJ245584 and AJ245585, respectively.

RESULTS

Analysis of STEC strains for chromosomal and plasmid-encoded determinants. All STEC strains were tested by PCR with the primers specific for the *stx*, *eae*, and EHEC *hly* genes, respectively, shown in Table 2. The presence of the genes encoding potential virulence characteristics in 206 strains is shown in Table 1. All strains harbored one or more *stx* genes, including *stx*$_1$, *stx*$_2$, and *stx*$_2$ variants (*stx*$_{2c}$ or *stx*$_{2d}$). However, there were marked differences among the serotypes regarding the types of the *stx* genes. Whereas the majority of O157:H7/H$^-$ and O145:H$^-$ isolates harbored the *stx*$_2$ and/or *stx*$_{2c}$ genes, all strains belonging to serotypes O103:H2 and O111:H$^-$ harbored *stx*$_1$, usually as the only *stx* gene, and none of the strains of these two serotypes possessed the *stx*$_2$ gene only. Within the serotype O26:H11/H$^-$, isolates containing the *stx*$_1$ and *stx*$_2$ gene occurred with similar frequency. Ten of 12 isolates of serotype O128:H2/H$^-$ harbored genes encoding a new *stx*$_2$ variant, *stx*$_{2d}$. With the exception of one strain, the *stx*$_{2d}$ gene was generally present in combination with the *stx*$_1$ gene. The *stx*$_{2d}$ gene was not found in any of the isolates belonging to the serotypes O157:H7/H$^-$, O26:H11/H$^-$, O103:H2, O111:H$^-$, or O145:H$^-$. In the heterogeneous group of 86 STEC strains comprising 50 different serotypes, more than one-third of the strains harbored the *stx*$_1$ gene only; among 49 strains with the *stx*$_2$ and/or the *stx*$_2$ variant genes, 22 strains contained *stx*$_{2d}$ alone or in combination with *stx*$_1$, and nine strains contained *stx*$_{2c}$. The 22 *stx*$_{2d}$-positive isolates belonged to 16 different serotypes.

The *eae* and EHEC *hly* genes were used as markers for the presence of the LEE PAI and the large EHEC plasmid, respectively. As demonstrated in Table 1, all strains of serotypes O157:H7/H$^-$, O26:H11/H$^-$, O103:H2, and O145:H$^-$, and all but one strain of serotype O111:H$^-$, harbored both *eae* and EHEC *hly* genes. One additional O111:H$^-$ strain possessed the *eae* but not the EHEC *hly* gene. In contrast, all 12 strains of serotype O128:H2/H$^-$ lacked the *eae* gene, and only seven contained the EHEC *hly* gene. Of the 86 strains belonging to 50 different serotypes, 21 and 45 isolates harbored the *eae* and EHEC *hly* genes, respectively, but only 13 isolates possessed both genes.

Presence of HPI in STEC strains. In order to test whether STEC strains carry the HPI, PCRs specific for *irp2* and *fyuA* genes were performed by using primers shown in Table 2. The distribution of the *irp2* and *fyuA* genes in strains of different serotypes and the correlation of these genes with the presence of the *eae* gene are shown in Table 3. All 31 *eae*-positive O26:H11/H$^-$ STEC strains were positive for both *irp2* and *fyuA* genes. Moreover, 7 of 12 *eae*-negative strains of serotype O128:H2/H$^-$ contained the HPI-specific genes. An additional 18 STEC strains that harbored *irp2* and *fyuA* included four *eae*-positive and 14 *eae*-negative isolates that belonged to nine different serotypes (Table 3). In total, the HPI-specific genes were found in 56 (27.2%) of 206 STEC strains. However, none

