

# The SHI-3 Iron Transport Island of *Shigella boydii* 0-1392 Carries the Genes for Aerobactin Synthesis and Transport

GEORGIANA E. PURDY<sup>1</sup> AND SHELLEY M. PAYNE<sup>1,2\*</sup>

*Institute of Cellular and Molecular Biology<sup>1</sup> and Department of Molecular Genetics and Microbiology,<sup>2</sup>  
The University of Texas at Austin, Austin, Texas 78712-1095*

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**In *Shigella boydii* 0-1392, genes encoding the synthesis and transport of the hydroxamate siderophore aerobactin are located within a 21-kb iron transport island between *lysU* and the *pheU* tRNA gene. DNA sequence analysis of the *S. boydii* 0-1392 island, designated SHI-3 for *Shigella* island 3, revealed a conserved aerobactin operon associated with a P4 prophage-like integrase gene and numerous insertion sequences (IS). SHI-3 is present at the *pheU* tRNA locus in some *S. boydii* isolates but not in others. The map locations of the aerobactin genes vary among closely related species. The association of the aerobactin operon with phage genes and mobile elements and its presence at different locations within the genomes of enteric pathogens suggest that these virulence-enhancing genes may have been acquired by bacteriophage integration or IS element-mediated transposition. An *S. boydii* aerobactin synthesis mutant, 0-1392 *iucB*, was constructed and was similar to the wild type in tissue culture assays of invasion and intercellular spread.**

Iron is essential for the growth of most bacterial pathogens, and the ability to acquire iron is associated with bacterial virulence. To obtain iron, *Shigella* spp. can use host iron sources such as heme directly, and they have the ability to remove iron from host sources via siderophore-mediated uptake systems (22, 47). Siderophores are low-molecular-weight, high-affinity iron chelators synthesized and secreted into the environment. The iron-siderophore complex is transported back into the cell using specific receptors. Two different siderophore-mediated iron transport systems have been observed in *Shigella* spp. and clinical *Escherichia coli* isolates. The catechol siderophore enterobactin is produced by *E. coli* (39) and some, but not all, *Shigella* spp. (36, 38), while the hydroxamate siderophore aerobactin is synthesized by *Shigella flexneri* and *Shigella boydii* (20) and *Shigella sonnei* (34), as well as some *E. coli* clinical isolates (11, 34). The aerobactin operon encodes the IucABCD enzymes for aerobactin synthesis and Iut, the outer membrane receptor for aerobactin. Expression of the aerobactin operon is negatively regulated by the iron-binding repressor protein Fur (3). Under low-iron conditions, expression of the aerobactin operon is derepressed, and the siderophore synthesis proteins and receptor are produced to facilitate iron acquisition.

The aerobactin genes are found on the pColV and F1me plasmids in some strains of *E. coli* and *Salmonella*, respectively, and are found chromosomally in *Shigella* and other *E. coli* strains (11, 20, 22, 26, 46). While the aerobactin genes were shown to be located in the SHI-2 pathogenicity island downstream of the *selC* tRNA gene in *S. flexneri* and *S. sonnei* (30, 43), their location in *S. boydii* remained unknown. In this report, we show that the aerobactin operon is located in a 21-kb iron transport island between *lysU* and the *pheU* tRNA

gene in *S. boydii*. While the sequence of the aerobactin genes is conserved, the sequences flanking the genes are distinct, and the aerobactin island in *S. boydii* has been designated SHI-3 for *Shigella* island 3.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, and growth conditions.** Clinical isolates of *S. boydii*, *S. dysenteriae*, *S. flexneri*, and *S. sonnei* were obtained from the Texas Department of Health. Enteroinvasive *E. coli* (EIEC) strains were obtained from J. H. Crosa, Oregon Health Science University. *E. coli* 1017 (HB101 *entF::Tn5*) has been described previously (12). The iron chelator EDDA [ethylenediamine di(*o*-hydroxyphenylacetic acid)] was deferrated as described previously and used at a concentration of 300 µg/ml to induce iron starvation (19). Strains were grown in L broth or on L-agar plates with the addition of antibiotics at the following concentrations when necessary: 250 µg of carbenicillin/ml, 30 µg of chloramphenicol/ml, and 200 µg of streptomycin/ml.

