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Insertion and Excision of *Bacteroides* Conjugative Chromosomal Elements

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Many strains of *Bacteroides* harbor large chromosomal elements that can transfer themselves from the chromosome of the donor to the chromosome of the recipient. Most of them carry a tetracycline resistance (Tc^r) gene and have thus been designated Tc^r elements. In the present study, we have used transverse alternating field electrophoresis to show that all but one of the Tc^r elements screened were approximately 70 to 80 kbp in size. The exception (Tc^r Em^r 12256) was 150 to 200 kbp in size and may be a hybrid element. All of the Tc^r elements inserted in more than one site, but insertion was not random. The Tc^r elements sometimes cotransfer unlinked chromosomal segments, or nonreplicating *Bacteroides* units (NBUs). Transverse alternating field electrophoresis analysis showed that insertion of NBUs was not random and that the NBUs did not insert near the Tc^r element. Although attempts to clone one or both ends of a Tc^r element have not been successful, ends of a cryptic element (XBU4422) were cloned previously and shown to be homologous to the ends of Tc^r elements. We have obtained DNA sequences of junction regions between XBU4422 and its target from several different insertions. Comparison of junction sequences with target sequences showed that no target site duplication occurred during insertion and that XBU4422 carried 4 to 5 bp of adjacent chromosomal DNA when it excised from the chromosome and inserted in a plasmid. We identified a short region of sequence similarity between one of the ends of XBU4422 and its target site that may be important for insertion. This sequence contained an 8-bp segment that was identical to the recombinational hot spot sequence on Tn*21*. XBU4422 could excise itself from plasmids into which it inserted. In most cases, the excision left a single additional A behind in the target site, but precise excision was seen in one case.

A family of novel self-transmissible elements has been found in *Bacteroides*, a genus of gram-negative obligate anaerobes. These elements are inserted in the *Bacteroides* chromosome and transfer themselves from the chromosome of the donor to the chromosome of the recipient (12). Most of these *Bacteroides* chromosomal elements carry a tetracycline resistance (Tc^r) gene and are thus designated Tc^r elements. Some elements also carry an erythromycin resistance (Em^r) gene. Recently, a set of overlapping cosmid clones that covered most of the interior of one of these elements, Tc^r Em^r DOT, was obtained (12). Southern hybridization experiments with these clones as probes demonstrated that the Tc^r elements in different *Bacteroides* strains were closely related but not identical. Accordingly, for purposes of identification, each element has been designated by the resistances it carries and the name of the *Bacteroides* strain in which it was originally found (Table 1). Not all members of this family of conjugal elements carry antibiotic resistance genes. A cryptic self-transmissible chromosomal element (XBU4422) was found recently in *Bacteroides uniformis* 0061 (15). XBU4422 contained DNA that hybridized to the Tc^r Em^r DOT cosmids outside the Tc^r and Em^r regions. In particular, there was appreciable homology between the ends of XBU4422 and the ends of the Tc^r elements (15).

Although the *Bacteroides* Tc^r elements have been called conjugative transposons, it was not known whether they inserted randomly in the chromosome or duplicated the target site when they inserted. In this study, we have used transverse alternating field electrophoresis (TAFE) to assess the number of insertion sites in the *Bacteroides thetaiotaomicron* chromosome and to estimate the sizes of the Tc^r elements. To determine whether insertion of the elements was accompanied by target site duplication, we needed to obtain the DNA sequences of the ends of an element and its target site. Previous attempts to clone the ends of the Tc^r elements or to trap the elements on plasmids were unsuccessful. However, some insertions of XBU4422 into plasmids had been isolated (15). Since the ends of XBU4422 appeared to share a high degree of homology with the ends of the Tc^r elements, it seemed likely that the characteristics of the XBU4422 insertion site might be similar to characteristics of the Tc^r element insertion sites. In this paper, we report the DNA sequences of the ends of XBU4422 and four of its target sites. Also, we show that XBU4422 is capable of nearly precise excision.

In addition to transferring themselves, Tc^r elements cotransfer 10- to 11-kbp segments called nonreplicating *Bacteroides* units (NBUs). NBUs are normally located in the chromosome and are excised from the chromosome by the Tc^r elements (14, 19). Recent experiments (16) have shown that the NBUs not only are excised by the Tc^r elements but are also transferred by them and integrate into the chromosome of the recipient. Accordingly, in this study we have also used TAFE to characterize NBU insertions into the *B. thetaiotaomicron* chromosome.

