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Structural and Genetic Organization of IS*232*, a New Insertion Sequence of *Bacillus thuringiensis*

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In the *Bacillus thuringiensis* strains toxic for the lepidopteran larvae, the δ-endotoxin genes *cryIA* are frequently found within a composite transposonlike structure flanked by two inverted repeat sequences. We report that these elements are true insertion sequences and designate them IS*232*. IS*232* is a 2,184-bp element and is delimited by two imperfect inverted repeats (28 of 37 bp are identical). Two adjacent open reading frames, overlapping for three codons, span almost the entire sequence of IS*232*. The potential encoded polypeptides of 50 and 30-kDa are homologous to the IstA and IstB proteins of the gram-negative insertion sequence IS*21*. The N-terminal part of the 50-kDa polypeptide contains a helix-turn-helix DNA-binding motif. The junctions at the insertion sites of three IS*232* elements were analyzed. Each case was different, with 0, 4, or 6 bp of the target DNA being duplicated. Transposition of IS*232* in *Escherichia coli* was demonstrated by using a genetic marker inserted upstream of the two open reading frames.

Bacillus thuringiensis is a gram-positive bacterium well known for its insecticidal activity toward larvae of insects, mainly lepidoptera, diptera, and coleoptera. Several strains with different entomopathogenic spectra have been described. The pattern of larvicidal activity is dependent on the production of different toxin proteins (δ-endotoxins). There are four major classes of δ-endotoxins, designated CryI, -II, -III, and -IV (11). Although these proteins display different insecticidal specificities, they all contain conserved regions. This characteristic strongly suggests that they are homologous proteins descended from a single ancestral protein (11, 16). The diversity of toxin genes found in *B. thuringiensis* is a striking example of divergent evolution, which confers to this bacterium a strong adaptative potential to proliferate among several insect species.

Recently two IS*240* insertion sequences were found flanking the *cryIVA* gene, which codes for the 134-kDa toxin active against dipteran larvae (1, 5). It was previously shown that the lepidoptera-specific *cryIA* genes were located in the vicinity of at least two types of mobile elements: the transposon Tn*4430* (17, 19) and the insertion sequence IS*231* (20, 21). Moreover, another set of inverted repeated sequences (IS*232*), initially designated IR2 or IR2150, was shown to flank this structure (12, 18). The structural organizations found in conjugative plasmids of *B. thuringiensis* subsp. *thuringiensis* Berliner 1715 and *B. thuringiensis* subsp. *kurstaki* HD73 are presented in Fig. 1. It appears, therefore that many δ-endotoxin genes are organized in transposonlike structures in which flanking insertion sequences might be the active elements in transposition. This structural characteristic suggests a possible mechanism for the creation of the diversity of the δ-endotoxin genes in *B. thuringiensis* species.

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The sequence of the left-terminal inverted repeat of IS*231*B (Fig. 1) is very different from the ends of the other iso-IS*231* elements (20). This rearrangement could have resulted from the insertion of the adjacent element (IS*232*A), consistent with a transpositional role for these external sequences.

Here we report the detailed analysis of one of these elements (IS*232*A), including its entire nucleotide sequence and results of transposition assays in *Escherichia coli*. We show that this element exhibits all of the characteristic features of an insertion sequence (7).

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* HB101 [F⁻ *hsdS20* (r_B^- m_B^-) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 supE44* λ⁻] (2) and LC916 (*thy thi pro recA* Rif^r) (3) were used in the mating experiments. *E. coli* K-12 strain TG1 [Δ(*lac-proAB*) *supE thi hdsD5*(F′ *traD36 proA*$^+$ *proB*$^+$ *lacI*^{q} *lacZ*ΔM15)] (T. J. Gibson, Ph.D. thesis, University of Cambridge, Cambridge, United Kingdom, 1984) was used as a host for the sequencing vectors M13mp19 and M13mp18 (27). *E. coli* CJ236 [*dut ung thi relA*(pGT105) (Cm^r)] and MV1190 [Δ(*lac-proAB*) *thi supE* Δ(*srt-recA*)*306*::Tn*10*(Tet^r) (F′ *traD36 proAB lacI*^{q} *lacZ*ΔM15)] (Bio-Rad Laboratories, Richmond, Calif.) were used for oligonucleotide-directed mutagenesis. *B. thuringiensis* subsp. *thuringiensis* Berliner 1715 (serotype 1) and *B. thuringiensis* subsp. *kurstaki* HD73 (serotype 3) were obtained from the World Health Organization Collaborating Center for Entomopathogen Bacillus (Institut Pasteur, Paris).