**Isolation of the *S. boydii* aerobactin genes.** A *Sau3AI* partial library of *S. boydii* 0-1392 was constructed in the cosmid vector pLAFR3 (13) and screened by colony hybridization using a probe to the *S. flexneri iucA* gene. Two overlapping cosmids, pGEP1 and pGEP2, were isolated, and their ability to confer aerobactin synthesis to *E. coli* 1017 was confirmed by the hydroxamate assay and siderophore bioassays (19, 35).

**Construction of the *iucB* mutation.** An *iucB* mutation was constructed in *S. boydii* 0-1392 by allelic exchange using the suicide plasmid pGP704 (27). In *S. boydii* 0-1392 *iucB*, the wild-type allele is replaced with one containing a chloramphenicol resistance cassette inserted into the *SmaI* site of *iucB*.

**Tissue culture, cell invasion, and plaque assays.** The ability of *S. boydii* 0-1392 and 0-1392 *iucB* to invade Henle cells was determined by the procedure of Hale and Formal (16). Plaque assays were performed as described by Oaks et al. (32).

**Nucleotide sequence analysis.** DNA sequencing was performed using an ABI Prism 377 automatic sequencer. *Bam*HI, *Hind*III, *Pst*I, and *Eco*RI fragments of pGEP1 and pGEP2 were subcloned into either pBluescript SK(–) (Stratagene) or pWKS30 (44) plasmid vectors for sequencing. Routine sequence analysis was performed using MacVector software (33) (Oxford Molecular). Sequence homology to known genes and proteins was analyzed using the BlastN and BlastX algorithms, respectively, through the National Center for Biotechnology Information database (1, 2, 14).

**PCR.** PCRs were performed in a GeneAmp PCR system 2400 (Perkin-Elmer). The primers used to amplify the SHI-3 *int3* and *int3-yjdC* junction are as follows: primer 1 (5'-CGCTGGAGATGGTTGCTGAAC-3'), primer 2 (5'-GAATCAG GTTTGTGGTCC-3'), and primer 3 (5'-GGGTTATTACCTGCTCTC-3'). The primers used to amplify the SHI-2 *int2* and *int2-selC* junction are as follows: primer 6 (5'-GCGGCGGTATGTATCTAC-3') and primers 7 and 8, which correspond to primers 1 and 2, respectively, described by Vokes et al. (43). The

\* Corresponding author. Mailing address: Institute of Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX 78712-1095. Phone: (512) 471-9258. Fax: (512) 471-7088. E-mail: payne@mail.utexas.edu.