MATERIALS AND METHODS

Strains and growth conditions. The *Bacteroides* and *Escherichia coli* strains used in this study are listed in Table 1. *Bacteroides* strains were grown in Trypticase (BBL Microbiology Systems, Cockeysville, Md.)-yeast extract-glucose

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TABLE 1. Strains and plasmids used in this study

Strain, plasmid, or conjugal element	Relevant phenotype(s)[a]	Description, reference, and/or source
Plasmids		
pEG920	(Ap^r Tc^r) Em^r	Shuttle vector containing pUC19, pB8-51, and the 4.0-kbp *Eco*RI fragment from Tn*4400* (13)
pEG920::XBU4422	(Ap^r Tc^r) Em^r Tra$^+$	pEG920 containing an insertion of the cryptic element, XBU4422 (15)
pE5-2	(Su^r Tc^r) Em^r	Shuttle vector containing RSF1010, pB8-51, and the 3.8-kbp *Eco*RI fragment from Tn*4351* (13)
pE5-2::XBU4422	(Su^r Tc^r) Em^r Tra$^+$	pE5-2 containing an insertion of the element XBU4422 (15)
pNIL51	(Ap^r Su^r Tc^r) Em^r	Shuttle vector pE5-2 with pBR322 derivative, pDG5 added (15)
pNIL51::XBU4422	(Ap^r Su^r Tc^r) Em^r Tra$^+$	pNIL51 containing an insertion of the cryptic element, XBU4422 (15)
NBU1, NBU2		Integrated 10- to 11-kbp segments that can be excised and mobilized by the *Bacteroides* Tc^r elements (14)
NBU3		Integrated DNA segment in *B. fragilis* 12256 that hybridizes with NBU1 and NBU2, size unknown (8)
R751-INS1		IncP plasmid R751 with an insertion of NBU1 and NBU2 (14), hybridizes with NBU3
E. coli strains		
E. coli HB101	(RecA$^-$ Str^r)	1
E. coli SF8	(RecA$^+$ Str^r)	W. Reznikoff
Bacteroides conjugal elements		
XBU4422	Tra$^+$	Cryptic conjugal element originally found in *B. uniformis* 1001 (15)
Tc^r V479	Tc^r Tra$^+$	Element originally found in *B. fragilis* V479 (20)
Tc^r Em^r CEST	Tc^r Em^r Tra$^+$	Element originally found in *B. fragilis* CEST (20)
Tc^r ERL	Tc^r Tra$^+$	Element originally found in *B. fragilis* ERL (20)
Tc^r Em^r ERL	Tc^r Em^r Tra$^+$	Element originally found in *B. fragilis* ERL (20)
Tc^r Em^r DOT	Tc^r Em^r Tra$^+$	Element originally found in *B. thetaiotaomicron* DOT (20)
Tc^r Em^r 12256	Tc^r Em^r Tra$^+$	Element originally found in *B. fragilis* 12256 (20)
B. thetaiotaomicron BT 4001	Rif^r	Rifampin-resistant derivative of *B. thetaiotaomicron* 5482

[a] Phenotypes in parentheses are expressed only in *E. coli* and not in *Bacteroides* spp. All phenotypes not in parentheses are expressed in *Bacteroides* species. Abbreviations: Ap, ampicillin; Su, sulfonamide; Tc, tetracycline; Str, streptomcyin; Em, erythromycin; Rif, rifampin; Tra, ability to self-transfer.

broth or on Trypticase-yeast extract-glucose agar medium (3). *E. coli* strains were grown in Luria broth or on Luria broth agar plates.

Matings. The Tc^r elements used in this study are listed in Table 1. *B. thetaiotaomicron* 4001 was chosen as the recipient in these matings because it does not contain any NBUs or Tc^r elements. Moreover, its DNA does not cross-hybridize with XBU4422 or any of the probes used in this study. The *Bacteroides*-to-*Bacteroides* mating procedure has been described previously (13, 20). Recipients containing NBU insertions were identified by colony hybridization with [^{32}P]R751-INS1 (14) as the probe. R751-INS1 carries all of NBU1 and NBU2. This probe also hybridizes with NBU3, a newly detected NBU (16), and could thus be used to detect all three NBUs. In some experiments, different transconjugants from the same mating were compared. In other experiments, to ensure that the transconjugants represented independent insertion events, at least 10 independent matings were done.

XBU4422 excision events were obtained by conjugal transfer of the XBU4422 plasmid chimeras from *B. uniformis* 1001 to *E. coli* SF8. Previously, we had found that in such matings XBU4422 was lost in all cases and only the plasmid into which XBU4422 had inserted was recovered (15). Matings were done with insertions of XBU4422 into one of three different plasmids, pEG920::XBU4422, pE5-2::XBU4422, or pNIL51::XBU4422. The procedure for *Bacteroides-E. coli* matings has been described previously (13, 20).

