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Complete Nucleotide Sequence of Insertion Element IS*4351* from *Bacteroides fragilis*

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The nucleotide sequence and genetic analyses of one of the directly repeated sequences flanking the macrolide-lincosamide-streptogramin B drug resistance determinant, *ermF*, from the *Bacteroides fragilis* R plasmid, pBF4, suggested that this region is an insertion sequence (IS) element. This 1,155-base-pair element contained partially matched (20 of 25 base pairs) terminal-inverted repeats, overlapping, anti-parallel open reading frames, and nine promoterlike sequences, including three that were oriented outward. Analysis of this sequence revealed no significant nucleotide homology to 13 other known IS elements. Inasmuch as Southern blot hybridization analysis detected homologous sequences in chromosomal DNA and its G+C content (42 mol%) was similar to that of *B. fragilis*, the data suggested that this element is of *Bacteroides* origin. Transposition promoted by this element was demonstrated in *recA E. coli*. Recombinants were recovered by selecting for the activation of a promoterless chloramphenicol resistance gene on the plasmid pDH5110 and were characterized by restriction endonuclease mapping and Southern blot hybridization. We propose that this IS element be designated IS*4351*.

Transposable elements are defined DNA segments that are capable of moving from one genetic locus to another. One class of transposable elements is insertion sequence (IS) elements, which are short segments of DNA (0.75 to 1.6 kilobases [kb]) that are believed to carry the genes required for transposition. IS elements have been identified in a variety of bacteria and are the subject of several recent reviews (3, 9, 14, 17, 18, 40).

Within the obligately anaerobic *Bacteroides* spp., three independently isolated R plasmids, pBF4 (44), pBFTM10 (41), and pBI136 (36), have been described which confer resistance to macrolide, lincosamide, and streptogramin B (MLS)-type antibiotics. In each of these plasmids, the MLS resistance genes are flanked by directly repeated (DR) DNA sequences of approximately 1.1 kb (12, 31, 35). The MLS resistance determinant and the flanking DR sequences on pBF4 (33, 34) and pBFTM10 (28) have been demonstrated to undergo transposition in both *Escherichia coli* and *Bacteroides* spp. and have been designated transposons Tn*4351* and Tn*4400*, respectively. A similar observation has been noted with the analogous region of pBI136 (C. J. Smith, submitted for publication).

During the course of studying the genetic basis of MLS resistance in the *Bacteroides fragilis* plasmid pBF4, we determined that this resistance gene, *ermF*, is transcriptionally dependent on an adjacent DR sequence (27). In the present study, we report the nucleotide sequence of one member of the DR segments of pBF4. Computer analyses of this nucleotide sequence revealed structural similarities of this element to procaryotic IS elements, although no significant homology was seen at the nucleotide level. We also examined the biological activity of this element in *E. coli* and present evidence that it can undergo *recA*-independent transposition. We propose to designate this element IS*4351*.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. Bacterial strains and plasmids are shown in Table 1. *B. fragilis* V479-1 or V598, V600, and V689 were maintained on supplemented brain heart infusion broth (22) containing 5 μg of clindamycin per ml or 10 μg of tetracycline per ml as appropriate. *E. coli* JM101 was maintained on YT medium (23). Other *E. coli* strains were maintained on LB medium (23) with antibiotics as follows: V831, 10 μg of tetracycline per ml; V1317, 50 μg of ampicillin per ml; V1337, 300 μg of erythromycin per ml; and V1348, 50 μg of ampicillin per ml, 50 μg of chloramphenicol per ml, and 10 μg of tetracycline per ml.

Genetic transformation of *E. coli*. *E. coli* strains JM101 and HB101 were transformed with bacteriophage and plasmid DNA, respectively, as previously described (27).

Plasmid DNA and M13 replicative-form DNA isolation. Preparations of covalently closed circular plasmid or phage replicative-form DNA were obtained by the method of Guerry et al. (10) adjusted to 100 ml of cell culture. Amplification and isolation of plasmid and phage DNA were done as previously described (27).

Construction of M13 clones. Restriction endonuclease fragments from pVA831 (22) and pBF4, pBF4Δ1, or pBFΔ2 (44) were ligated with appropriately cleaved M13, mp8, or mp9 replicative-form DNA. T4 DNA ligase (New England BioLabs, Inc., Beverly, Mass.) was used at concentrations of 2 and 400 U per reaction for cohesive and blunt-end ligations, respectively. Purified recombinant phage was used for the preparation of overlapping deletion derivatives as described by Poncz et al. (25). Recombinant plaques were identified by a modification of the plaque-screening method of Benton and Davis (2). Nitrocellulose (Schleicher & Schuell, Inc., Keene, N.H.) or Gene-Screen (New England Nuclear Corp., Boston, Mass.) filters were used to lift phage from agar plates. Phage-laden filters were treated as described by Maniatis et al. (21) except that incubations were extended for 10 min, followed by a 1-min rinse in 2× SSC (pH 7.0) (1× SSC is 0.15 M NaCl plus 0.015 M sodium