The *E. coli* strains were grown at 37°C in Luria broth. Cultures for mating experiments were grown in brain heart infusion (Difco). *B. thuringiensis* strains were cultured as previously described (15).

Antibiotic concentrations for bacterial selection were as follows: ampicillin, 100 μg/ml; chloramphenicol, 10 μg/ml; kanamycin 25 μg/ml; and rifampin, 100 μg/ml.

Plasmids. The plasmids used are listed in Table 1.

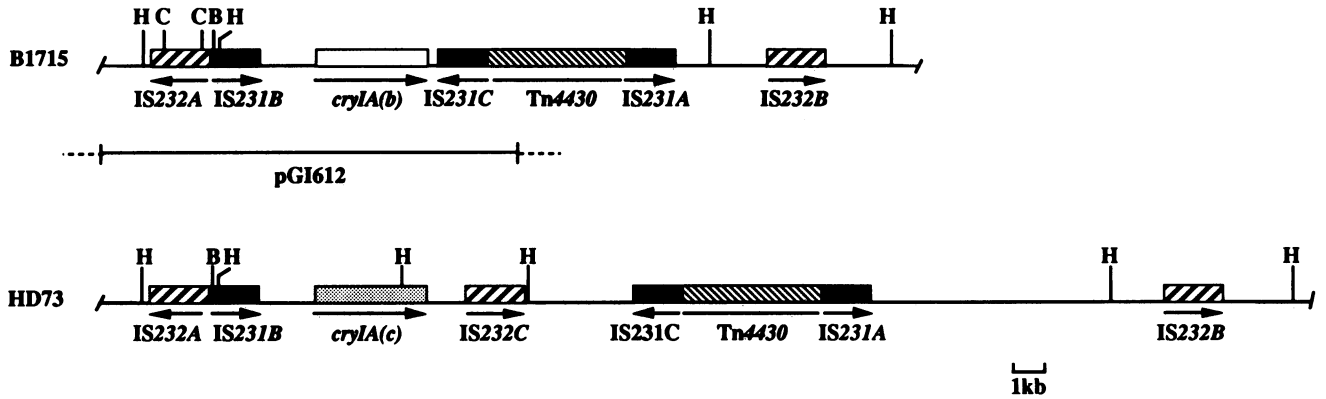

FIG. 1. Structural organizations of the transposable elements flanking δ-endotoxin genes in *B. thuringiensis*. The model summarizes the different organizations of DNA segments of two plasmids of 65 and 75 kb from *B. thuringiensis* subsp. *thuringiensis* Berliner 1715 (B1715) and subsp. *kurstaki* HD73 (HD73). These structural organizations are deduced from Kronstad and Whiteley (12, 13) and Lereclus et al. (18). The segment labeled pGI612 represents the 15-kb DNA fragment cloned from the plasmid of *B. thuringiensis* subsp. *thuringiensis* Berliner 1715 (20). Arrows above the *cryIA(b)* and the *cryIA(c)* genes indicate the direction of transcription. Arrows above IS*231* and IS*232* represent the relative orientations. IS, Insertion sequence; Tn, transposon. Restriction sites: H, *Hin*dIII; C, *Cla*I; B, *Bgl*II.

Preparation and manipulation of DNA. The basic recombinant DNA procedures were performed as described by Maniatis et al. (22). All enzymes were used as recommended by the manufacturers. Transfection of M13 recombinant phage DNA and purification of single-stranded DNA were performed as described in the Amersham "M13 Cloning and Sequencing Handbook." Nucleotide sequencing by the dideoxy-chain termination method (31) was carried out by using recombinant M13mp19 or M13mp18 phage as the template. The Sequenase sequencing kit was purchased from U.S. Biochemical Corp. (Cleveland, Ohio), and [α-^{35}S]dATP (24.1 TBq/mmol) was supplied by Amersham (Amersham, United Kingdom).