Preparation and digestion of chromosomal DNA for TAFE. Cultures were grown in 10 ml of Trypticase-yeast-extract-glucose broth to a final concentration of about 4×10^8/ml (optical density at 650 nm = 0.4). Bacteria were harvested by centrifugation and resuspended in 0.8 ml of distilled water. Anaerobic conditions were maintained up to this point. An equal volume of molten 1% SeaPlaque low-melting-point agarose (FMC BioProducts, Rockland, Maine) equilibrated at 37°C was added to the cell suspension, and the mixture was immediately dispensed into a mold to form 0.1-ml inserts (2 by 5 by 10 mm). Steps after this point followed the procedure of Smith et al. (17). In initial experiments, *Not*I, *Sfi*I, *Pac*I, *Sgr*A1, and *Asc*I were tested, but *Not*I was chosen because it produced a number of well-spaced bands (see Fig. 1). *Sfi*I gave partial digests, and the other enzymes produced numerous small fragments. Approximately 10 U of enzyme was used in each reaction, and buffers were those recommended by the manufacturer.

Electrophoresis and Southern blot analysis. Plugs were placed into the wells of a SeaKem LE (FMC BioProducts) agarose gel and electrophoresed in the GeneLine TAFE system (Beckman Instruments, Inc., Fullerton, Calif.). Two types of agarose gel were used. Standard 1% agarose gels were electrophoresed for 30 min at 170 V with a 4-s switch time and then for 18 h at 150 V with a 90-s switch time. Step gradient gels consisted of 4.2 cm of 0.8% agarose in the upper portion and 5.0 cm of 1.1% agarose in the lower portion. These gels were electrophoresed for 30 min at 170 V with a 4-s switch time and then for 18 h at 150 V with a 75-s switch time. Gels were stained with ethidium bromide and

photographed under short-wavelength UV light. For Southern blots, chromosomal DNA was obtained as described by Saito and Miura (10). DNA was transferred to Optibind or BAS-NC membranes (Schleicher & Schuell, Inc., Balley Park, Mo.) by capillary action (5). Probes were labelled with [^{32}P]CTP by using a nick translation system (Life Technologies Inc., Gaithersburg, Md.). Hybridization and washes were performed as described previously (5, 12).

DNA sequence analysis. Since pEG920::XBU4422, pE5-2::XBU4422, and pNIL51::XBU4422 were not stable in *E. coli*, junction fragments were sequenced from XBU plasmid DNA, which had been isolated from *B. uniformis* 1001. The region into which XBU4422 inserted in pEG920, upstream of the *tetX* gene, has recently been sequenced (16). DNA primers used for sequencing were constructed on the basis of this DNA sequence information. The primers were 5'GAT AGAAGTCCAAGGTCTGCTG3' and 5'ACTTTCCTGTC TTACG3'. These primers were also used to sequence the junctions of the XBU4422 insertion into pE5-2. Once the DNA sequences at the ends of XBU4422 had been obtained, primers were constructed for sequencing the insertion in pNIL51. The primers were 5'CAAGTAACGGAGCTCCGT CTTGATTTGGC3' for the right end of XBU4422 and 5'GCAGTAGATAACTAACGAATTCGTAACC3' for the left end. DNA sequencing was done with the Sequenase 2.0 kit (U.S. Biochemicals) according to the manufacturer's directions.

The junctions between the ends of XBU4422 and the *B. uniformis* 1001 chromosome were obtained by cloning junction region DNA which had been amplified by anchored polymerase chain reaction. The amplification procedure was a modification of the procedure of Roux and Dhanarajan (9). *Hae*II-digested *B. uniformis* 1001 DNA (1 ng) was mixed with *Hae*II anchor primer (2 μg) and a primer internal to one of the ends of XBU4422 (100 to 200 ng) and amplified with *Taq* polymerase. The DNA primers for the XBU4422 ends were constructed on the basis of DNA sequence information from the sequencing of pEG920::XBU4422 junctions. The two primers were the same as those used to obtain the DNA sequence of the XBU4422-plasmid junctions in pNIL51. The *Hae*II anchor primer sequence was 5'GATACTCGAGCTC AAGCTTGAATTCGCGC3'. Amplification conditions consisted of an initial cycle of 94°C for 1 min, annealing at 37°C for 1 min, and polymerization at 72°C for 2 min and then 30 cycles of denaturation (94°C for 1 min), annealing (55°C for 1 min), and polymerization (75°C for 2 min). Amplified DNA was purified from a SeaPlaque GTG agarose (FMC BioProducts) gel and then digested with either *Eco*RI (left end of XBU4422) or *Sst*I (right end of XBU4422) and cloned into the multiple cloning site of pUC19. More than one amplified product was obtained in each case. Several different clones were sequenced to find the correct clone, which was identified by its identity to the known XBU4422 end sequence. Three different clones of the right-end junction and three different clones of the left-end junction were sequenced.