TABLE 1. Strains and plasmids used

Bacterium	Strain	Plasmid	Relevant description[a]	Reference or source
B. fragilis	V479-1	pBF4	MLS^r Tc^r	44
	V600	pBF4Δ1[b]	MLS^s Tc^r	44
	V689	pBF4Δ2[b]	MLS^s Tc^r	44
	V598	None	MLS^s Tc^r	22
E. coli	V831	pVA831(pBR325::*Eco*RI-D)	*hsdR4* Gal$^-$ Tc^r Ap^r	22
	V1338	pDH5110 and pVA1337	HB101 background, Ap^r Em^r	42; this study
	V1348	pVA1348	HB101 background, Ap^r Cm^r Tc^r	This study
	HB101	None	*recA13 hsdS20*	
	JM101	F′	Δ(*lac pro AB*)/F′ *traD36* *proA*$^+$*B*$^+$ *lacI*^{q}ZΔM13	New England BioLabs

[a] Abbreviations: MLS^r, macrolide, lincosamide, streptogramin B resistant, operationally, 10 μg of clindamycin or erythromcin per ml; Tc^r, tetracycline resistant (10 μg/ml); *hsd*, host-specific restriction deficient; Gal$^-$, galactose nonfermenting; Ap^r, ampicillin resistant (25 μg/ml); Em^r, erythromycin resistant (300 μg/ml); Cm^r, chloramphenicol resistant (50 μg/ml); *rec*, recombination deficient; Δ*pro-lac*, deletion of proline and lactose region; *traD36*, conjugation deficient; *proA*$^+$B$^+$, proline auxotroph.

[b] pBF4Δ1 and pBF4Δ2, Independent spontaneous deletion derivatives of pBF4 missing the MLS^r gene.

citrate). Treatment of filters and hybridization with ^{32}P-labeled probes were according to Maniatis et al. (21). Nick translation kits were obtained from New England Nuclear and were used as recommended by the supplier.

DNA sequencing. Single-stranded DNA from recombinant phage was isolated and used as a template in the dideoxy sequencing method of Sanger et al. (30) and used in sequencing reactions as previously described (27).

Southern blot hybridization. The transfer of DNA from agarose gels to nitrocellulose paper (Schleicher & Schuell) and subsequent DNA-DNA hybridization were as previously described (21, 38).

Transposition of IS*4351*. Transposition mediated by IS*4351* was demonstrated in *recA E. coli* HB101 by using the plasmid pVA1337 as a delivery vehicle and the plasmid pDH5110 (42) as a target (see Fig. 5). Plasmid pVA1337 was construct-