TABLE 2. PCR primers and conditions used in this study

Primer designation	Nucleotide sequence of primers	Target	PCR conditions						Length of PCR product (bp)	Reference for PCR
			Denaturing		Annealing		Extension			
			Temp[a]	T[b]	Temp[a]	T[b]	Temp[a]	T[b]		
KS7 KS8	5′-CCCGGATCCATGAAAAAAACATTATTAATAGC-3′ 5′-CCCGAATTCAGCTATTCTGAGTCAACG-3′	*stx$_1$B*	94	30	52	60	72	40	285	42
GK3 GK4	5′-CCCGGATCCATGAAGAAGATGTTTATGGCG-3′ 5′-CCCGAATTCTCAGTCATTTATTAAACTGCAC-3′	*stx$_2$B* *stx$_{2c}$*B	94	30	52	60	72	40	260	14
LP43 LP44	5′-ATCCTATTCCCGGGAGTTTACG-3′ 5′-GCGTCATCGTATACACAGGAGC-3′	*stx$_2$A* and variants	94	30	57	60	72	60	584	10
VT2-cm VT2-f	5′-AAGAAGATATTTGTAGCGG-3′ 5′-TAAACTGCACTTCAGCAAAT-3′	*stx$_{2d}$*	94	30	55	60	72	60	256	35
SK1 SK2	5′-CCCGAATTCGGCACAAGCATAAGC-3′ 5′-CCCGGATCCGTCTCGCCAGTATTCG-3′	*eae*	94	30	52	60	72	60	863	43
Hly A1 Hly A4	5′-GGTGCAGCAGAAAAAGTTGTAG-3′ 5′-TCTCGCCTGATAGTGTTTGGTA-3′	EHEC *hlyA*	94	30	57	60	72	90	1,551	45
asnU int2	5′-TTTCGCTGTTAAGATGTGCC-3′ 5′-TGCTTCCAGATAATCCGACCAC-3′	*asnU/int*	94	30	53	60	72	60	1,500	This study[c]
asnV int2	5′-GACAGCAAACAAACAAAAA-3′ 5′-TGCTTCCAGATAATCCGACCAC-3′	*asnV/int*	94	30	53	60	72	60	1,500	This study
ybtEup fyuybtE (IX)	5′-GCAAGATAGACAAAAAACGCC-3′ 5′-GCTGACAACGGTAGACGAGA-3′	*ybtE/fyuA*	94	60	52	60	72	60	359	This study
fyuA FP fyuA RP (X)	5′-GCGACGGGAAGCGATTTA-3′ 5′-CGCAGTAGGCACGATGTTGTA-3′	*fyuA*	94	60	57	60	72	60	780	49
50A 50B (XI)	5′-ATTGATCCACCGTTTTACTC-3′ 5′-CGAACGAAAGCATGAAACAA-3′	IS*100*	94	60	50	60	72	60	100	This study
int5 ybtSlp (XII)	5′-ATGGAATCGGGTTTATGGG-3′ 5′-GCTATTGCTGAACTGGAGG-3′	*int/ybtS*	94	60	54	60	72	60	830	This study
ybtSup ybtQ3lp (XIII)	5′-GAAACAGCACGGTAAACGCA-3′ 5′-ACGCGGCAGGAGGTAGAAG-3′	*ybtS/ybtQ*	94	30	55	60	72	180	2,797	This study