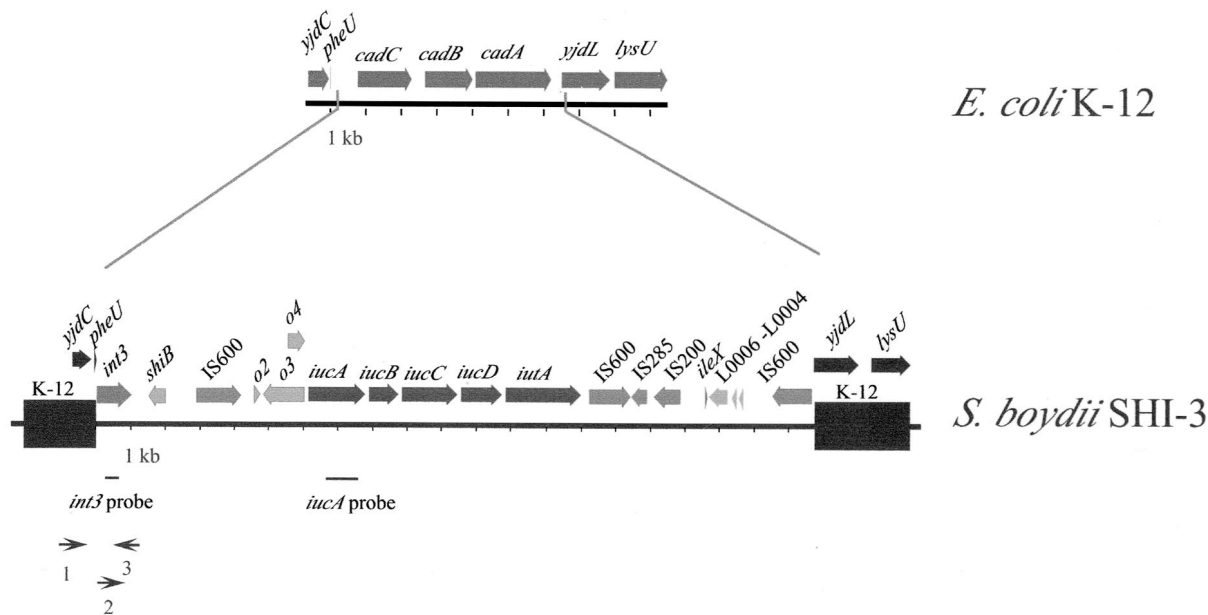


FIG. 1. Map of the *S. boydii* 0-1392 aerobactin island, SHI-3, and the corresponding *E. coli* K-12 region between *pheU* and *lysU* at min 94. Sequences present in both *E. coli* K-12 and *S. boydii* are indicated by the black bars and are designated K-12 on the SHI-3 map. The ORFs, indicated as *shiB* and *o2* to *o4*, were inferred from sequence analysis. The *iucA* probe and the *int3* probe, as well as the approximate positions of primers used for PCR amplification of the junctions, are positioned below the SHI-3 map.

SHI-3 *int3* probe was generated by PCR using primer 2 and primer 3. The *iucA* probe was generated by PCR using primer 4 (5'-GGCAGCCCATACAGACA G-3') and primer 5 (5'-CATCCCACGCTTCACTTC-3'). PCRs consisted of 30 cycles with an annealing temperature of 50°C and extension times of 30 s for primer pairs 2-3 and 6-8, 1 min for primer pair 4-5, and 2 min for primer pairs 1-3 and 7-8.

**Southern hybridizations.** Genomic DNA was isolated using Qiagen Genomic-tip DNA isolation columns according to the manufacturer's instructions. Probe labeling, hybridization, standard stringency washes, and detection were performed as described in the ECL Direct Nucleic Acid Labeling Kit (Amersham Pharmacia).

**Nucleotide sequence accession number.** The GenBank accession number for the sequence described here is AF335540.

**RESULTS**

**Isolation of the *S. boydii* aerobactin genes.** Strains of *S. boydii*, like those of *S. flexneri*, produce the siderophore aerobactin, but the *S. boydii* aerobactin synthesis genes do not map to the same location as those in *S. flexneri* (43). To map the location of the *S. boydii* 0-1392 aerobactin genes, cosmids containing the aerobactin genes were isolated from a library of strain 0-1392 by DNA hybridization using a probe to *iucA*. Two overlapping *iucA*-positive cosmids, pGEP1 and pGEP2, conferred the ability to synthesize aerobactin upon *E. coli* 1017 as shown by positive hydroxamate tests and siderophore bioassays (data not shown). The entire region containing the aerobactin genes was sequenced, revealing a 21-kb island between *pheU* and *yjdL* at min 93 to 94 of the *E. coli* K-12 map (Fig. 1). Aerobactin genes have not previously been mapped to this location in any species.

To confirm that the aerobactin genes at this locus are responsible for hydroxamate synthesis, an *iucB* mutation was constructed by inserting a chloramphenicol resistance cassette into the cloned *iucB* gene and transferring the mutation to

0-1392 by allelic exchange. 0-1392 *iucB* did not synthesize aerobactin, indicating that there is a single aerobactin operon in 0-1392. 0-1392 *iucB* invaded Henle cells at wild-type levels and produced plaques in a standard plaque assay (data not shown).

**Structure of the *S. boydii* aerobactin island.** The *S. boydii* aerobactin island, which we have designated SHI-3, contains a functional aerobactin operon. SHI-3 is demarcated by a putative integrase gene inserted 200 bp downstream of the *pheU* tRNA at min 94 and by an IS600 interruption of *yjdL*, an uncharacterized open reading frame (ORF) adjacent to *lysU* (Fig. 1). Sequence scanning did not reveal direct repeats or any known sequence in the 84 bp between the conserved intergenic sequence downstream of *pheU* and the start of the integrase gene. The genes, ORFs, and insertion elements present in *S. boydii* SHI-3 are summarized in Table 1. The second ORF of the *S. boydii* aerobactin island shares 97% nucleotide identity with the *S. flexneri* M90T SHI-2 ORF of unknown function, *shiB* (30). The region between *shiB* and the aerobactin genes in *S. boydii* contains ORFs of unknown function with sequence similarity to ORFs upstream of aerobactin in *S. flexneri* SHI-2 and *E. coli* pColV-K30 (Table 1). There is 100% nucleotide identity between *orf2* of 0-1392 and *orf24* of the *S. flexneri* SA100 SHI-2 aerobactin island. The adjacent region is 99% identical to *S. flexneri* SA100 SHI-2 *rorf25*, an ORF transcribed off the minus strand, but the insertion of a thymine codon at base 5333 in the *S. boydii* SHI-3 island generates a stop codon, creating the defective *rorf3* (43). The 398-bp *orf4* is 99% homologous at the nucleotide and amino acid levels to SHI-2 *orf27* and shares 93% nucleotide identity to sequence upstream of the aerobactin genes in *E. coli* pColV-K30. The SHI-3 *iucA*, *-B*, *-C*, and *-D* and *iutA* genes share 99% nucleotide identity with the SHI-2 aerobactin genes (30, 43) and 92 to 95% iden-