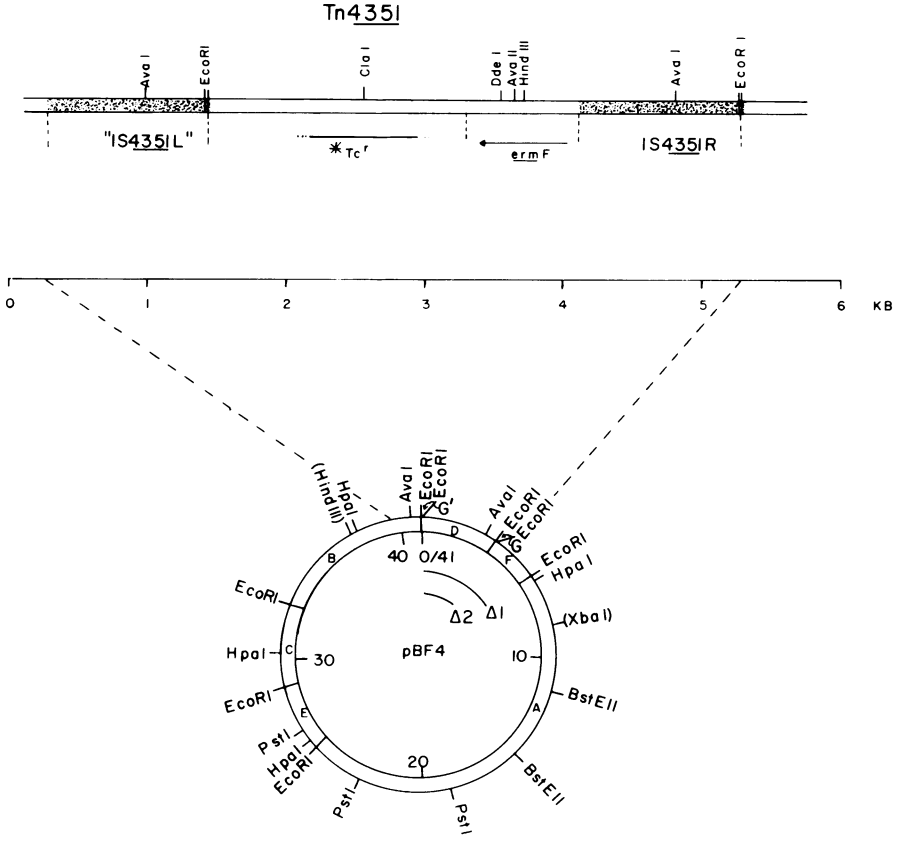

FIG. 1. Restriction endonuclease maps of pBF4 and Tn*4351* from *B. fragilis* V479-1. Lines within the pBF4 map designate the approximate regions missing from the independently isolated deletion derivatives pBF4Δ1 and pBF4Δ2 (44). The *Eco*RI fragments of pBF4 are indicated by the capital letters (A thru G). In Tn*4351*, the directly repeated copies of IS*4351* (L, left; R, right) are represented by the shaded areas. IS*4351*L is in quotation marks because it has been only partially sequenced and has not yet been tested for transposition. The relative positions of the MLS^r gene, *ermF* (27), and the cryptic tetracycline resistance gene, *Tc^r (11), are indicated below the Tn*4351* map. The enzymes *Cla*I, *Dde*I, *Ava*II, and *Hin*dIII recognize multiple sites within pBF4 in addition to those shown on the Tn*4351* map.

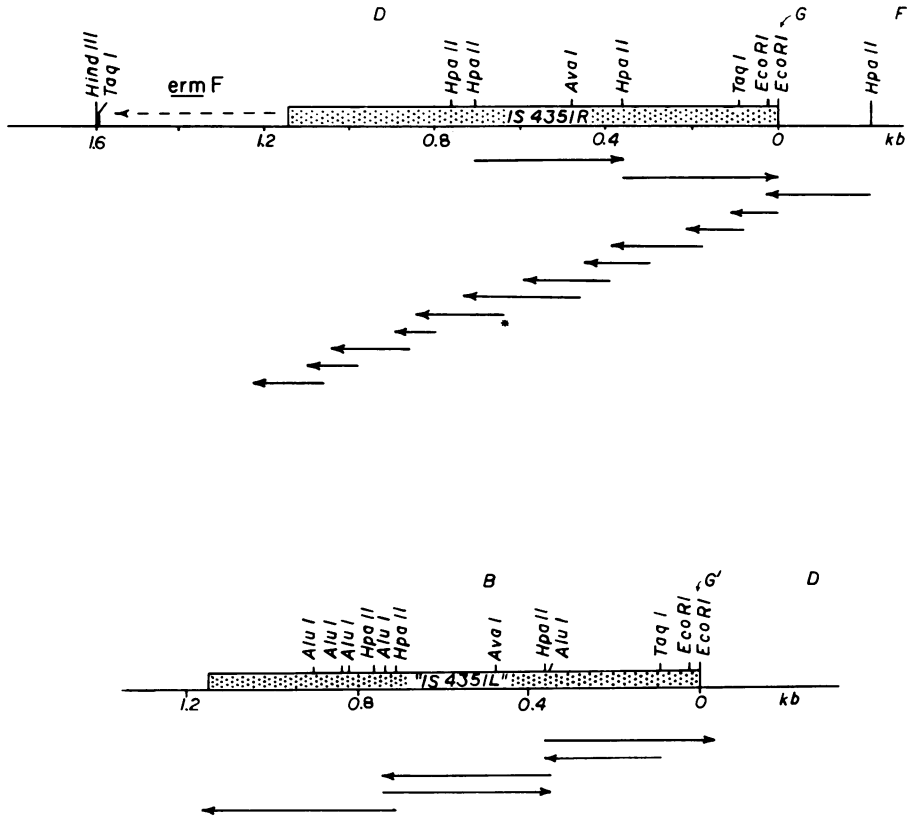

FIG. 2. DNA sequence analysis strategy for IS*4351* from pBF4 (upper panel) and pBF4Δ2 (lower panel). The *Eco*RI fragments of pBF4 (Fig. 1) are indicated by the capital letters above the maps. Arrows below the maps indicate the direction and extent of DNA sequence analysis. In the upper panel, the direction of transcription of *ermF* is indicated by the dashed arrow (27). The asterisk indicates DNA sequence data obtained by using a synthetic oligonucleotide primer.