Overlapping deletions were obtained by using the technique of Dale et al. (4). These deletions were made by using the Cyclone system as directed by the manufacturer (International Biotechnologies Inc., New Haven, Conn.). We synthesized two oligonucleotides deduced from preliminary sequencing results, 5′-CAAATTCTGTTTGAATATCTAG C-3′ and 5′-GTAGGACAATCCTATCG-3′, matching at nucleotide positions 126 to 104 and 2080 to 2096, respectively, in Fig. 2. These oligonucleotides were used as primers to sequence the two extremities of different copies of IS*232* elements.

Oligonucleotide site-directed mutagenesis. The mutagene Phagemid kit (Bio-Rad) was used, and the method was based on the procedure described by Kunkel et al. (14). The 2.8-kb *Hin*dIII-*Bgl*II DNA fragment carrying an entire copy of IS*232*A was purified from plasmid pGI612 (Fig. 1). This DNA fragment was subcloned between the *Hin*dIII and *Bam*HI sites of the vector pTZ19U (25) to give plasmid pHTZ232. pHTZ232 DNA was isolated from the *dut ung* double mutant strain CJ236, which incorporates uracil in the place of thymine. Superinfection of this strain with the helper phage M13K07 (35) leads to single-stranded DNA production from the f1 origin of pHTZ232. The uracil-containing strand is then used as the template for the in vitro synthesis of a complementary strand, primed by an oligonucleotide containing the desired mutation. This synthetic oligoprimer, containing a *Bam*HI site (in italics), 5′-CTGAC

TABLE 1. Plasmids used

Plasmid	Phenotype	Relevant characteristics[a]	Source or reference
pGI612	Ap	Plasmid containing a 15-kb DNA fragment from *B. thuringiensis* subsp. *thuringiensis* Berliner 1715. This DNA fragment harbors a *cryIA(b)* gene, two copies of IS*231*, and one copy of IS*232*	20
pTZ19U	Ap	Phagemid containing both the f1 and pBR322 origins of replication for generation of double-stranded and single-stranded DNA	25
pHTZ232	Ap	pTZ19UΩIS*232*A (2.8-kb *Hin*dIII-*Bgl*II fragment from pGI612)	This work
pHTZ232B	Ap	pHTZ232 in which IS*232*A was modified by in vitro insertion of a *Bam*HI site	This work
pAT21	Ap Tc Km	pBR322Ω*aphA3* (1.5-kb *Cla*I fragment)	34
pHSG575	Cm	Low-copy-number plasmid carrying a *cat* gene	32
pHTG232B	Cm	pHSG575ΩIS*232*A (2.8-kb *Hin*dIII-*Sma*I fragment from pHTZ232B)	This work
pHTG232K	Cm Km	pHTG232BΩ*aphA3* (1.35-kb *Cla*I-*Hin*dIII fragment from pAT21). The *aphA3* gene and *orf1* and *orf2* of IS*232* have the same direction of transcription	This work
pHTG223K	Cm Km	pHTG232BΩ*aphA3* (1.35-kb *Cla*I-*Hin*dIII fragment from pAT21). The *aphA3* gene and *orf1* and *orf2* of IS*232* are in opposite orientations	This work
pOX38	Tra$^+$	Conjugative F derivative	9

[a] The *cat* gene encodes a chloramphenicol acetyltransferase (Cm^r) and the *aphA3* gene encodes an aminoglycoside phosphotransferase (Km^r).

FIG. 2. Nucleotide sequence of IS*232* and deduced amino acid sequences of *orf1* and *orf2*. Proposed ribosome-binding sites for *orf1* and *orf2* are in bold characters. Potential ATG start codons and the 14-bp directly duplicated upstream of *orf1* are boxed. Asterisks represent stop codons. The terminal inverted repeats are underlined with arrows. The potential −10 and −35 regions found at either end of IS*232* are discussed in the text and are overlined with brackets.

TCTG*GGATCC*AGAAAGGTGGTTAG-3', matches the DNA sequence located at positions 73–87 in Fig. 2.

The resulting double-stranded DNA was introduced into *E. coli* MV1190, which contains uracil *N*-glycosylase. The uracil-containing strand was therefore inactivated with high efficiency, leaving the newly synthesized non-uracil-containing DNA strand to replicate. The resulting recombinant plasmid was named pHTZ232B.

Mating procedures. Conjugation experiments between *E. coli* strains were performed in brain heart infusion medium as previously described (17) except that the HB101 and LC916 cells were mixed at a ratio of 1:10.

Computer analysis. DNA and protein sequences were analyzed as previously described (21).