ybtQup ybtA1lp (XIV)	5′-GCCGCCAGTCTATCCACA-3′ 5′-GAATCGGCCACAATAGGA-3′	*ybtQ/ybtA*	94	30	52	60	72	180	2,805	This study
ybtAup irp2 RP (XV)	5′-GGTATGGATATTTTGCTCTGG-3′ 5′-TCGTCGGGCAGCGTTTCTTCT-3′	*ybtA/irp2*	94	60	54	60	72	60	1,340	This study
irp2-1up irp1-1lp (XVI)	5′-ACCTCTTCACCCACCCTTCT-3′ 5′-TTCAGGAAAATGGCAGGCGT-3′	*irp2/irp1*	94	60	54	60	72	60	300	This study
irp1-1up ybtTlp (XVII)	5′-TTCCGGTCCCCTGTCTCA-3′ 5′-ATCCGCCAATGTCTATCA-3′	*irp1/ybtT*	94	30	52	60	72	120	1,762	This study
asnT int2	5′-GACAGACAAGGTACCGCTAA-3′ 5′-TGCTTCCAGATAATCCGACCAC-3′	*asnT/int*	94	60	52	60	72	60	1,500	This study
asnT1 int3 (I)	5′-CACGATTCCTCTGTAGTTCA-3′ 5′-TCCTTTTTCGTGTCGTAACCC-3′	*asnT/int*	94	60	52	60	72	60	1,255	This study
asnT1 int2 (II)	5′-CACGATTCCTCTGTAGTTCA-3′ 5′-TGCTTCCAGATAATCCGACCAC-3′	*asnT/int*	94	60	52	60	72	60	1,500	This study
int1 int2 (III)	5′-TCCCTTACCGACGCAAAAATCC-3′ 5′-TGCTTCCAGATAATCCGACCAC-3′	*int*	94	60	58	60	72	60	1,203	This study
ybtSup ybtSlp (IV)	5′-GAAACAGCACGGTAAACGCA-3′ 5′-GCTATTGCTGAACTGGAGG-3′	*ybtS*	94	60	52	60	72	60	160	This study
ybtQup ybtQ1lp (V)	5′-CGGGCGGCCTCTTCTACCT-3′ 5′-GCGATGCGGCGACAAATGC-3′	*ybtQ*	94	30	59	60	72	90	797	This study
ybtAup ybtAlp (VI)	5′-GGTATGGATATTTTGCTCTGG-3′ 5′-GGTAATGCTCTCGAATCGG-3′	*ybtA*	94	60	52	60	72	60	233	This study
irp2 FP irp2 RP (VII)	5′-AAGGATTCGCTGTTACCGGAC-3′ 5′-TCGTCGGGCAGCGTTTCTTCT-3′	*irp2*	94	60	60	60	72	60	280	49
irp1up irp1lp (VIII)	5′-TGAATCGCGGGTGTCTTATGC-3′ 5′-TCCCTCAATAAAGCCCACGCT-3′	*irp1*	94	60	56	60	72	60	240	34
ybtTup fyuA1lp (XVIII)	5′-TGCAAAAACAGCCCAGTA-3′ 5′-CATTCCATCCCACATAGG-3′	*ybtT/fyuA*	94	30	50	60	72	180	2,518	This study