ed by ligation of a *Hin*dIII-*Xba*I 7-kb fragment containing one copy of IS*4351* (from pBF4Δ2; Fig. 1) with similarly digested pVA856 (19). This plasmid conferred resistance to erythromycin (300 μg/ml) in *E. coli*. Plasmid pDH5110 was obtained from C. D. Tu (Pennsylvania State University) and is a pBR322 derivative containing a promoterless chloramphenicol acetyltransferase (CAT) gene inserted into the *Cla*I site of pBR322 (39) in the same orientation as the tetracycline resistance gene. This plasmid conferred resistance to ampicillin (50 μg/ml) in *E. coli*. An *E. coli* strain (HB101) containing both pVA1337 and pDH5110 was constructed by transformation (8). An overnight culture of this strain was diluted to 10^{-6} and then used to inoculate 20 separate broth cultures. After incubation, cultures were plated on LB agar containing 50 μg of ampicillin per ml and 100 to 200 μg of chloramphenicol per ml. After single-colony isolation, chloramphenicol-resistant clones were screened for erythromycin and tetracycline resistance and then used for preparation of plasmid DNA (10).

Enzymes and chemicals. Restriction endonucleases were purchased from Bethesda Research Laboratories, Inc., Gaithersburg, Md., Boehringer Mannheim Biochemicals, Indianapolis, Ind.; International Biotechnologies, Inc., New Haven, Conn.; and New England BioLabs and were used as recommended by the suppliers. Exonuclease BAL 31 was purchased from Boehringer Mannheim. *E. coli* DNA polymerase I large fragment, M13 oligonucleotide sequencing primer (17-mer), and T4 DNA ligase were purchased from New England BioLabs. Deoxy- and dideoxynucleotide triphosphates were obtained from Pharmacia Fine Chemicals, Piscataway, N.J. Radiolabeled nucleotides ([α-^{32}P]dATP, 800 Ci/mmol; [α-^{32}P]dCTP, 800 Ci/mmol) were purchased from New England Nuclear.

Computer analysis. DNA sequence data were analyzed by using the Intelligenetics, Inc. (Palo Alto, Calif.) IFIND program, the programs of Conrad and Mount (6), or the programs of Queen and Korn (26). In general, DNA sequences were compared by using a window size of 20 nucleotides and a word length of 4 or 5 nucleotides.

RESULTS

DNA sequence analysis of IS*4351*. We previously determined that the transcriptional control of the pBF4 MLS^r gene (*ermF*) is contained within an adjacent DR (27). We subsequently sequenced this copy of the DR as well as the single copy present on the pBF4 deletion derivative, pBF4Δ2. Computer analysis of the identical sequences along with demonstration of *recA*-independent transpositional recombination (see below) suggested that at least one copy of the DR of pBF4 is an active insertion element. We have designated this element IS*4351*R (Fig. 1). The left repeat, designated IS*4351*L, appears to be similar to IS*4351*R on the basis of restriction mapping data (35, 44) and partial sequence analysis (data not shown). It is not known whether these elements are completely homologous or whether IS*4351*L is a functional IS element.

Sequence analysis of IS*4351*R from pBF4 made use of the chimeric plasmid pVA831 [pBR325::*Eco*RI-D of pBF4 (22)]