RESULTS

Sizes of the Tc^r elements. We estimated the sizes of the different Tc^r elements by comparing the *Not*I digest patterns of *B. thetaiotaomicron* 4001 transconjugants containing these Tc^r elements with the *Not*I digest pattern of *B. thetaiotaomicron* 4001 containing no Tc^r element. Fragments containing Tc^r elements were identified initially by their altered mobility on the stained TAFE gel. A typical stained gel is shown in Fig. 1. This identification was

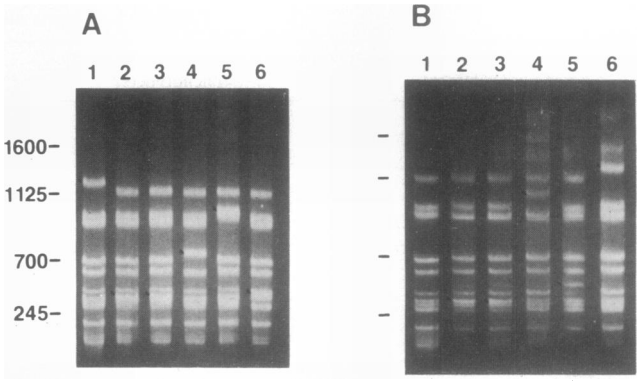

FIG. 1. Stained TAFE gels of *Not*I-digested chromosomal DNA from *B. thetaiotaomicron* transconjugants. (A) Lanes 1 through 6, DNA from transconjugants that had an insertion of the Tc^r Em^r DOT element. (B) Lane 1, wild-type *B. thetaiotaomicron* DNA (no Tc^r element insertions); lanes 2 through 6, DNA from transconjugants that had insertions of both Tc^r Em^r DOT and NBU2. The sizes of DNA size standards are given in kilobase pairs at the left sides of the gels.

confirmed by Southern hybridization with [^{32}P]pEG920::XBU4422 as a probe. A typical Southern blot is shown in Fig. 2A and B. Most of the Tc^r elements were 70 to 80 kbp in size, except Tc^r Em^r 12256, which was 150 to 200 kbp in size (Fig. 3).

Distribution of Tc^r element insertions. The numbers of insertions in each *Not*I fragment are summarized in Table 2. All of the elements tested inserted into more than one *Not*I fragment. In our initial experiments, we analyzed different transconjugants produced in one or two separate matings. To determine whether these insertions represented the full range of possible insertions, we analyzed transconjugants from 12 independent matings in which *Bacteroides fragilis* 12256 (Tc^r Em^r 12256) was the donor and from 11 independent matings in which *B. thetaiotaomicron* DOT (Tc^r Em^r DOT) was the donor. From this, we concluded that these two elements had at least one insertion site in almost every *Not*I fragment (Table 2). Most of the Tc^r elements cotransferred NBUs in some fraction of the matings (16). In Table 2, matings in which NBUs were cotransferred are listed separately from matings in which the NBU was not cotransferred. There was no evidence that cotransfer of an NBU affected insertion specificity.

The results in Table 2 show that the Tc^r elements inserted into more than one site in the chromosome. To determine whether insertions occurred randomly, we hybridized ^{32}P-labeled pEG920::XBU4422 with Southern blots of *Hin*dIII-digested chromosomal DNA. This probe detects the ends of the Tc^r elements (15). Insertions of the same Tc^r element in the same site in the same orientation should have the same restriction pattern. In all but one case, insertions of the same Tc^r element in the same *Not*I band had the same restriction pattern (data not shown). The one exception was an insertion of the Tc^r Em^r CEST element in the 310-kbp *Not*I band, which had a restriction pattern different from those of the other two Tc^r Em^r CEST insertions in the same *Not*I fragment. Thus, the Tc^r elements appear not to insert randomly in the chromosome. The fact that multiple insertions were obtained in the same site, even in the cases in which many independent matings were done, indicates that enough transconjugants were screened to provide an accurate picture of insertion specificity.

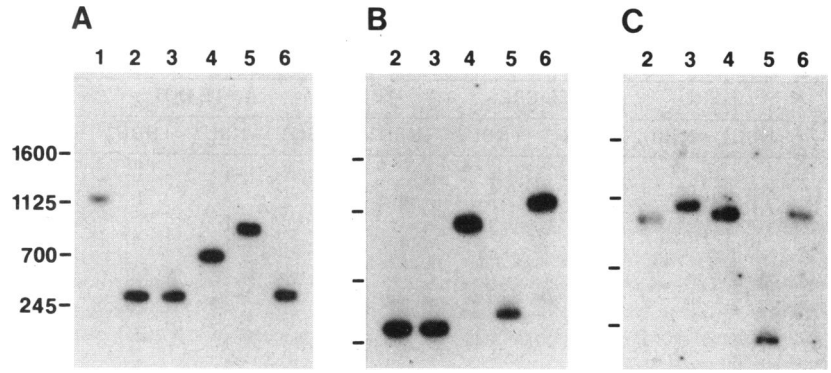

FIG. 2. Southern blots of the TAFE gels shown in Fig. 1. (A) Southern blot of the gel in Fig. 1A that has been hybridized with [^{32}P]pEG920::XBU4422, a probe that hybridizes with the ends of the Tc^r Em^r DOT element; (B) Southern blot of the gel in Fig. 1B that has been hybridized with [^{32}P]pEG920::XBU4422 to detect the Tc^r Em^r DOT insertions in matings in which both Tc^r Em^r DOT and NBU2 transferred; (C) Southern blot of the gel in Fig. 1B hybridized with [^{32}P]R751-INS1, a probe that hybridizes with NBU2 but not with the Tc^r Em^r DOT element. The sizes of DNA size standards are given in kilobase pairs at the left sides of the blots.