[a] Temperature in degrees Celsius.
[b] Time in seconds.
[c] The primers asnU and asnV were designed according to DNA sequences published for the respective genes in *E. coli* K-12 (1); the primer pair asnT/int2 and the primer pairs targeting the regions I to VI, IX, and XI to XVIII were designed according to published sequences of *Y. pestis* HPI (13).

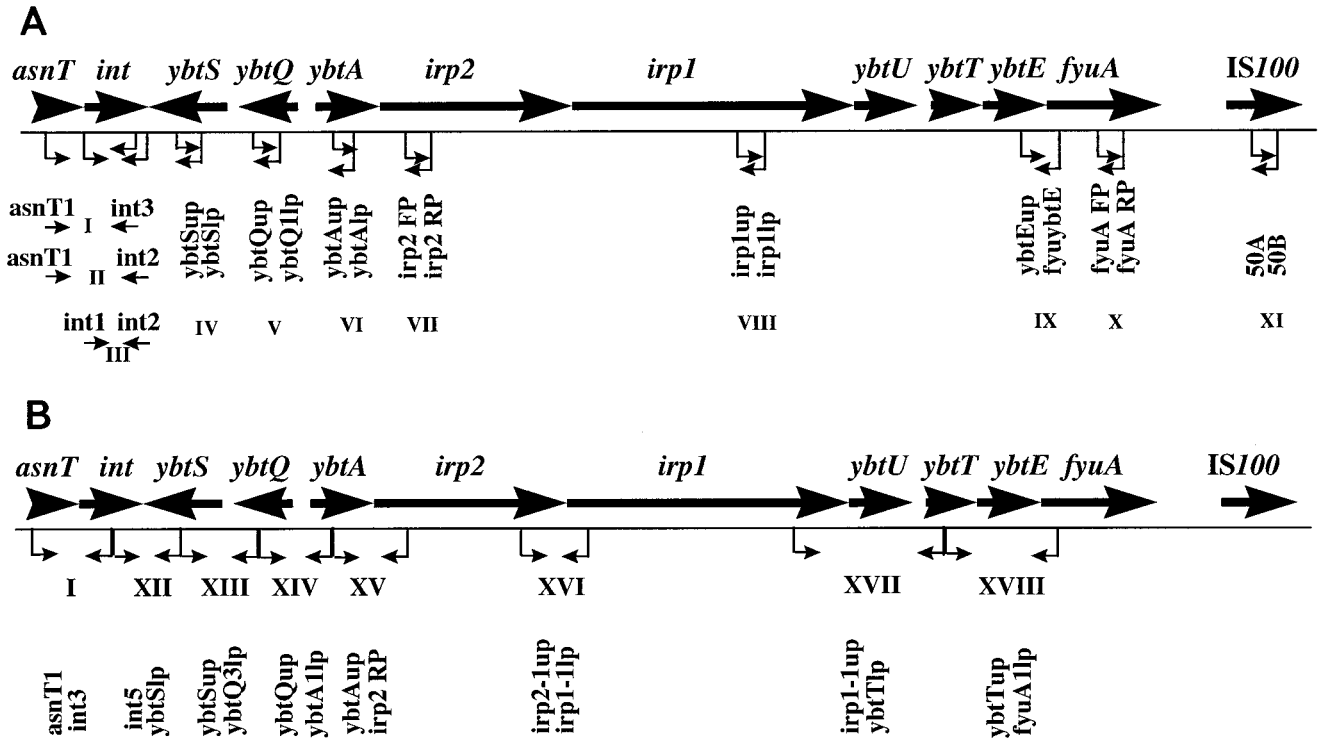

FIG. 1. Physical map of the HPI element of pathogenic yersiniae. Important genes are indicated by large black arrows and include the following: *asnT* and *int* boundary genes; *ybtS*, *ybtQ*, *ybtA*, *irp2*, *irp1*, *ybtU*, *ybtT*, and *ybtE*, constituting the siderophore yersiniabactin biosynthetic gene cluster; *fyuA*, encoding the receptor for yersiniabactin and pesticin; and IS*100* insertion element (7, 13, 34, 38). PCR primers used to target single HPI genes (panel A, regions III to VIII, X, and XI) or to link consecutive genes (panel A, regions I, II, and IX, and panel B) are indicated by small arrows, and nucleotide sequences of the primers are given in Table 2.

of the STEC strains of serotypes O157:H7/H$^-$, O103:H2, O111:H$^-$, and O145:H$^-$, which were all *eae*-positive, harbored either *irp2* or *fyuA*.

Two STEC strains, including 3172/97 (O128:H2) and 5720/96 (O26:H$^-$), were subjected to 11 different PCRs with primers targeting the genes described to occur in the HPI of pathogenic yersiniae in order to determine whether these genes are present in the investigated *E. coli* strains. The target regions (I to XI) and the corresponding primer pairs are indicated in Fig. 1A and in Table 2, respectively. As demonstrated in Table 4, besides an asparagine tRNA gene and the integrase gene, sequences similar to *ybtS*, *ybtQ*, *ybtA*, *irp2*, *irp1*, *ybtE*, and *fyuA* could be detected in both *E. coli* strains. The sizes of the PCR products obtained from O128 STEC strain 3172/97 were close to the sizes of the corresponding HPI regions in *Y. pestis* and *Y. enterocolitica* determined from published sequence data (13, 34). In O26 STEC strain 5720/96, however, the sizes of PCR products with the primers homologous to the *int* gene were smaller (Table 4).

TABLE 3. Presence of the HPI and LEE in STEC strains

Serotype	Total no. of strains	No. of strains positive for:	
		LEE (*eae*)	HPI (*irp2*, *fyuA*)
O157:H7/H$^-$	37	37	0
O103:H2	13	13	0
O111:H$^-$	14	14	0
O145:H$^-$	13	13	0
O26:H11/H$^-$	31	31	31
O128:H2/H$^-$	12	0	7
Others	21	21	4[a]
	65	0	14[b]
Total	206	129	56

[a] Serotypes O118:H (two strains), O4:H (one strain), and O121:H10 (one strain).

[b] Six different serotypes, including O3:H2 (one strain), O3:H10 (one strain), O41:H$^-$ (one strain), O60:H$^-$ (one strain), O78:H$^-$ (three strains), and O152:H4 (one strain); three additional strains were not typeable with the O antisera used, and three strains were rough.