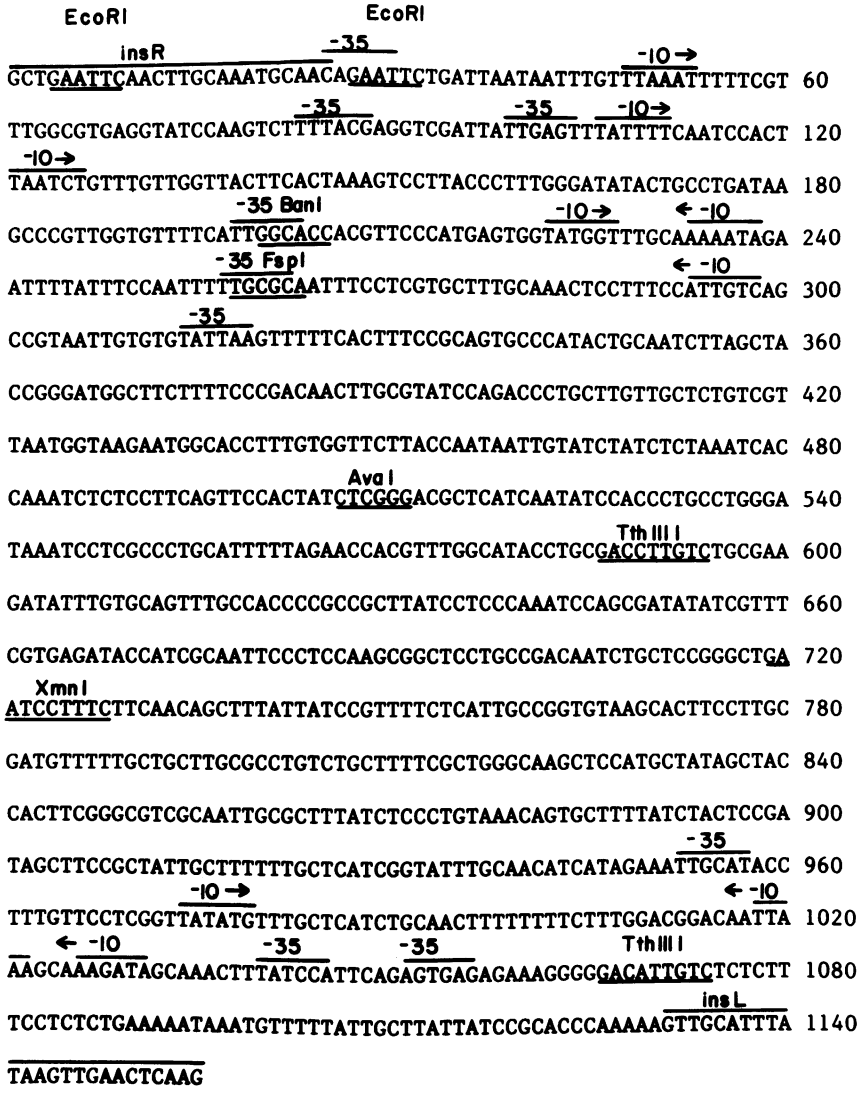

FIG. 3. Complete nucleotide sequence of the 1,155-bp insertion element IS*4351*. Sequence shown 5′ → 3′. Unique restriction endonuclease sites are underlined. Terminal inverted repeats (*insL* and *insR*) and putative RNA polymerase-binding sites (−10 and −35) are indicated over the sequence.

as well as an *Hpa*II fragment from pBF4 (Fig. 2, upper panel). A series of overlapping BAL 31-generated deletion derivatives then were used for DNA sequence analyses (Fig. 2, upper panel). Additionally, a portion of IS*4351* was analyzed by using a synthetic oligonucleotide as a primer (see asterisk in Fig. 2).

Partial sequence determination of IS*4351*L from pBF4Δ2 was accomplished by analysis of subcloned restriction fragments (Fig. 2, lower panel). Identification of the termini of IS*4351* was made on the basis of DNA sequence analysis of restriction fragments from a plasmid containing a transposed copy of IS*4351* (see below). These analyses showed that IS*4351* was a 1,155-base-pair (bp) element with a moles percent G+C content of 42.

Computer analysis of IS*4351*. The structural organization of IS*4351*, on the basis of computer analysis of the DNA sequence, is indicated in Fig. 3 and 4. IS*4351* was found to terminate in imperfect inverted repeats with a match of 20 of 25 bp. We have designated these sequences inverted sequence left (*insL*) and inverted sequence right (*insR*).

Several open reading frames were found within IS*4351* (Fig. 4). The largest open reading frame (ORF I) extended from nucleotide 988 to nucleotide 11. This 978-bp sequence could code for a 326-amino acid polypeptide of 39,120 daltons. The nonanucleotide sequence 5′-AAAAAAAGT-3′ situated just upstream from ORF I showed a 6- and 9-bp match with the 3′ terminus of *B. fragilis* 16S rRNA (43), suggestive of a ribosome-binding site (32).

Two smaller open reading frames (ORF II and ORF III, Fig. 4) were found on the opposite strand. ORF II extended from nucleotide 366 to nucleotide 662 and encoded a 99-amino acid polypeptide of 11,800 daltons. ORF III (nucleotides 782 to 1018) could encode a 79-amino-acid polypeptide of 9,480 daltons. Putative ribosome-binding sites upstream from ORF II (5′-AATCTAGCT-3′) and ORF III (5′-GTAAGCA-3′) matched the 3′ terminus of *B. fragilis* 16S rRNA at 5 of 9 bp and 5 of 7 bp, respectively.

The entire 1,155-bp sequence of IS*4351* also was analyzed by computer for hexanucleotide sequences that corresponded to −35 and −10 regions of known *E. coli* promoter