In general, insertions of the same Tc^r element in different *Not*I sites had different restriction patterns (Fig. 4). An unexpected finding was that this was not true of the Tc^r Em^r 12256 element. Insertions of this element in different *Not*I fragments all had exactly the same banding pattern on Southern blots (data not shown). This indicated that the regions on Tc^r Em^r 12256 which hybridized with the ends of XBU4422 were interior to the element and not located at its ends, in contrast to the other Tc^r elements.

Distribution of NBU2 and NBU3 insertions. The NBUs, like the Tc^r and Tc^r Em^r elements, appeared to have a limited number of insertion sites (Fig. 2C and Table 3). NBU2 appeared to have four insertion sites in the *B. thetaiotaomicron* chromosome (Fig. 2C and Table 3). With one exception, all NBU2 insertions in the same *Not*I had the same *Hin*dIII banding pattern when probed with the NBU probe [^{32}P]R751-INS1, i.e., they were in the same site in the same orientation. NBU3, a newly discovered NBU that is cotransferred with the Tc^r Em^r 12256 element inserted only in one *Not*I band. NBU3 was identified as DNA that cross-hybridized with R751-INS1, which contains both NBU1 and NBU2, but NBU3 itself has not been isolated. Since it is not clear whether the R751-INS1 probe hybridizes to the ends of NBU3 or only to an interior segment, it was not possible to ascertain whether all of the NBU3 insertions in the 960-kbp *Not*I band were in the same site.

Sequence analysis of XBU4422 insertions. Previously, we had isolated many insertions of XBU4422 into pEG920 (15) but only one insertion each in two other plasmids, pE5-2 and pNIL51. We sequenced junction regions of five insertions of XBU4422 in pEG920, each of which was obtained from a different mating. We also sequenced junction regions of the single insertion in pE5-2 and the single insertion in pNIL51. A comparison of the ends of XBU4422 and the different target sites is given in Fig. 5. All of the insertions into pEG920 and the single insertion in pE5-2 occurred at the same site, which lies 650 bp upstream of the start codon of *tetX*. The *tetX* upstream region on pEG920 was cloned from Tn*4400*, whereas the *tetX* upstream region on pE5-2 was cloned from the closely related transposon Tn*4351*. The entire *tetX* upstream region (over 1 kb) is identical on the two transposons, except for 4 bp (18). The XBU4422 insertions occurred at one edge of this 4-bp region.

An alignment of the target site on pEG920 with the ends of XBU4422 revealed 24 identical bases, 14 of which were grouped close together on the left side of the insertion point (Fig. 5). Not all of the insertions in pEG920 were identical. One insertion (Ω7) had the 4-bp sequence CCCG immedi-

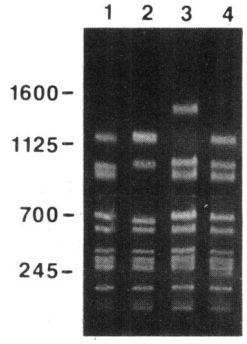

FIG. 3. Ethidium bromide-stained TAFE gels of *Not*I-digested DNA from *B. thetaiotaomicron* transconjugants that had insertions of the Tc^r Em^r 12256 element. Lane 1, DNA from wild type (no insertions); lanes 2 through 4, DNA from transconjugants carrying the Tc^r Em^r 12256 element. The sizes of DNA size standards are given in kilobase pairs at the left side of the gel.

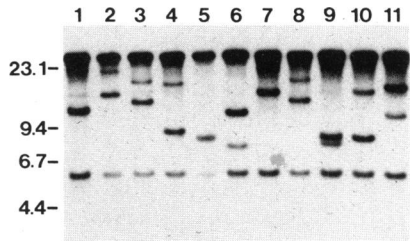

FIG. 4. Southern blot of *Hin*dIII-digested chromosomal DNA from *B. thetaiotaomicron* transconjugants containing Tc^r Em^r DOT insertions. Lanes 1, 4, 5, and 6, DNA from Tc^r Em^r DOT insertions in different *Not*I bands. Lanes 2 and 7 and lanes 3 and 8, DNA from insertions in the same *Not*I band. The blot was hybridized with [^{32}P]pEG920::XBU4422. This probe hybridizes with two internal *Hin*dIII fragments of Tc^r Em^r DOT (6 and >23 kb), as well as with the ends of the element (variable bands). The junction bands are identical in lanes 2 and 7 and in lanes 3 and 8. The sizes of DNA size standards in kilobase pairs are indicated at the left of the blot.