Nucleotide sequence accession number. The nucleotide sequence data reported have been submitted to GenBank/EMBL (accession number M38370).

RESULTS

Nucleotide sequence analysis of an IS*232* element. IS*232* was obtained from the recombinant plasmid pGI612, itself from a plasmid DNA library of *B. thuringiensis* subsp. *thuringiensis* Berliner 1715 (20). pGI612 contains a 15-kb DNA fragment carrying a *cryIA(b)* gene and two copies of IS*231*. Figure 1 represents the structural organizations of the IS*232* copies on the plasmids of *B. thuringiensis* subsp. *thuringiensis* Berliner 1715 and subsp. *kurstaki* HD73 as previously described by Kronstad and Whiteley (12, 13) and Lereclus et al. (18). The IS*232* elements were designated A, B, and C according to their locations on the plasmids. As an IS*232*A element was known to be adjacent to IS*231*B, we subcloned the 2.8-kb *Hin*dIII-*Bgl*II DNA fragment bearing the DNA region located upstream of the IS*231*B element into phages M13mp18 and M13mp19.

The nucleotide sequence of the 2.8-kb DNA fragment was determined on both strands (Fig. 2). The true positions of the ends of IS*232*A were precisely defined by comparing the nucleotide sequences of the extremities of different IS*232* copies, as described below.

IS*232*A is 2,184 bp long and is delimited by two 37-bp imperfect inverted repeats (28 of 37 bp are identical). Nucleotide sequence analysis revealed two long open reading frames (ORFs) on two frames of the same DNA strand. No ORF of >80 codons was detected in the four other possible frames.

The two adjacent ORFs, *orf1* and *orf2*, overlap for 8 bp. Their ATG start codons are preceded by the potential ribosome-binding sites 5'-GAGGTGG-3' (*orf1*) and 5'-GGTGAGGT-3' (*orf2*). Since no information on the 16S RNA from *B. thuringiensis* was available, the sequence of the putative ribosome-binding sites was compared with that of the 16S RNA of *Bacillus subtilis* (24). The free energies of interaction, according to Tinoco et al. (33), are −57.7 kJ/mol (*orf1*) and −56.8 kJ/mol (*orf2*). *orf1* encodes 431 amino acids, putatively protein ORF1, starting at position 93 and stopping at position 1385. The putative *orf2* product is encoded between positions 1378 and 2127 and is a 250-amino-acid polypeptide, designated ORF2. The deduced molecular weights of ORF1 and ORF2 are 49,836 and 29,198, respectively.

The organizations of the two ORFs suggest that the two genes of IS*232* are transcribed as a single message. However, no obvious promoterlike sequences that match known consensus sequences of *B. subtilis* promoters (26) were found upstream of *orf1*. However, a −10 region was found at one end of the IS*232* sequence, and −35 region was found at the other (Fig. 2). These sequences are discussed below.

The nucleotide sequence of IS*232* contains a 14-bp direct duplication upstream of *orf1*. One of these 14-bp repeats (boxed in Fig. 1) is an internal part of the terminal inverted repeat of the IS*232* element.

Comparison of the IS*232* sequence with known insertion sequences. The nucleotide sequence of IS*232* and the deduced amino acid sequences of its two putative gene products were compared with those of other known transposable elements. Two insertion sequences appear to be homologous to IS*232*: IS*21* isolated from *Pseudomonas aeruginosa* (30) and IS*640* isolated from *Shigella sonnei* (23). IS*640* is shorter than the two others but is almost identical to the first half of IS*21*, differing at only a few nucleotide positions, including a frameshift. Furthermore, the DNA immediately downstream of and adjacent to the ORF of IS*640* is identical to the corresponding region of IS*21*.

No significant nucleotide sequence similarity was found between IS*232* and IS*21*; their terminal inverted repeats are not similar in size or in base composition. However, IS*232* and IS*21* share features of molecular organization, including the sizes and amino acid sequences of encoded polypeptides. In common with IS*232*, IS*21* is a long insertion sequence (2,131 bp) encoding two proteins, IstA (46 kDa) and IstB (30 kDa). The TGA stop codon of the *istA* gene overlaps with the ATG start of *istB* (30).