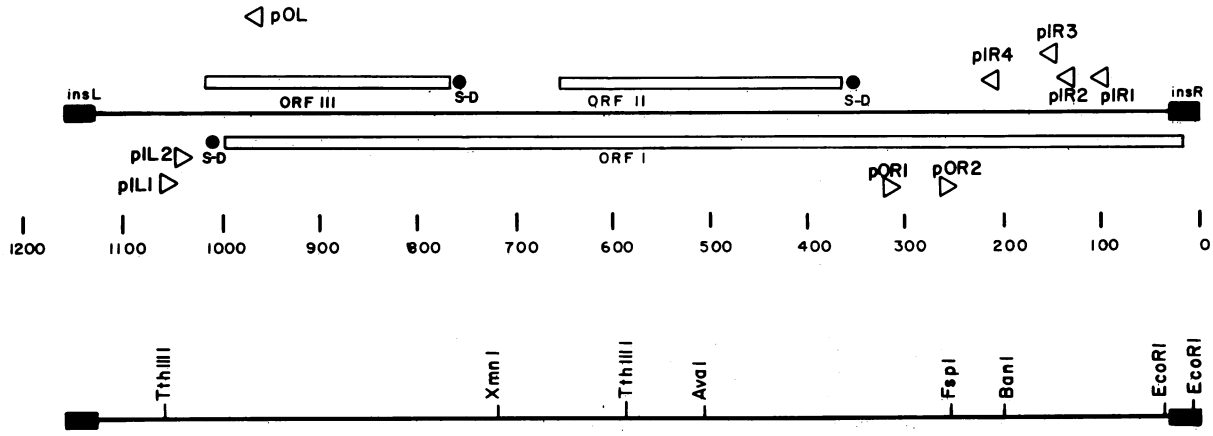

FIG. 4. Linear map of IS element IS*4351*. Potential open reading frames (ORF I, ORF II, and ORF III) and corresponding Shine-Dalgarno sequences (S-D) are indicated on the upper map. The nine possible RNA polymerase-binding sites (pOL, pIR1, pIR2, pIR3, pIR4, pIL1, pIL2, pOR1, and pOR2) are indicated by triangles pointing in the hypothetical direction of transcription. *insL* and *insR*, The 25-bp terminal inverted repeats. Unique restriction endonuclease sites are indicated on the lower map.

sequences (13). Such sequences that occurred within IS*4351* in the correct orientation (i.e., a −35 sequence upstream from a −10 sequence) and with a spacing of 17 ± 2 bp are identified in Fig. 3 and 4. Four inwardly directed promoter-like sequences (pIR1, pIR2, pIR3, pIR4) were located just upstream from ORF II, and two inwardly directed promoter-like sequences (pIL1 and pIL2) were located just upstream from ORF I. Putative promoter sequences that were directed toward each of the termini of the element (pOR1, pOR2, and pOL) were also identified.

The sequence of IS*4351* was compared with the sequences of transposable elements that were available on the Intelligenetics, Inc., data base. These were IS*1*, IS*2*, IS*4*, IS*5*, IS*10*R, IS*10*L, IS*0*-IS*1*, IS*102*, IS*H1*, IS*H2*, Tn*3*, Tn*5*, Tn*7*, and Tn*903*. Additional comparison to other IS DNA sequences ([IS*3*, [42]; IS*26*R, [24]; IS*30*, [7]) were accomplished with the computer program of Conrad and Mount (6). None of these IS elements showed significant nucleotide homology with IS*4351*.

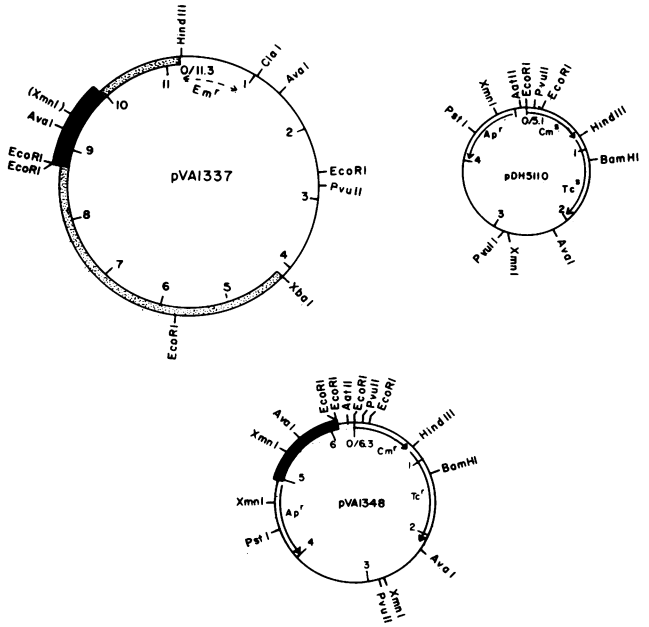

FIG. 5. Plasmids relevant to transpositional recombination assay. See the text for details. Restriction endonuclease maps of the delivery plasmid, pVA1337 (11.3 kb); the target plasmid, pDH5110 (5.1 kb) (42); and a recombinant plasmid, pVA1348 (6.3 kb). The dark rectangle represents IS*4351*. The stippled area in pVA1337 represents *Bacteroides* pBF4Δ2 DNA. Restriction endonuclease sites in parentheses indicate the presence of other unmapped sites.

Transposition of IS*4351*. Our general strategy for testing transposition promoted by IS*4351* made use of the plasmid pVA1337 (Fig. 5) as a delivery vehicle and the plasmid pDH5110 (Fig. 5) as a target (see Materials and Methods). We hypothesized that the putative promoters in IS*4351* could activate the CAT promoterless gene in pDH5110, resulting in a chloramphenicol resistance phenotype.