TABLE 2. Distribution of insertions of different Tc^r and Tc^r Em^r elements in the *B. thetaiotaomicron* chromosome

*Not*I fragment size (kbp)[a]	No. of insertions in *B. thetaiotaomicron* chromosome[b]													
	T-V479	TE-CEST	T-ERL		TE-ERL		TE-DOT		TE-DOT[c]		TE-12256		TE-12256[c]	
			−NBU2	+NBU2	−NBU2	+NBU2	−NBU2	+NBU2	−NBU2	+NBU2	−NBU3	+NBU3	−NBU3	+NBU3
1,100									1	1	2			2
960	1		1			3	4	5		1	1			
900	2					1			1		1	1		1
670					1		1		1				1	1
550		2											4	
380						1				1				
340													1	
310		2, 1[d]	3						1					
280[e]									2	2				
200	1		1	6	4									
<200	1		1											2
<200														

[a] Sizes of *Not*I fragments in the wild-type BT4001 chromosome.
[b] The number of insertions in each *Not*I fragment is given. A blank indicates that there were no insertions in that fragment. Abbreviations: T-V479, Tc^r V479; T-ERL, Tc^r ERL; TE-ERL, Tc^r Em^r ERL; TE-DOT, Tc^r Em^r DOT; TE-12256, Tc^r Em^r 12256; −NBU, no NBU cotransferred; +NBU, NBU cotransferred. See Table 3 for locations of NBU insertions. Unless otherwise indicated, all insertions of a particular element in the same *Not*I band are in the same site.
[c] Results of screening 11 to 12 insertions, each from different matings, as opposed to multiple insertions from one to two matings.
[d] Insertions in different sites within the same *Not*I fragment: two in one site and one in a second site.
[e] Doublet. Two of the insertions in this doublet are in one site and two are in a second site.

ately to the left of the insertion point rather than the 5-bp sequence, GAAAA, which was seen in all other insertions. In the case of pNIL51, less identity was seen than with the other insertions.

DNA sequence of the chromosomal insertion of XBU4422 is also shown in Fig. 5. In this case, we do not have an independent sequence of the target region. An alignment of the presumed chromosomal target site with the ends of XBU4422 revealed that 12 bp in the 14-bp region identified to the left of the insertion site in pEG920 were conserved. The sequence of the junctions between the ends of XBU4422 and the *B. uniformis* chromosome suggested an explanation for our finding that one of the junction sequences of the pEG920 insertions had CCCG next to the insertion point rather than GAAAA. The chromosomal insertion of XBU4422 had CCCG at one end and GAAAA at the other. A more extended sequence of the two ends of XBU4422 is shown in Fig. 6. No open reading frames larger than 100 bp were found. Comparison of the two ends revealed a 23-bp imperfect indirect repeat.

TABLE 3. Distribution of NBU insertions in the *B. thetaiotaomicron* chromosome

*Not*I fragment size (kbp)[a]	No. of insertions in chromosome[b]					
	T-ERL	TE-ERL	TE-DOT	TE-DOT[c]	TE-12256	TE-12256[c]
1,100						
960		4, 1[d]	4	1	1	6
900	6			3		
670						
550						
380						
340						
310						
280						
200						
<200			1	1		
<200						

[a] Sizes of *Not*I fragments in the wild-type BT4001 chromosome.
[b] The number of insertions in each *Not*I fragment is given. A blank indicates that there were no insertions in that fragment. Abbreviations: T-ERL, Tc^r ERL; TE-ERL, Tc^r Em^r ERL; TE-DOT, Tc^r Em^r DOT; TE-12256, Tc^r Em^r 12256. Unless otherwise indicated, all insertions of a particular NBU in the same *Not*I band are in the same site.
[c] Results of screening insertions from independent matings, as opposed to multiple insertions from one to two matings.
[d] Insertions in different sites within the same *Not*I fragment: four in one site and one in a second site.

Sequence analysis of XBU4422 excision events. To determine whether XBU4422 excision was precise, we sequenced the region around the XBU4422 insertion site in at least one excision event from each of five independently obtained pEG920::XBU4422 chimeras, which had GAAAA at the insertion point. We also sequenced three independently obtained excisions from the one example of pE5-2::XBU4422. This insertion also had GAAAA at the insertion site. In all cases, excision of XBU4422 left an additional A behind at the site where the XBU4422 had originally inserted. In one pEG920::XBU4422, CCCG rather than GAAAA was at the end of the pEG920::XBU4422 insertion (Ω7). We sequenced three independently obtained excision events from this chimera. In all three cases, the excision was precise. Despite numerous attempts to obtain an excision of XBU4422 from pNIL51::XBU4422 by mating this chimera into *E. coli* SF8, no transconjugants were ever obtained. Thus, XBU4422 appeared not to be able to excise itself from its insertion site on pNIL51.