The amino acid sequence of ORF1 of IS*232* is 23% identical to the sequence of IstA of IS*21*, and the similarities are found in several short stretches (Fig. 3). If conservative amino acid changes are accepted, the similarity is 30%. The putative protein ORF2 of IS*232* and the protein IstB of IS*21* presented more striking similarities (Fig. 3). The identity was about 39% and increased to 55% if conservative amino acid changes are included.

Although no function has been ascribed to the two putative proteins ORF1 and ORF2, the highly basic amino acid composition of ORF1 (75 arginines, lysines, or histidines and 43 aspartic or glutamic acids) is consistent with it being a DNA-binding protein. Moreover, ORF1 has a potential helix-turn-helix motif in the N-terminal region (Fig. 3). This motif can be deduced from amino acid sequence similarities with Cro-like DNA-binding regions. Alignments between IS*232* and domains of defined DNA-binding proteins show striking homology (Fig. 4)

According to the Dodd-Egan system (6), this region scores 1720, which places the deduced polypeptide ORF1 among the DNA-binding proteins implicated in transposition, in regulation of gene expression, or in site-specific recombination.

Insertion sites of IS*232* and target duplications. Both to define the true ends of IS*232* (Fig. 2) and to determine the lengths of the target duplications at the insertion site, we sequenced and compared the borders of different IS*232* elements naturally present on the plasmids of *B. thuringiensis* subsp. *thuringiensis* Berliner 1715 and subsp. *kurstaki* HD73. The three *Hin*dIII DNA fragments containing IS*232*B of *B. thuringiensis* subsp. *thuringiensis* Berliner 1715 and IS*232*A and -C of *B. thuringiensis* subsp. *kurstaki* HD73 (Fig. 1) were each cloned at the *Hin*dIII site of pUC vectors, using a ^{32}P-labeled internal *Cla*I fragment of the IS*232*A from *B. thuringiensis* subsp. *thuringiensis* Berliner 1715 as a radioactive probe (results not shown). The nucleotide sequences of the borders of the IS*232* elements were determined from the recombinant plasmids, using synthetic oligonucleotides (see Materials and Methods) as primers for the

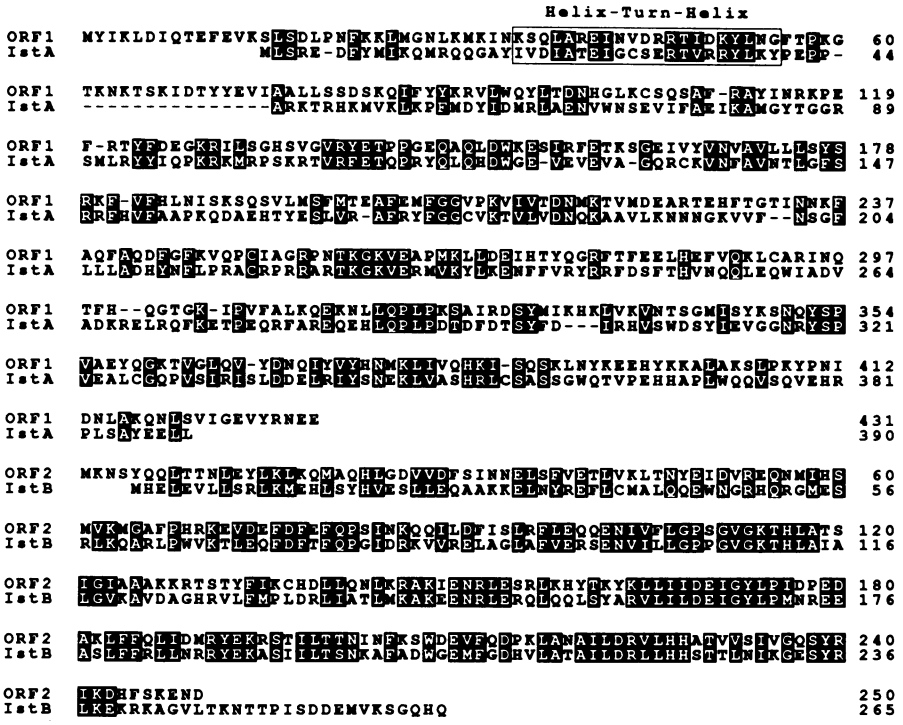

FIG. 3. Comparison of the amino acid sequences of the polypeptides encoded by IS*232* and IS*21*. Sequences of the putative ORF1 and ORF2 proteins of IS*232* and the proteins IstA and IstB of IS*21* are aligned. Boxes indicate Cro-like, potential α-helix–turn–α-helix DNA-binding domains of ORF1 and IstA, as defined by Pabo and Sauer (28). Identical and similar residues are indicated by black boxes. Accepted conservative substitutions are as follows: I, L, V, and M; D and E; N and Q; A and G; R and K; S and T; and F and Y. Numbers indicate the positions in the amino acid sequences.

sequencing reactions. The nucleotide sequences of the extremities and of the insertion sites of the different IS*232* elements are shown in Fig. 5.