In addition, a second set of primer pairs was used to analyze the order of the HPI-specific genes in both STEC strains. For this purpose, primer pairs derived from sequences of *Y. pestis* HPI were constructed that enabled us to link consecutively arranged genes. The location of the target regions (XII to XVIII) and the primers used are shown in Fig. 1B and in Table 2, respectively. As seen from Table 4, PCR products were obtained from all investigated regions of both STEC strains; the sizes of these PCR products closely corresponded to the sizes of the respective regions in the *Y. pestis* and *Y. enterocolitica* HPIs determined according to the published sequence analysis (13, 34).

Determination of the integration site of HPI in STEC. The HPI element in all *Yersinia* species tested is located in the vicinity of an asparagine-specific tRNA gene. A recent study (7) demonstrated that the HPI in *Y. pseudotuberculosis* is located not only adjacent to *asnT*, as is the case in *Y. enterocolitica* and *Y. pestis*, but also adjacent to two other *asn* tRNA loci, *asnU* and *asnV*. In order to analyze the location of the HPI in STEC strains, PCRs specific for each of the three asparagine-specific tRNA location sites were performed. In 17 representative *irp2-fyuA*-positive STEC strains of different serotypes, the insertion site of the HPI is next to the tRNA gene *asnT*, as demonstrated by employing primer pair asnT and int2

TABLE 4. Comparison of the HPI in STEC and *Y. pestis* and *Y. enterocolitica*

Strain	Serotype	Size of PCR product (bp) from region[a]:																	
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII
STEC 3172/97	O128:H2	1,200	1,400	1,200	160	800	230	280	240	360	780	—[b]	800	2,800	2,800	1,300	300	1,700	2,500
STEC 5720/96	O26:H$^-$	900	1,100	900	160	800	230	280	240	360	780	—	800	2,800	2,800	1,300	300	1,700	2,500
E. coli DH5α		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Y. pestis KIM6$^+$		1,255	1,500	1,203	160	797	233	286	237	359	780	100	830	2,797	2,805	1,340	300	1,762	2,518
Y. enterocolitica WA-314	O8	1,255	1,500	1,203	160	797	233	286	237	359	780	NP[c]	830	2,797	2,930	1,340	300	1,762	2,518

[a] I to XI are regions shown in Fig. 1A; XII to XVIII are regions shown in Fig. 1B. The sizes of *Yersinia* PCR products derived from regions I to XVIII are according to the published HPI sequences (13, 34, 39); the sizes of *E. coli* PCR products derived from the corresponding regions of *E. coli* strains were determined after agarose gel electrophoresis.
[b] —, no PCR product obtained.
[c] NP, not present.

in PCR. A PCR product was detected in all *E. coli* strains. Whereas all five *E. coli* O26:H11/H$^-$ strains and an O60:H$^-$ strain demonstrated a 1,200-bp product, a 1,500-bp product was obtained from strains of other serotypes, including 128:H2, O3:H10, ONT:H$^-$, ONT:H8, and Orough:H$^-$ (data not shown). No PCR products were obtained with primer pairs asnU and int2 and asnV and int2 specific for a probable HPI insertion adjacent to the tRNA genes *asnU* and *asnV*, respectively.

Characterization of boundary genes in STEC. The 17 representative *irp2-fyuA*-positive STEC isolates were further used to characterize HPI boundary genes in STEC. PCRs performed with the primer pair asnT1 and int2 specific for the left junction of the HPI, including *asnT* and the integrase gene *int*, revealed products of 1,100 bp in all O26:H11/H$^-$ strains and the O60:H$^-$ strain and products of 1,400 bp in all STEC strains of the other serotypes (data not shown). Sequence analysis of the 1,400-bp PCR product found in O128 STEC strain 3172/97 revealed that the integrase gene was intact, as seen in other *E. coli* isolates and *Y. pseudotuberculosis* and *Y. pestis* strains (7, 13). The integrase PCR product of strain 3172/97 showed 94.5% identity with the corresponding sequence from *Y. pestis* strain 6/69 (7). In O26 STEC strain 5720/96, however, a deletion of 347 bp was found in the integrase gene which resulted in a frameshift introducing a premature stop codon 36 bp downstream. Figure 2 shows an alignment of the deduced amino acid sequences of the integrases of the two STEC strains as compared with those of *Y. pestis* and *Y. pseudotuberculosis*.