DISCUSSION

Except for Tc^r Em^r 12256, all of the Tc^r elements examined in this study appeared to be approximately 70 to 80 kbp in size. This size is similar to the size of the cryptic element, XBU4422, which is approximately 60 kbp (14). Previous comparisons of Tc^r Em^r 12256 with the other Tc^r Em^r elements by using Southern analysis had indicated that the elements were all very similar in the regions around the Tc^r and Em^r genes and in other internal portions of the elements (10a, 12, 19). In the present study, we found that the regions of Tc^r Em^r 12256 which hybridized to the ends of XBU4422

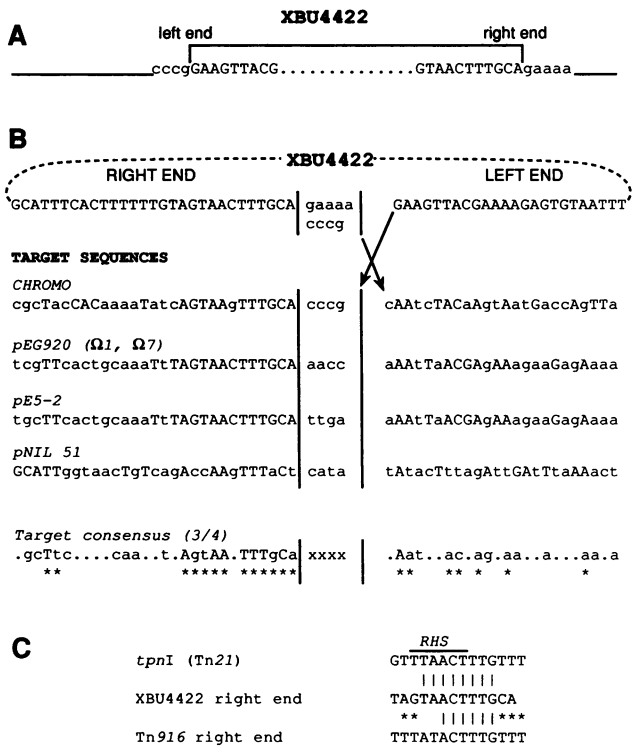

FIG. 5. Comparison of the DNA sequences of the ends of XBU4422 and the different targets into which XBU4422 inserted. (A) Ends of XBU4422 in the *B. uniformis* 1001 chromosome. XBU4422 DNA is indicated by capital letters. This chromosomal insertion was the source of the XBU4422 that inserted in the plasmid targets. (B) XBU4422 drawn as a circle to show the alignment between its right end and the target sequences. We do not know whether XBU4422 forms a circle, as do Tn*916* and Tn*1545* when they transfer. The target sequences in the *B. uniformis* 1001 chromosome (CHROMO) and in the three plasmids are shown below XBU4422. Five independently obtained insertions in pEG920 were sequenced, but all were identical to the one shown. Note that the target sequence in pE5-2 is identical to that in pEG920 except for the 4 bp between the vertical lines. Arrows indicate the junctions seen when XBU4422 inserted. Capital letters indicate identity between the corresponding end sequences of XBU4422 and the target sequence, aligned as indicated with respect to the vertical line. Bases that were identical in three of the four target sites are shown as target consensus. Capital letters indicate identity in all four target sequences. Stars under the target site consensus indicate identity between the target consensus and the ends of XBU4422. (C) Alignment of the highly conserved sequence on the right end of XBU4422 with the recombinational hot spot (RHS) from Tn*21* (7) and with the right end of Tn*916* (8). Stars indicate the bases in the Tn*916* sequence that are identical to the sequence in Tn*21* but not to the sequence in XBU4422.

were not at the ends of Tc^r Em^r 12256 as they were in the other Tc^r elements. Thus, the large size of the Tc^r Em^r 12256 could be due to the insertion of a 70 to 80 kbp Tc^r element into another element to create a hybrid conjugal element. If so, the element into which the original Tc^r element inserted may represent another family of *Bacteroides* chromosomal elements, because it did not hybridize with any known *Bacteroides* chromosomal element, including XBU 4422.

The possibility that the Tc^r Em^r 12256 element may be a hybrid is interesting in connection with other differences between the Tc^r Em^r 12256 element and the other Tc^r and Tc^r Em^r elements. For one thing, the Tc^r Em^r 12256 is the only Tc^r element to exhibit constitutive transfer. Transfer of the other Tc^r elements is enhanced 100- to 1000-fold if the donor is pregrown in tetracycline (13, 18). The constitutivity of Tc^r Em^r 12256 could indicate that the transfer functions are being provided by the extra DNA. The extra DNA could also be affecting host range, because in a previous study, we found that the Tc^r Em^r 12256 element was the only Tc^r element capable of transferring DNA from the human colonic *Bacteroides* organism to the ruminal anaerobe *Prevotella ruminicola* (11).