TABLE 1. ORFs within the *S. boydii* aerobactin island

ORF, gene, or IS	Location	Length (bp)	Similar sequence (reference)	% Nucleotide (protein) identity
<i>int3</i>	84–1345	1,261 <sup>a</sup>	P4 prophage integrase (37)	(63)
<i>shiB</i>	1800–2249	449	<i>S. flexneri</i> M90T <i>shiB</i> gene in SHI-2 (30)	97 (94 over 143 of 153 aa) <sup>b</sup>
IS600	3198–4459	1,261	<i>S. sonnei</i> IS600 (10, 24)	98
<i>orf2</i>	4815–5081	266	<i>S. flexneri</i> SA100 <i>orf24</i> in SHI-2 (43)	100 (100)
<i>rorf3</i> <sup>c</sup>	5037–6229	1,192	<i>S. flexneri</i> SA100 <i>rorf25</i> in SHI-2 (43)	99 (89 for 297 of 397 aa) <sup>b,d</sup>
<i>orf4</i>	5877–6305	428	<i>S. flexneri</i> SA100 <i>orf27</i> in SHI-2 (43)	99 (99)
<i>iucA</i> , -B, -C, -D	6308–12112	5,804	<i>S. flexneri</i> M90T aerobactin synthesis genes in SHI-2 (30)	99 (99)
<i>iutA</i>	12115–14313	2,198	<i>S. flexneri</i> M90T <i>iutA</i> in SHI-2 (30)	99 (99)
IS600	14542–15809	1,267	<i>S. sonnei</i> IS600 (10, 24)	98 (93)
Partial IS285	15803–16159	356	<i>Y. pestis</i> IS285 (21)	86 (79 for 88 of 270 aa) <sup>b</sup>
Partial IS200	16374–17106	732	<i>E. coli</i> IS200 (4)	95 (87)
<i>ileX</i>	17706–17778	72	<i>E. coli</i> <i>ileX</i> tRNA gene (5)	87
L0006	17928–18590	662	<i>E. coli</i> LEE pathogenicity island prophage gene L0006 (37)	93 (95)
L0005	18604–18786	182	<i>E. coli</i> LEE pathogenicity island prophage gene L0005 (37)	92 (78)
Partial L0004	18799–19047	248	<i>E. coli</i> LEE pathogenicity island prophage gene L0004 (37)	90 (95 for 82 of 116 aa) <sup>b</sup>
IS600	19699–20961	1262	<i>S. sonnei</i> IS600 (10, 24)	98 (93)

<sup>a</sup> Three ORFs corresponding to the integrase-like sequence were corrected for apparent frameshifts and are presented here as a single ORF.

<sup>b</sup> Amino acid identity for the partial ORF present in SHI-3.

<sup>c</sup> *rorf3* is an ORF transcribed off the minus stand.

<sup>d</sup> Due to a premature stop codon, *rorf3* is defective.

tity with the *E. coli* aerobactin genes (17, 18, 23). Thus, the aerobactin biosynthesis and transport genes and the immediate upstream region appear to be highly conserved among the different *Shigella* and *E. coli* strains that carry them.