Expression of *fyuA* gene in non-O157 STEC strains. In order to analyze the expression of *fyuA* in non-O157 STEC strains

```
MSLTDAKIRT LKPSDKPFKV SDSHGLYLLV KPGGSRHWYL KYRISGKESR IALGAYPAIS
MSLTDAKIRT LKPSDKPFKV SDSHGLYLLV KPGGSRHWYL KYRISGKESR IALGAYPAIS
MSLTDAKIRT LKPSDKPFKV SDSHGLYLLV KPGGSRHWYL KYRINGKESR IALGAYPAVS
MFLTDAKIRT LKPSDKPFKV SDSHGLYLLV KPGGSRHWYL K--------- ----------

LSDARQQREG IRKMLALNIN PVQQRAAERG SRTPEKVFKN VALAWHKSNR KWSQNTADRL
LSDARQQREG IRKMLALNIN PVQQRAAERG SRTPEKVFKN VALAWHKSNR KWSQNTADRL
LSDARQQREG VRKMLALNIN PVQQRAAERG SRTPDKVFKN VALAWHKSNR KWSQNTADRL
---------- ---------- ---------- ---------- ---------- ----------

LASLNNHIFP VIGNLPVSEL KPRHFIDLLK GIEEKGLLEV ASRTRQHLSN IMRHAVHQEL
LASLNNHIFP VIGNLPVSEL KPRHFIDLLK GIEEKGLLEV ASRTRQHLSN IMRHAVHQEL
LASMNNHIFP VIGNLPVSEL KPRHFIDLLK GIEEKGLLEV ASRTRQHLSN IMRHAVHQGL
---------- ---------- ---------- -------LEV ASRTRQHLSN IMRHAVHQEL

IDTNPAANLG GVTTPPVRRH YPALPLERLP ELLERIGAYH QGRELTRHAV LLMLHVFIRS
IDTNPAANLG GVTTPPVRRH YPALPLERLP ELLERIGAYH QGRELTRHAV LLMLHVFIRS
IDTNPAANLG GVTTPPVRRH YPALPLERLP ELLERIGAYH QGRELTRFAV LLMLHVFIRS
IDTNPAANLG GVTTPPVRRH YPALPLERLP ELLERIGAYH QGRELTRHAV LLMLHVFIRS

SELRFARWSE IDFTNRVWTI PATREPIIGV RYSGRGAKMR MPHIVPLSEQ SIAILKQIKD
SELRFARWSE IDFTNRVWTI PATREPIIGV RYSGRGAKMR MPHIVPLSEQ SIAILKQIKD
SELRFARWSE IDFTNRVWTI PATREPIIGV RYSGRGAKMR MPHIVPLSEQ SIAILKQIKD
SELRFARWSE IDFTNRVWTI PATREPIIGV RYSGRGAKMR MPHIVPLSEQ SIAILKQIKD

ITGNNELIFP GDHNPYKPMC ENTVNKALRV MGYDTKKDIC GHGFRAMACS ALMESGLWAK
ITGNNELIFP GDHNPYKPMC ENTVNKALRV MGYDTKKDIC GHGFRAMACS ALMESGLWAK
ITGNNELIFP GDHNPYKPMC ENTVNKALRV MGYDTKKDIC GHGFRAMACS ALMESGLWAK
ITGNNELIFP GDHNPYKPMC ENTVNKALRV MGYDTKKDIC GHGFRAMACS ALMESGLWAK

DAVERQMSHQ EHNTVRMAYI HKAEHLEARK AMMQWWSDYL EACRESYAPP YTIGKNKFIP
DAVERQMSHQ ERNTVRMAYI HKAEHLEARK AMMQWWSDYL EACRESYAPP YTIGKNKFIP
DAVERQMSHQ ERNTVRMAYI HKAEHLEARK AMMQWWSDYL EACRESYAPP YTIGKNKFIP
DAVERQMSHQ ERNTVRMAYI HKAEHLEARK AMMQWWSDYL EACRESYAPP YTIGKNKFIP
```

FIG. 2. Alignment of the deduced amino acid sequences of the integrases of *Y. pestis* (first line), *Y. pseudotuberculosis* (second line), STEC strain 3172/97 (third line), and STEC strain 5720/96 (fourth line). Translation of the latter sequence was performed without consideration of the frameshift resulting from the deletion of 347 bp. Bold letters represent differences in the amino acid sequence from the sequence of *Y. pestis* in the first line. Dashes in the last line indicate amino acid residues that are not present in this sequence (deletions). The deduced amino acid sequences of the *Y. pestis* and *Y. pseudotuberculosis* integrases are based on references 7 and 13.