Twenty-eight Cm^r clones recovered in these experiments were screened for plasmids. On the basis of restriction endonuclease mapping, 13 of these clones appeared to contain IS elements other than IS*4351* inserted into pDH5110 (data not shown). However, two of the independently isolated Cm^r clones did contain IS*4351* inserted into

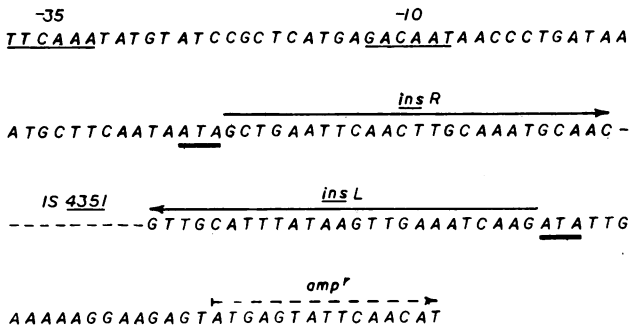

FIG. 6. Duplication of target sequences upon transposition of IS*4351* from pVA1348 into pDH5110. A partial sequence of pDH5110 (39) corresponding to the region containing IS*4351* is shown. The terminal inverted repeats (*insR* and *insL*) of IS*4351* are indicated by arrows. ----, Remainder of IS*4351*. The 3-bp duplication (ATA) is indicated by the thick underline. The promoter sequences (−35 and −10) and the start of the ampicillin resistance gene (amp^r) are also indicated.

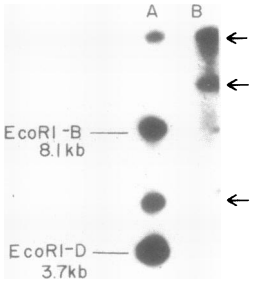

FIG. 7. Southern blot hybridization of *Eco*RI-digested bulk cellular DNA from *B. fragilis* strains V479-1 (MLR^r, containing pBF4) and V598 (an MLS^s, plasmidless derivative of V479-1) to an IS*4351* probe. The probe was pVA1348 containing one copy of IS*4351* (Fig. 5). Lanes: A, *Eco*RI-digested cellular DNA from V479-1; B, *Eco*RI-digested cellular DNA from V598. The pBF4 *Eco*RI B and D hybridizing fragments, both carrying IS*4351* sequences (Fig. 1) are noted at the left. The nonplasmid IS*4351*-hybridizing sequences found in V479-1 and V598 are indicated by arrows.

pDH5110. One of these clones was chosen for further studies and contained a recombinant plasmid, pVA1348 (Fig. 5), with one copy of IS*4351* inserted upstream from the chloramphenicol gene. This plasmid conferred resistance to ampicillin, tetracycline, and chloramphenicol in *E. coli* and was characterized by restriction endonuclease mapping and Southern blot hybridization (data not shown). Plasmid pVA1348 was also used for examination of the target site of IS*4351* (see below).

Analysis of target site sequences. pVA1348 was analyzed to determine whether duplication of target sequences of pDH5110 had occurred upon insertion of IS*4351*. *Taq*I-generated fragments of pVA1348 were cloned into M13 vectors and used for DNA sequence analysis. Comparison of sequence data from fragments containing either the left or right terminus of IS*4351* (and adjacent pDH5110 sequences) to the pBR322 sequence (39) showed that a 3-bp duplication (5′-ATA-3′) of target sequences had occurred (Fig. 6). IS*4351* inserted into pDH5110 just upstream from the ampicillin resistance gene and was found to be situated between the start codon of the ampicillin resistance gene and its promoter (39).

Demonstration of the presence of IS*4351*-related sequences in the chromosome of *B. fragilis*. To determine whether IS*4351* was found in the *Bacteroides* chromosome, plasmid pVA1348 (Fig. 5) containing one copy of IS*4351*R was used to probe bulk cellular DNA from *B. fragilis* V479-1 MLS^r containing pBF4 and *B. fragilis* V598, a spontaneous MLS^s variant of strain V479-1 lacking plasmid pBF4 (22). Southern blot analyses of *Eco*RI-cleaved bulk cellular DNA from each strain probed with pVA1348 are shown in Fig. 7. As expected, hybridization to the *Eco*RI B and *Eco*RI D IS*4351*-containing fragments of pBF4 was detected (lane A). However, two other *Eco*RI fragments (Fig. 7) that did not correspond to pBF4-generated *Eco*RI fragments showed homology to the IS*4351* probe. Two *Eco*RI fragments that hybridized to the IS*4351* probe were also detected in DNA from strain V598 (lane B). Only one of these fragments appeared to be similar in size to the nonplasmid fragments of V479-1.