*S. boydii* SHI-3 contains genes and mobile elements that suggest either bacteriophage- or insertion sequence (IS) element-mediated horizontal transfer of the genes. The SHI-3 integrase, *Int3*, appears to be a member of the P4 prophage integrase family, which has been implicated in the integration of other iron transport and pathogenicity islands (30, 37, 43). *Int3* shares 64% amino acid homology with the P4 prophage integrase of *E. coli*, 54% with the putative P4-like prophage integrase of the *E. coli* O157:H7 locus of enterocyte effacement (LEE) pathogenicity island, and 40% with the SHI-2 integrase, *Int2* (8, 30, 37, 43). The SHI-3 integrase gene sequence has nucleotide insertions or deletions that generate frameshifts, preventing the translation of a functional integrase. In addition to the integrase gene, SHI-3 also contains the putative O157:H7 LEE prophage genes L0004, L0005, and L0006 (37) downstream of the aerobactin operon. L0004 and L0006 both resemble transposase genes, but the function of L0005 is unknown. While some prophage-like genes are present within SHI-3, this island does not encode an entire putative P4-like prophage.

IS elements, which potentially can mobilize intervening sequence as composite transposons when present in multiple copies, are also present in SHI-3. There are two intact copies of the *S. sonnei* IS600 downstream of *iutA*. A third IS600 is located upstream of the aerobactin operon, but it is unlikely to be functional, as the transposase gene contains premature stop codons. A partial copy of the *E. coli* IS200 transposase gene and a portion of the *Yersinia pestis* IS285 transposase gene are also present within SHI-3. No direct or inverted repeats are observed in the sequence flanking these IS elements. It is possible that IS elements may have been involved in the assembly of the *S. boydii* aerobactin island and that the present island evolved through several insertions or deletions mediated

by IS elements. The presence of a region with 85% nucleotide identity to *ileX* tRNA between the putative prophage genes and the partial copies of IS200 and IS285 is indicative of the mosaic structure of the SHI-3 island. It is possible that the *ileX* tRNA gene was the target of a bacteriophage integration event and then was incorporated into SHI-3 via other bacteriophage- or IS element-mediated events.

**Association between SHI-3 and loss of *cadA*.** In *S. boydii* 0-1392, there is a deletion of more than 6 kb relative to the *E. coli* K-12 sequence at the site occupied by SHI-3, including the lysine decarboxylase gene, *cadA* (Fig. 1). Cadavarine, produced by the decarboxylation of lysine, inhibits *Shigella* enterotoxin activity, and deletion of *cadA* has been shown to enhance the virulence of *Shigella* and EIEC (25). Thus, in *S. boydii*, the acquisition of SHI-3 may have resulted not only in the enhanced ability to scavenge iron from the host using the siderophore aerobactin but also in the loss of a gene whose absence is associated with an increase in virulence.

**Distributions of SHI-3 *int3* and SHI-2 *int2* among enteric bacteria.** PCR was used to determine the distribution and location of the SHI-3 integrase gene, *int3*, among other *Shigella* strains, and hydroxamate tests were performed to assess the possible correlation of *int3* presence with aerobactin production (Table 2). Primer pair 2-3 (Fig. 1) amplified a 395-bp product internal to *int3* in several *S. boydii*, *S. flexneri*, *S. sonnei*, and *S. dysenteriae* serotypes, as well as EIEC strains, illustrating the distribution of this putative P4-like prophage integrase among *Shigella* spp. and *E. coli*. In those strains positive for *int3*, PCR to detect the *yjdC-int3* junction was performed. Using primers 1 and 3, where primer 1 is in *yjdC*, the uncharacterized ORF upstream of *pheU*, and primer 3 is internal to *int3*, a 2.1-kb *yjdC-int3* fragment was amplified in *S. boydii* 0-1392, 0-1393, and 224860, as well as in *S. dysenteriae* 1-130 (Fig. 1). All four of these strains produced aerobactin, as determined by hydroxamate assays and siderophore bioassays (data not shown). Therefore, it is possible that SHI-3, carrying the aerobactin genes, is located downstream of *pheU* in each of

TABLE 2. Presence of aerobactin and linkage of SHI-3 *int* to *pheU* in *Shigella* and *E. coli* strains

Species and strain	Serotype	Aerobactin synthesis	<i>int3</i> <sup>a</sup> (linked to <i>pheU</i> ) <sup>b</sup>	<i>int2</i> <sup>c</sup> (linked to <i>selC</i> ) <sup>d</sup>
<i>S. boydii</i>				
1-1660	1	+	-	-
1-1097	2	+	-	-
0-1392	5	+	+	-
0-1393	5	+	+	-
224860	7	+	+	-
0-5353	9	+	-	-
0-1591	11	+	+	-
<i>S. flexneri</i> <sup>e</sup>				
SA100	2a	+	+	+
5 strains	1a	+	-	+
9 strains	1b	+	-	+
20 strains	2a	+	+	+
9 strains	2b	+	+	+
10 strains	3a	+	-	+
11 strains	3b	+	-	+
8 strains	3c	+	-	+
4 strains	4a	+	-	+
9 strains	4b	+	-	+
9 strains	5	+	+	+
6 strains	6	+	-	-
<i>S. dysenteriae</i>				
0-4576	1	-	-	+
1-130	2	+	+	-
0-4242	3	+	-	-
9-3485	4	+	-	-
217726	9	+	-	-
<i>S. sonnei</i>				
1-679		+	+	+
EN120		+	+	+
EIEC				
930-78	O124:H <sup>-</sup>	-	-	-
550-3076		+	+	-
1107-81		+	+	-