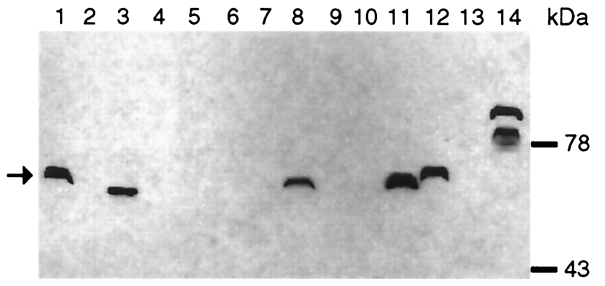

FIG. 3. Immunoblot of outer membrane proteins probed with anti-FyuA rabbit serum. The arrow indicates the FyuA protein band. Lane 1, *Y. pseudotuberculosis* O1 (HPI$^+$); lane 2, *E. coli* K-12 MG1655 (HPI$^-$); lane 3, EAEC strain 17-2 (HPI$^+$); lane 4, STEC strain O157:H7 3268/90 (HPI$^-$); lane 5, STEC strain O62:H$^-$ 4595/97 (HPI$^-$); lane 6, STEC strain O40:H$^-$ 4828/97 (HPI$^-$); lane 7, STEC strain O103:H$^-$ 4797/97 (HPI$^-$); lane 8, STEC strain O128:H2 3115/97 (HPI$^+$); lane 9, STEC strain O128:H2 3172/97 (HPI$^+$); lane 10, STEC strain ONT:H$^-$ 4941/97 (HPI$^+$); lane 11, STEC strain O3:H10 5726/96 (HPI$^+$); lane 12, STEC strain O60:H$^-$ 3357/98 (HPI$^+$); lane 13, STEC strain Orough:H$^-$ 0512E015 (HPI$^+$); lane 14, STEC strain O26:H11 6061/96 (HPI$^+$). Molecular mass is shown on the right.

carrying the *Yersinia* HPI, immunoblotting of outer membrane proteins was performed. As shown in Fig. 3, FyuA was detectable in four out of seven HPI-positive STEC strains, whereas none of the HPI-negative strains revealed expression of *fyuA*. In accordance with previous results, FyuA from three *E. coli* strains appeared to be the same size as *Yersinia* FyuA (67 kDa) (15, 20). However, in one *E. coli* isolate, two polypeptides were detected, both larger than the expected FyuA (Fig. 3). Polypeptides of apparently larger size have been previously observed in certain *Y. pseudotuberculosis* strains (36).

DISCUSSION

Horizontal gene transfer represents a key genetic mechanism in the evolution of pathogens (2, 12, 15, 26, 29). Genes encoding important virulence factors are often located on mobile genetic elements such as phages, plasmids, or transposons and can therefore be transferred from one cell to another. PAIs are discrete genetic units preferentially located in the chromosomes of pathogens which also carry virulence genes. Those genes may have been introduced into the genome of pathogens recently via lateral gene transfer (15, 17). Gene transfer processes such as these lead to a mosaic pattern of pathogenicity in many infectious agents. The STEC strains represent an example par excellence of pathogen development by lateral gene transfer. Important virulence factors such as Stx, the adherence factor intimin, and the EHEC hemolysin are encoded by phages, the LEE PAI, and the large plasmid, respectively (11, 30, 33, 45, 50). STEC strains are a heterogeneous group of pathogenic organisms with respect to their serotypes, *stx* genes, and the presence of additional virulence factors.

The majority of PAIs detected in enterobacteria are specific for particular species or even pathotypes. Thus, the LEE island, encoding virulence factors in diarrheagenic *E. coli*, has not been described in pathotypes other than STEC and EPEC (28). The so-called HPI, first described in pathogenic *Yersinia* (9), however, represents an exception, because the HPI element has been detected in many enterobacterial species and pathotypes, including both enteroaggregative and extraintestinal *E. coli* (49). In addition, more than 30% of *E. coli* isolates from physiological intestinal microflora also carry this island (49). The mobility of the HPI elements may be associated with an intact integrase gene located at the left junction of the HPI. The gene product, integrase, may be involved in the excision and mobilization of the HPI element (7, 16, 17).