DISCUSSION

Transposition in *E. coli* promoted by one copy of the DR sequence of pBF4 has allowed us to identify this sequence as an IS element, which we have designated IS*4351*. We infer that IS*4351* is able to mediate transposition in *Bacteroides* spp., on the basis of previously published results (28, 33, 34). IS*4351* showed a structural arrangement similar to other known IS elements, including the presence of terminal inverted repeated sequences with a match of 20 of 25 base pairs (9, 18).

Although the structural organization of IS*4351* is similar to other IS elements, computer analyses of this 1,155-bp sequence failed to reveal any significant nucleotide homology to 13 other IS elements, suggesting that this is a new element. We believe that IS*4351* is of *Bacteroides* origin, inasmuch as the moles percent G+C content of IS*4351* (42 mol%) is similar to that of chromosomal DNA of *Bacteroides* spp. (4) and copies of this element have been found in the chromosome of at least one *B. fragilis* strain (Fig. 7). Moreover, Southern blot analyses have demonstrated homology between IS*4351* and the DR sequences of two other independently isolated *Bacteroides* plasmids, pBFTM10 (31), similar if not identical to pCP-1 (12), and pBI136 (35), that also confer MLS resistance, suggesting widespread dissemination of this element on plasmids. IS*4351* has not yet been identified in independent MLS^s isolates of *Bacteroides* spp.

Although sequence anlayses of the DR elements of pBFTM10 and pBI136 are not yet available, it seems reasonable to assume that these repeats are also IS elements. Transposition by the DR elements of pBFTM10 (tentatively designated IS*4400*) has been observed in both *E. coli* and *Bacteroides* sp. (28). Moreover, the DR sequences and the intervening DNA of pBFTM10 (28) and pBI136 (Smith, submitted) both undergo transposition. The data reported in this paper support and extend the work of Shoemaker et al. (33, 34), who showed that Tn*4351* (Fig. 1) can transpose in both *E. coli* and *Bacteroides* sp. Our data suggest that in *E. coli* transposition of Tn*4351* might be mediated by IS*4351*.

IS elements were first identified in *E. coli* on the basis of polar mutations created by insertion into operons (15, 20). Some of these elements have also been shown to activate nearby genes by means of outwardly directed promoter sequences (1, 5, 16, 29, 42). A similar role has been observed with IS*4351*. In pBF4, the *ermF* gene is transcribed from a promoter within the adjacent IS*4351* sequence (27). Further evidence for the presence of functional outwardly directed promoter sequences within IS*4351* resulted from the transposition assay used in this study. The target plasmid, pDH5110, possessed promoterless chloramphenicol and tetracycline genes. Insertion of IS*4351* such that pOR1 and pOR2 (Fig. 4) were oriented toward the promoterless antibiotic resistance genes in pVA1348 (Fig. 5) resulted in a Tc^r Cm^r phenotype. We do not know whether the same promoters that function in *Bacteroides* spp. also operate in *E. coli*.

Sequence analyses of the IS*4351*-pDH5110 junctions in pVA1348 suggested that IS*4351* could also be controlling the ampicillin resistance gene; this IS element inserted between the start codon and the promoter of the ampicillin resistance gene (Fig. 6). Unfortunately, the actual promoter sites within IS*4351* that were driving transcription of the ampicillin, chloramphenicol, and tetracycline resistance genes in pVA1348 could not be identified from these data. High-resolution S1 mapping experiments are needed to clarify this issue.

DNA sequence analysis of the pDH5110-IS*4351* junctions in pVA1348 further showed that a 3-bp target sequence (5′-ATA-3′) was duplicated upon insertion of IS*4351*. The *ermF* transposon of pBI136 has also been shown to generate

a 3-bp duplication (Smith, submitted). On a similar note, IS*3* has also been shown to produce a 3-bp duplication upon transposition to a new site (37, 42).

The role of the putative proteins (ORF I, II, and III; Fig. 4) of IS*4351* has yet to be determined. These peptides have a high percentage of basic amino acid residues (data not shown) which could facilitate interactions with DNA. It seems reasonable that one or more of the ORFs could code for a transposase, a repressor, or an inhibitor such as is found with other IS elements (for a review, see reference 9).

The activation of foreign genes by IS elements has been documented in *E. coli* (1, 16, 42), and we have hypothesized that this was the basis for the IS*4351-ermF* association in the *B. fragilis* plasmid pBF4 (27). The presence of IS*4351*-like sequences in the chromosome of V479-1 (Fig. 7) further supports our model for the evolution of MLS^r in *B. fragilis*. It will be interesting to examine the dissemination of this element in *Bacteroides* sp. and to see whether IS*4351* is associated with other genes. Moreover, it seems likely that IS*4351* could be used in the future for expression of genes cloned into *Bacteroides* spp. as well as a tool for identifying silent genes and for introducing mutations in *Bacteroides* spp.

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