The terminal 37 bp of IS*232* are identical in every case. Target duplication at the insertion site appears to be variable: no target duplication is observed for the two IS*232*A sequences whereas duplications of 4 and 6 bp are present for IS*232*C and -B, respectively.

Transposition of IS*232* in *E. coli*. A *Bam*HI restriction site was introduced upstream of *orf1* by in vitro mutagenesis as described in Materials and Methods. The *Hin*dIII-*Sma*I DNA fragment of pHTZ232B containing the modified

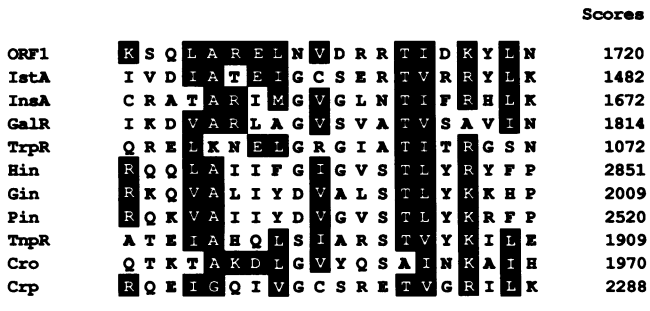

FIG. 4. Possible helix-turn-helix domain in the deduced ORF1 protein of IS*232*. Amino acid sequence similarities were found with other DNA-binding domains (7, 28, 30). Indicated scores correspond to predictions of λ Cro-like DNA-binding regions by using the Dodd-Egan weight matrix (6). Amino acids conserved between ORF1 and at least three other DNA-binding proteins are indicated by black boxes.

IS*232*AΩ*Bam*HI was cloned between the *Hin*dIII and *Sma*I sites of the low-copy-number plasmid pHSG575 (32) to give the recombinant plasmid pHTG232B. A 1.35-kb *Cla*I-*Hin*dIII DNA fragment of pAT21 (34) that contains the kanamycin resistance gene *aphA3* was subcloned into the *Bam*HI site of pHTG232B. Both the *Cla*I-*Hin*dIII fragment and the *Bam*HI-digested plasmid were filled in with the Klenow fragment of DNA polymerase I, ligated, and used to transform *E. coli* HB101. Recombinant plasmids obtained after transformation in *E. coli* were analyzed, and two plasmids (pHTG232K and pHTG223K) in which the *aphA3* gene was cloned in either orientation were selected. The relative orientation of the *aphA3* gene cloned in pHTG232K and pHTG223K was verified by sequencing the fusion junctions (results not shown). The *aphA3* gene and *orf1* and *orf2* of IS*232* have the same direction of transcription in pHTG232K, whereas they are in opposite orientations in pHTG223K (Table 1). The *Cla*I-*Hin*dIII fragment of pAT21 does not contain the potential terminator of the *aphA3* gene (34), thus allowing a transcriptional fusion with downstream genes in the case of pHTG232K.

These constructs were tested for transposition in *E. coli*, using a conjugative transposition assay based on the conjugative F derivative plasmid pOX38 (9), which is used as a recipient molecule for transposable elements. Transposition is detected by selecting for pOX38-mediated transfer of a genetic marker carried on the transposable element. Transposition of IS*232* was observed only with use of the donor strain HB101(pOX38, pHTG232K), in which the *aphA3* gene and *orf1* and *orf2* are in the same orientation (Table 2). Thus, in plasmid pHTG232K, the *aphA3* gene acts as a selectable