<sup>a</sup> PCR with primer pair 2-3 amplifies a 395-bp product internal to SHI-3 *int3*.

<sup>b</sup> Linkage determined by PCR with primer pair 1-3, where primer 1 is in *yjdC* and primer 3 is in SHI-3 *int3*.

<sup>c</sup> PCR with primer pair 6-8 amplifies a 120-bp product internal to SHI-2 *int2*.

<sup>d</sup> Linkage determined by PCR with primer pair 7-8, where primer 7 is in *selC* and primer 8 is in SHI-2 *int2*.

<sup>e</sup> Each serotype pool contains the indicated number of clinical isolates from the Texas Department of Health.

these strains. The presence of strains positive for *int3* but lacking the *yjdC-int3* junction, such as *S. boydii* 0-1591, suggests that another Int3-mediated bacteriophage integration event occurred at a different map location in these strains.

The presence of *int2*, the related but distinct integrase gene found in SHI-2, among the *Shigella* and EIEC clinical isolates was also determined through PCR (Table 2). Although the two integrases are related, no cross-amplification of *int3* and *int2* genes occurs with primer pair 2-3 and primer pair 6-8. Using primers 6 and 8, a 120-bp fragment internal to *int2* was amplified in all but one of the *S. flexneri* serotypes, in one of five *S. dysenteriae* strains, and in both *S. sonnei* strains. *int2* was absent from the seven *S. boydii* strains and from the three EIEC strains tested (Table 2). In *S. flexneri* serotypes 2a, 2b, and 5, as well as *S. sonnei* strains, PCR amplification with both the *int2* and *int3* primer pairs suggested two independent bacterio-

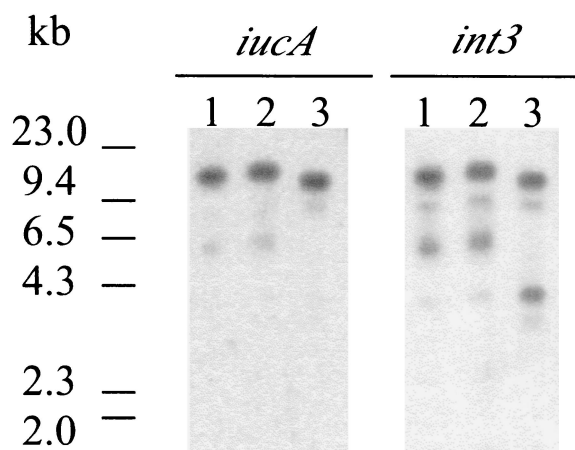


FIG. 2. The P4-like integrase and aerobactin genes are linked in several *S. boydii* serotypes. Genomic DNA was digested with *PvuII*, and the fragments were hybridized to a 1-kb *iucA* probe or a 395-bp *int3* probe. Locations of the probes are shown in Fig. 1. Lane 1, *S. boydii* 0-1392; lane 2, *S. boydii* 0-1393; lane 3, *S. boydii* 224860.

phage integration events. The *selC-int2* junction was present in the *int2*-positive strains as determined by an additional PCR with the primer pair 7-8, where primer 7 is located in *selC* and primer 8 is internal to *int2*. *int2* is linked to *selC* in all tested strains; however, in *S. dysenteriae* the *selC-int2* junction does not correlate with the presence of the SHI-2 aerobactin island, indicating that more than one pathogenicity island containing *int2* exists (43). It is possible that the 3' end of the *selC* tRNA gene was the target for a single Int2-mediated integration in *Shigella* and that different pathogenicity islands resulted from multiple horizontal gene transfers mediated by other mobile elements. Alternatively, other bacteriophage integration events mediated by *int2* occurred at the same map location, introducing different pathogenicity islands into different lineages.