Results of TAFE and Southern analysis of Tc^r element insertions indicated that insertion was neither site specific nor random. The finding that XBU4422 inserted into only a single site on the plasmids used to trap it also suggests that insertion is not random. From the number of different Tc^r insertions detected, particularly in cases in which many independent insertions were screened, it appears that each Tc^r element has three to seven sites in the *B. thetaiotaomicron* chromosome. The NBUs also appeared to be relatively site selective. However, because of the relative infrequency of NBU transfers, fewer insertions were available for screening in this case. Thus, the full range of NBU insertions might not have been seen. The insertion sites for the Tc^r elements appear to be different from the insertion sites of the NBUs they mobilize, because cotransfer of an NBU did not influence the distribution of Tc^r element insertions and the NBUs did not insert next to the mobilizing element.

XBU4422 insertions were not accompanied by a duplication of the target site, as is usually the case for transposons, including the relatively site-specific transposon Tn*7* (6). XBU4422 behaved similarly to the streptococcal conjugative transposons, Tn*916* and Tn*1545* (2, 8), and the integrative streptomyces plasmids, e.g., pSAM2 (4), which also do not duplicate their target sites. XBU4422, like the Tn*916*-Tn*1545* type of element, had short regions of identity between one of its ends and its target sites. Also, the two ends of the element formed imperfect indirect repeats of each other (Fig. 6A) (8). One difference between XBU4422 and the Tn*916*-Tn*1545* type of transposon, aside from differences in size, was that XBU4422 preferentially took the 5-bp sequence GAAAA at one of its ends when it left the *B. uniformis* chromosome and inserted in the plasmids. Only one insertion took the 4-bp sequence CCCG from the other side of XBU4422 in the *B. uniformis* chromosome. Tn*916* and Tn*1545* remove 6- to 7-bp sequences from either end with equal probability (8). Excision of XBU4422 left behind an intact DNA segment, but in most cases an extra A was left. Thus, excision was not precise. It will be interesting to determine whether the extra A causes the site no longer to function as a target for XBU4422 insertions.

A comparison of the different XBU4422 target sequences suggests some features of the target sequence that may be important. Although the 4 bp immediately to the left of the insertion point (as depicted in Fig. 5) was not conserved in the different target sequences and was not related to the end sequences of XBU4422, it nonetheless appeared to be important because a change in this 4-bp segment between pE5-2 and pEG920 (Fig. 5B) affected insertion frequencies by at least 10-fold. It is interesting that the 4-bp sequence in pEG920 is part of an 8-bp palindrome, TTTGCAAA. This palindrome is destroyed in the target sites on pE5-2 and pNIL51. It is also destroyed in the *B. uniformis* chromosomal target site (TTTGCACC), but we do not know how good a target site the chromosomal site is.

A 6-bp sequence on the right end of the XBU4422 target

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XBU4422: LEFT END
  1  (End) GAAGTTACGA AAAGAGTGTA ATTTATTGGA AATGAGCATT GGGTAATCGC
 51  TCATAATAGT AATAGGTTAC GAATTAGTTA GTTATCTACT GCATTATTCT
101  TCTGTGCGTT GCGTAACCTT CAAAGAAACTC TTCGTATGCA AAGTAACAAT
151  AAACGGGACA TTTAGAATGA ATCTCCTGAT TTT

XBU4422: RIGHT END
  1  ACTAATCCTT AAGGCCTTGT TTCGTTATTC GTGTCCATTT CGTCACCTAT
 51  TGGCGTGGTT ACGAACAAGT AACGTAGCTC CGTCTTGATT TGGCTTCGGC
101  GTGGATTGTA ATGGCTCTCA GAATAATTGG CAGCGTTAAT GAAACCCCTG
151  TAAATCAAAT CCATTACCAT ATTCTTCCGC ATTTCACTTT TTTGTAGTAA
451  CTTTGCA (End)
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FIG. 6. DNA sequences of the left and right ends of XBU4422. The DNA sequences of the left and right ends were deduced from sequences of the different junctions between XBU4422 and its targets. Lines under the left and right ends of XBU4422 indicate a large, imperfect, indirect repeat between the two ends.

site was identical with a 6-bp sequence near the right end of Tn*916*-Tn*1545* (Fig. 5C). Perhaps more important, the 8-bp sequence covering this region in the XBU4422 right end was identical to an 8-bp sequence from Tn*21*, which is known to contain a recombinational hot spot (Fig. 5C). The recombinational hot spot on Tn*21* and related transposons is thought to be involved in the evolution of transposons carrying multiple drug resistances (7). In Tn*21*, the sequence is within the transposon, whereas in XBU4422, this sequence falls near one of its ends. Also, in the case of Tn*21*, insertions occurred within this 8-bp segment and not at its end.

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