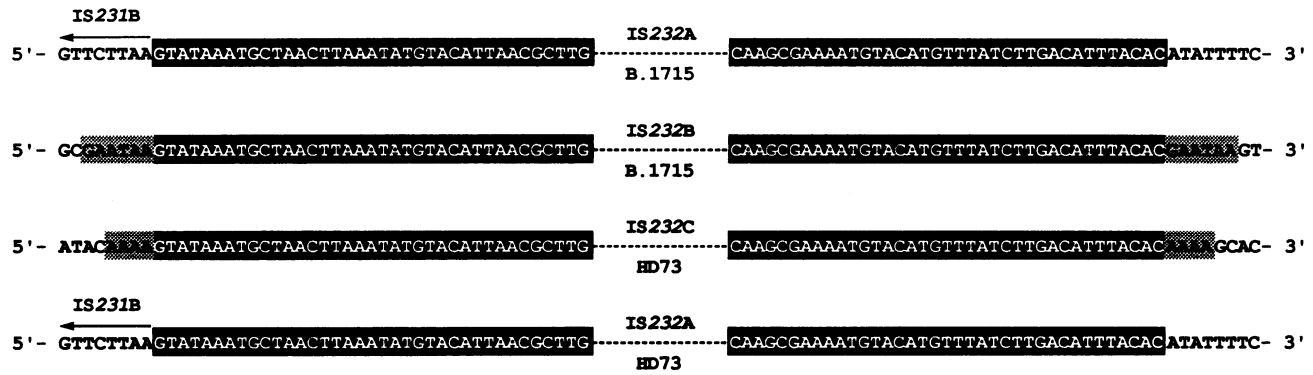

FIG. 5. Nucleotide sequences of the insertion sites of different IS*232* elements. The 37-bp terminal inverted repeats of the IS*232* elements are boxed in black. The target duplications are indicated with gray boxes. Arrows indicate the orientation of the IS*231*B as represented in Fig. 1. B1715 and HD73 indicate the origins of IS*232*A, -B, and -C from *B. thuringiensis* subsp. *thuringiensis* Berliner 1715 and subsp. *kurstaki* HD73, respectively.

marker and presumably forms a transcriptional fusion with the downstream genes.

The absence of Cm^r clones among the Km^r Rif^r exconjugants indicates that pOX38-pHTG232K cointegrates either did not form or were not stably maintained in the donor cells. The frequency of cointegration was estimated to be less than $3 \cdot 10^{-2}$ per Km^r Rif^r cell, since only 30 Km^r Rif^r clones were obtained, all of which were Cm^s.

Transposition of IS*232*Ω*aphA3* onto pOX38 was confirmed by the subsequent transfer of kanamycin resistance to the recipient strain from the LC916(pOX38::IS*232*Ω*aphA3*) donor strain. The relatively low frequency of transfer in one case (10^{-3}) could have resulted from the transposition of IS*232* into a site that impaired the conjugative properties of pOX38.

DISCUSSION

The *cryIA* genes encoding the δ-endotoxins of *B. thuringiensis* are located on conjugative plasmids and are included in a composite transposonlike structure. The results presented here demonstrate that the flanking elements of this structure are true insertion sequences, IS*232*. Three different copies of IS*232*, conventionally designated A, B, and C were cloned from *B. thuringiensis* subsp. *thuringiensis* Berliner 1715 and subsp. *kurstaki* HD73. Nucleotide sequencing of IS*232*A and comparison of the junction segments of the three elements at their insertion sites reveal that IS*232* is a genetic element of 2,184 bp delimited by two inverted repeats of 37 bp in which there are nine nucleotide mismatches. The terminal inverted repeats of mobile genetic elements frequently contain such variability (7).

Duplication of the target site and implications for the composite structures containing the *cryIA* genes. No sequence duplication was observed at the insertion site of IS*232*A, whereas 4 and 6 bp were duplicated at both ends of IS*232*C and -B, respectively. Although many transposable elements appear to induce a duplication of a fixed number of base pairs, several exceptions have been reported (7).

Variable target duplication length could be responsible for the situation observed with IS*232*B and -C. However, the absence of direct repeats at the ends of IS*232*A could result from molecular rearrangements after transposition, leading to different flanking regions for this element.

Taken together, these results are not consistent with the hypothesis that the IS*232* is the active element in the composite transposon containing the *cryIA* genes. Were this the case, duplication of the target DNA site at the left end of IS*232*A and at the right end of IS*232*B would be expected (see Fig. 1). However, these results suggest that the IS*232* elements have transposed independently.