**Distribution of SHI-3 among enteric bacteria.** To determine whether the aerobactin and P4-like integrase genes are linked in *S. boydii* strains containing the *yjdC-int3* junction, Southern hybridizations were performed using probes to *iucA* and *int3* (Fig. 2). In *S. boydii* 0-1392, both probes hybridized to an approximately 12.9-kb *PvuII* fragment, the size predicted from the DNA sequence analysis. In 0-1392 and 224860, the sizes of the *PvuII* fragments were slightly different than in 0-1392, but in each strain the *iucA* and *int3* probes both hybridized to fragments of the same size, suggesting linkage of the *int3* and *iuc* genes. Thus, an SHI-3-like island may be found in multiple *S. boydii* serotypes.

## DISCUSSION

SHI-3 is a 21-kb iron transport island carrying the aerobactin genes and is located downstream of the *pheU* tRNA gene in *S. boydii* 0-1392. SHI-3 has many characteristics of a pathogenicity island: it contains mobile elements, including a P4-like prophage integrase and IS elements; it is associated with a tRNA gene; and it may have been acquired via horizontal transfer. However, the role that this island plays in *Shigella* pathogenicity is unclear. The only potential virulence genes

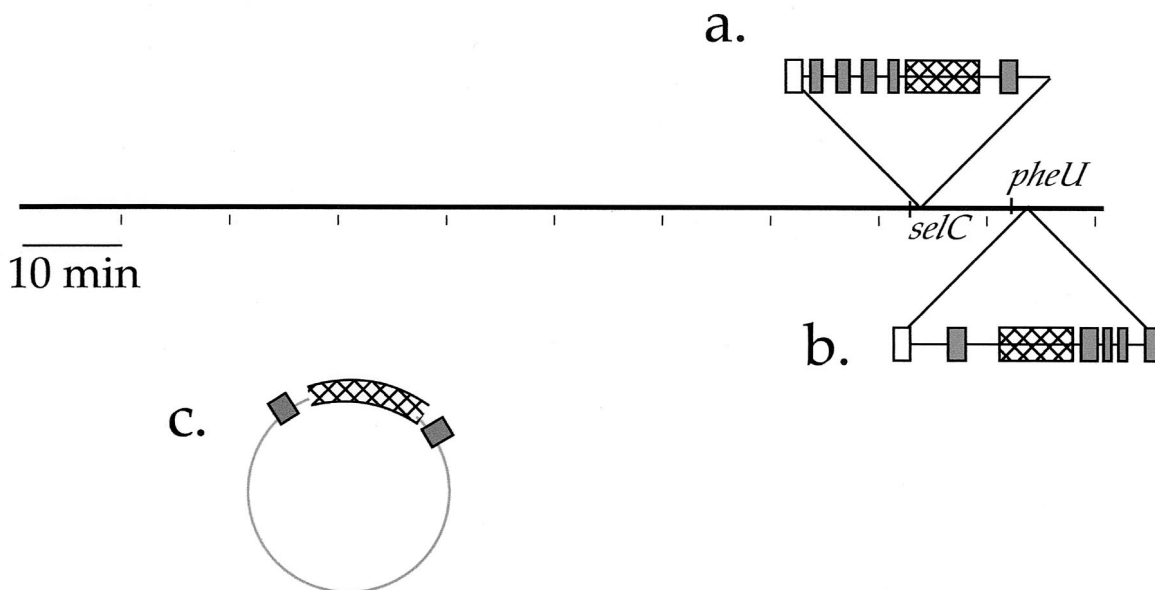


FIG. 3. Map locations of the aerobactin genes among the *Enterobacteriaceae*. The islands containing aerobactin genes are shown as insertions relative to the *E. coli* K-12 chromosome. Hatched boxes indicate the aerobactin operon, white boxes indicate P4-like prophage integrase genes, and dark grey boxes indicate ISs. (a) The SHI-2 aerobactin pathogenicity island at *selC* in some *S. flexneri* strains and in *S. sonnei*. (b) The SHI-3 aerobactin island at *pheU* in *S. boydii*. (c) The aerobactin genes are encoded on the pColV and F1me plasmids in some strains of *E. coli* and *Salmonella enterica*, respectively. On pColV, the aerobactin genes are flanked by IS1 elements.