It has been shown recently that the HPI elements are not present in the genome of STEC strains of serotype O157:H7/H$^-$ (49). We therefore analyzed 206 STEC strains to investigate the possibility that HPI elements are present in STEC strains of other serotypes. Although we could confirm that O157 strains do not carry the HPI element, it became apparent that STEC strains of other clonal lineages were HPI positive, including the O26:H11/H$^-$ group, which is currently regarded as the most common non-O157 group of STEC strains in Germany and in other European countries (5, 8). Detailed analysis of the HPI in two representative STEC strains demonstrated that with the exception of the IS*100* insertion element, all investigated genes were present and arranged in the order that was demonstrated for the HPI of pathogenic yersiniae (13, 34).

For each of the HPI-positive STEC strains, the presence of both *fyuA* and *irp2* genes was demonstrated. However, the yersiniabactin receptor FyuA was expressed in only about 60% of these strains. This may be due to partial deletions of the *fyuA* gene as has been previously shown for certain *E. coli* isolates (49). The fact that HPI-positive *E. coli* strains lack expression of FyuA may indicate that the yersiniabactin siderophore system is not the primary advantage of possessing the HPI. This hypothesis is supported by the observation that, in *E. coli*, deletions of the HPI are reported to affect solely the *fyuA* segment. However, the reason for the different expression of *fyuA* remains to be clarified.

The HPI of STEC shares common features with the HPI elements of other enterobacteria, including pathogenic yersiniae. It encodes FyuA proteins which may act as receptors for pesticin and the siderophore yersiniabactin (20, 21, 34). Other genes located on the HPI encode this particular iron uptake system. From an evolutionary point of view, the high degree of sequence identity between the homologous HPI-specific genes of various pathotypes and species including STEC suggests a recent transfer of the HPI from one species to another. As also shown for other enterobacteria, the HPI in STEC is located next to the tRNA gene *asnT*. The *asnT* locus is linked to a gene which is highly homologous with a phage-derived integrase determinant termed *int*. In *Y. pseudotuberculosis*, *Y. pestis*, and extraintestinal *E. coli*, the *int* gene seems to be intact, whereas in *Y. enterocolitica* the open reading frame has been destroyed by a frameshift mutation. In some STEC strains, the *int* open reading frame is intact, but in strains of the O26 group, a deletion in *int* has led to a truncation of the integrase. It therefore seems that the HPI of the STEC O26 group represents a new and unique type of HPI with a partially deleted *int*. The deletion in the *int* gene may result in a nonfunctional integrase and subsequent fixation of the HPI in the genome of this STEC clonal lineage. In pathogenic yersiniae, the HPIs are flanked by two direct repeats of 16 bp which may be involved in HPI mobility. In *E. coli*, however, only one direct repeat is present. Therefore, insertion and excision events may be prevented, even when the integrase gene is intact.

The HPI elements code for a particular iron uptake system, termed yersiniabactin. Iron uptake in general increases the metabolic fitness of bacteria and does not directly contribute to host damage and infection. The question arises whether the HPI elements in *E. coli* indeed represent PAIs or whether they contribute to the survival of the strains in certain ecological niches. Although, at the present time, we are unable to answer this question in regard to the STEC strains analyzed here, the fact that the HPI-positive as well as HPI-negative STEC strains

differ little in their pathogenic potential supports the view that the HPI in *E. coli* is a form of fitness island rather than a PAI. This idea is corroborated by the fact that more than 30% of nonpathogenic fecal *E. coli* strains are also HPI positive (49). From an evolutionary point of view, and because of the occurrence of examples like these, we can assume that all these genetic elements are variations of genomic islands (17). Since genomic islands show similar structural features, it is likely that they have been transferred in recent times by horizontal processes. The genomic islands may contribute to the fitness (fitness islands) or metabolic flexibility (metabolic islands) of the organisms, or they may increase their pathogenic potential (PAIs). The particular function of an island will thus depend strongly on the genetic background of the individual strains. Further experiments are necessary in order to define the exact role of the HPI element in the life cycle of STEC strains.

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