Homology between IS*232* and IS*21*. Comparisons between the putative polypeptides ORF1 and ORF2 encoded by IS*232* and the proteins IstA and IstB of IS*21* clearly indicate that these two insertion sequences are derived from a common ancestor. IS*21* transposition requires the *istA* and *istB* gene products (30). By analogy, both ORF1 and ORF2 are, presumably, involved in IS*232* transposition.

Transposases bind the ends of their respective elements (7). The fact that ORF1 has an α-helix–turn–α-helix motif is compatible with the possibility that this putative protein binds to the terminal inverted repeats of IS*232*.

The homologous pairs of proteins of IS*232* and IS*21* do not

TABLE 2. Transposition of IS*232*Ω*aphA3* in *E. coli*

Plasmid(s)[a]	Donor strain	Recipient strain	Antibiotic selection	Transposition frequency,[b] Km^r cells/recipient cells	Frequency of cointegration,[c] Km^r Cm^r cells/Km^r cells
pHTG232K	HB101	LC916	Rif, Km	$<5 \cdot 10^{-9}$	—
pHTG223K	HB101	LC916	Rif, Km	$<5 \cdot 10^{-9}$	—
pOX38 + pHTG232K	HB101	LC916	Rif, Km	$5 \cdot 10^{-8}$	$<3 \cdot 10^{-2}$[d]
pOX38 + pHTG223K	HB101	LC916	Rif, Km	$<5 \cdot 10^{-9}$	—
pOX38::IS*232*Ω*aphA3*	LC916	HB101	Sm, Km	10^{-3}, $4 \cdot 10^{-1}$	—

[a] Descriptions are given in Table 1.
[b] Results are averages of three independent mating experiments except for plasmid pOX38::IS*232*Ω*aphA3*, for which the extreme values obtained are indicated. In our experimental conditions, the calculated frequency of conjugation of pOX38 is $4 \cdot 10^{-1}$ per recipient cell.
[c] Calculated by testing the Km^r transposition products for resistance to chloramphenicol. —, Not tested.
[d] The 30 Km^r Rif^r clones obtained were sensitive to chloramphenicol.

display the same degree of similarity. The greater divergence of ORF1 and IstA could reflect the dissimilarity of the terminal inverted repeats of IS*232* and IS*21*. However, there are no data on the insertional functions of the protein IstB and the putative polypeptide ORF2, which appear to be highly conserved.

Reimmann and Haas (29) have shown that a single copy of IS*21* transposes infrequently, whereas a tandem repeat of IS*21* with little intervening DNA is highly active for transposition. More recently, Reimmann et al. (30) have clearly demonstrated that the IS*21*-IS*21* junctions form a functional promoter that directs the transcription of the *istAB* operon. The upstream element provides the −35 region of the promoter, and the downstream element provides the −10 region.

An analogous phenomenon is possible for IS*232*, since a potential −10 region (TATAAAT) is found at the start of the DNA sequence and a potential −35 region (TTGACA) is present at the end of the element (Fig. 2). We could expect that an IS*232* tandem with an intervening DNA sequence of about 10 bp would result in a functional promoter for the downstream *orf1* and *orf2*.

Transposition of IS*232*. Introduction of a genetic marker upstream of and in the same transcriptional orientation as *orf1* led to transposition of IS*232* in *E. coli*. This result indicates that a transcriptional fusion between the two genes is required for transposition. The chimeric insertion sequence transposed at low frequency ($5 \cdot 10^{-8}$ transposition events per recipient cell). This is a poor guide to the insertional activity of IS*232* in its original host. However, the absence of cointegrate molecules among the transposition products suggests that IS*232* transposes via a conservative mechanism (8). Indeed, the absence of Cm^r clones among the Km^r Rif^r transconjugants (Table 2) indicates that the plasmid DNA did not transfer during conjugation. This result suggests that IS*232*Ω*aphA3* transposes alone on pOX38 without formation of a stable intermediate cointegrate between the donor and the recipient molecules.

The best model for IS*232*, therefore is a cut-and-paste mode of transposition, as proposed for IS*21* (30). However, it is possible that IS*232* transposes via the formation of an unstable cointegrate that could not be detected by the conjugative transposition assay.

IS*232* was isolated from a large conjugative plasmid from *B. thuringiensis* as IS*21*, which originates from the broad-host-range IncP plasmid R68 (10). In gram-negative bacteria, IS*21* mediates the mobilization of other replicons (29). IS*232* may have a similar function in gram-positive bacteria such as *B. thuringiensis*.

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