within this region encode enzymes for aerobactin synthesis and the outer membrane receptor Iut. Aerobactin is known to be important for bacterial survival in low-iron conditions and also may be important in the host. *S. flexneri* aerobactin mutants show reduced fluid accumulation in the rabbit ileal loop model of infection (31), yet *iuc* mutants are capable of wild-type invasion, form plaques in cultured epithelial cells, and are positive in the Serény test. In this study we have shown that *S. boydii* aerobactin mutants are also capable of wild-type invasion and plaque formation, most likely due to the presence of additional iron uptake systems. The presence of multiple functional iron transport systems in *Shigella* suggests that there is selection for the acquisition and maintenance of these genes. These iron acquisition systems may benefit *Shigella* in the different environments it encounters within the host, making the contribution of one iron transport system to virulence difficult to assess. The SHI-3 island also is associated with the absence of the gene encoding lysine decarboxylase activity, the loss of which contributes to *Shigella* pathogenicity (25). Thus, the aerobactin island may contribute to both the survival and the pathogenicity of these bacteria.

The acquisition of SHI-3 may have been bacteriophage mediated, as SHI-3 contains various phage genes, including that for an integrase similar to the LEE P4-like prophage integrase of *E. coli*. *S. boydii* 0-1392 SHI-3 appears to be stable (data not shown), although spontaneous deletions of the aerobactin genes in *S. flexneri* have been observed (19), suggesting the instability of the aerobactin genes in certain strains. The G+C content of the 21-kb SHI-3 island is similar to the observed base composition of the chromosome (51%). Either *S. boydii* acquired this island early in its evolution and the base composition has become similar to that of the chromosome, or SHI-3

was transferred from an organism with a base composition similar to that of *Shigella*.

Many pathogenicity islands are associated with tRNA genes or tRNA-like loci, and the 3' ends of tRNA genes may act as sites for integration of foreign DNA (15). The *S. boydii* SHI-3 island is the second island to be associated with *pheU* in enteric pathogens. The LEE pathogenicity island is found at *pheU* in clinical isolates of enterohemorrhagic and enteropathogenic *E. coli* strains expressing  $\beta$ -intimin, while it is found immediately downstream of *selC* in strains expressing  $\alpha$ - or  $\gamma$ -intimin (41, 45). The *selC* tRNA gene is also the site of the *S. flexneri* and *S. sonnei* SHI-2 aerobactin islands (30, 43).

SHI-3 is distinct from the previously described *S. flexneri* SHI-2, although the aerobactin genes are highly conserved. The G+C content of the 30-kb *S. flexneri* SHI-2 is slightly lower (46%) than that of the *S. boydii* SHI-3 (51%) and contains a colicin immunity gene (43). Additionally, SHI-3 contains three copies of IS600 and incomplete copies of IS200 and IS285, as well as the putative prophage genes *L0004* to *L0006*, which are not present in SHI-2. The presence of different genes and IS elements and the difference between SHI-2 and SHI-3 in G+C content indicate that SHI-2 may have been acquired at a later time, or from another source, than SHI-3.

Horizontal gene transfer involves the introduction of genes into a single lineage via plasmid, bacteriophage, or IS elements, resulting in a scattered phylogenetic distribution among closely related species. Mapping of several iron transport loci among the *Enterobacteriaceae* suggests horizontal transfer of these genes. The various locations of the aerobactin genes, the distribution of the SHI-2 and SHI-3 aerobactin islands among enteric bacteria, and the association of aerobactin genes with bacteriophage or mobile elements suggest that genes for aer-

obactin synthesis and transport have been acquired through horizontal transfer (Fig. 3). Similarly, the presence of the *shu* heme transport locus in *S. dysenteriae* type 1 and various *E. coli* strains, but not in other *S. dysenteriae* serotypes or *Shigella* spp., suggests that the *shu* genes also spread via one of these transfer mechanisms (28, 29, 42, 47). Finally, the genes encoding the siderophore yersiniabactin and its receptor are present in the high-pathogenicity islands of *Yersinia enterocolitica*, *Y. pestis*, and *Y. pseudotuberculosis* (6, 7, 9). High-pathogenicity islands also have been found in the chromosomes of some pathogenic *E. coli* strains, suggesting horizontal transfer between these two species (40). The horizontal transfer of iron acquisition systems effectively alters the ecological and pathogenic characteristics of the recipients by allowing their survival in low-iron conditions. The aerobactin iron transport system may allow *Shigella* to compete for iron in certain iron-limited environments, including the host.

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