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A New *Bacteroides* Conjugative Transposon That Carries an *ermB* Gene

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The erythromycin resistance gene *ermB* has been found in a variety of gram-positive bacteria. This gene has also been found in *Bacteroides* species but only in six recently isolated strains; thus, the gene seems to have entered this genus only recently. One of the six *Bacteroides ermB*-containing isolates, WH207, could transfer *ermB* to *Bacteroides thetaiotaomicron* strain BT4001 by conjugation. WH207 was identified as a *Bacteroides uniformis* strain based on the sequence of its 16S rRNA gene. Results of pulsed-field gel electrophoresis experiments demonstrated that the transferring element was normally integrated into the *Bacteroides* chromosome. The element was estimated from pulsed-field gel data to be about 100 kb in size. Since the element appeared to be a conjugative transposon (CTn), it was designated CTnBST. CTnBST was able to mobilize coresident plasmids and the circular form of the mobilizable transposon NBU1 to *Bacteroides* and *Escherichia coli* recipients. A 13-kb segment that contained *ermB* was cloned and sequenced. Most of the open reading frames in this region had little similarity at the amino acid sequence level to any proteins in the sequence databases, but a 1,723-bp DNA segment that included a 950-bp segment downstream of *ermB* had a DNA sequence that was virtually identical to that of a segment of DNA found previously in a *Clostridium perfringens* strain. This finding, together with the finding that *ermB* is located on a CTn, supports the hypothesis that CTnBST could have entered *Bacteroides* from some other genus, possibly from gram-positive bacteria. Moreover, this finding supports the hypothesis that many transmissible antibiotic resistance genes in *Bacteroides* are carried on CTns.

Erythromycin resistance was once rare in *Bacteroides* strains, and few strains isolated before 1970 carried any of the *erm* genes that encode this type of resistance. The first *erm* gene found in a *Bacteroides* strain was an *ermF* gene, which was located on a self-transmissible plasmid (14). The *erm* genes encode enzymes that modify the 23S rRNA by adding one or two methyl groups. This modification reduces the binding to the ribosome of three classes of antibiotics, macrolides, lincosamides, and streptogramin B, resulting in resistance to all three classes. Two genes with ≥80% amino acid identity are assigned the same class, which is given a letter designation. More recently, the *ermG* gene has been found in *Bacteroides.* Some *ermF* genes are located on plasmids, but both *ermF* and *ermG* genes have also been found on integrated self-transmissible elements called conjugative transposons (CTns) (8). In a recent survey of *Bacteroides* resistance genes, it was noted for the first time that a third type of *erm* gene, *ermB*, had begun to appear in *Bacteroides* strains (18). This observation was of interest because *ermB* genes have been found mainly in gram-positive bacteria.

Earlier studies had revealed that CTns carrying *ermF* and the tetracycline resistance gene *tetQ* were all closely related to a CTn called CTnDOT. CTnDOT-type CTns have now spread so widely among *Bacteroides* strains that over 80% of recent isolates carry a CTn of this type (18). Yet this type of CTn seems to be transferring mainly among members of the *Bacteroides* phylogenetic group, which includes *Bacteroides* species and *Porphyromonas* species. Finding *ermB* genes in *Bacteroides* species raised the possibility that transmissible elements might also be transferring to *Bacteroides* species from much more distantly related phylogenetic groups, such as gram-positive bacteria.

Since the human colon is thought to be one of the sites where horizontal gene transfer is likely to occur, it is of interest to learn more about the diversity of transmissible elements found in the species that numerically predominate in the colon, such as *Bacteroides* species, and about the extent to which such elements are actually transferring in that setting. In this paper, we report that the *ermB* gene in one of the *Bacteroides* strains found in our survey can be transferred by conjugation and is located on a CTn that is unrelated to any of the *Bacteroides* CTns described so far.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. *Bacteroides* strains were grown anaerobically at 37°C in prereduced Trypticase-yeast extract-glucose (TYG) broth (9) or TYG agar plates incubated in BBL GasPak jars. *Escherichia coli* strains were grown aerobically at 37°C in Luria-Bertani (LB) broth or plated on LB agar. *Bacteroides* strains BT4001 and BT4100 are spontaneous mutants of *Bacteroides thetaiotaomicron* 5482A (ATCC 29148) that are rifampin resistant and trimethoprim resistant, respectively. *E. coli* strain DH5αMCR (Gibco BRL) was used as a host for plasmid construction and, in many cases, as a recipient in filter mating assays.

DNA isolation and molecular methods. DNA preparations were made by a modification of the method of Saito and Miura (15). Plasmid DNA was isolated from *E. coli* and *Bacteroides* either by the method of Ish-Horowitz as described by Sambrook et al. (17) or with the QIAGEN plasmid midi kit. Restriction digests and ligations were performed as specified by the manufacturer (New England Biolabs, Promega, or Invitrogen).

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Marker[a]	Description and/or source
Strains		
Escherichia coli		
DH5αMCR	$RecA^-$	Gibco BRL
S17-1	$RecA^-$ Tp^r	Contains the transfer functions of RP4 integrated into the chromosome
Bacteroides sp.		
BT4001	(Rif^r)	Rif^r derivative of *B. thetaiotamicron* 5482
BT4100	(Thy^- Tp^r)	Thy^- Tp^r derivative of *B. thetaiotamicron* 5482
WH207	(Em^r)	A community isolate with the *ermB* element
WH207 Thy^-	(Em^r Thy^- Tp^r)	Thy^- Tp^r derivative of WH207
BT4020	(Em^r Rif^r)	BT4001 with a copy of the *ermB* element from WH207 Thy^- donor
BT4021	(Em^r Thy^- Tp^-)	BT4100 with a copy of the *ermB* element from BT4020 donors
Plasmids		
pUC19	Ap^r	*E. coli* cloning vector
pAG1	Ap^r	3-kb *Hin*dIII-*Hin*dIII fragment containing the *ermB* gene and flanking regions from WH207 cloned into pUC19; this study
pLYL001A	Ap^r (Tc^r)	Bacteroides suicide vector contains *tetQ* and *oriT* (RK2) cloned into pUC19
pAG2	Ap^r (Tc^r)	1.5-kb *Kpn*I-*Bam*HI fragment from pAG1 cloned into pLYL001A; this study
pGEM-T	Ap^r	*E. coli* PCR cloning vector (Promega)
$pLYL7_{oriT(RK2)}$	Ap^r (Cfx^r)	Can be mobilized by IncP plasmid RP4 but not by *Bacteroides* conjugative transposons
pLYL01	Ap^r (Tc^r)	*Bacteroides-E. coli* shuttle vector with pBI143 *mob* and *oriT*
pLYL11	Ap^r (Cfx^r)	*Bacteroides-E. coli* shuttle vector with NBU1 *mob* and *oriT*
pT-COW	Ap^r Tc^r (Tc^r)	*Bacteroides-E. coli* shuttle vector obtained by cloning pB8-51 and *tetQ*
pAG4	Ap^r (Tc^r Em^r)	*ermB* gene and upstream sequence from WH207 cloned into pLYL01; this study

[a] *Bacteroides* markers are indicated within parentheses.

Filter mating assays. The filter mating assays have been described previously in detail (20). For matings between *Bacteroides* donors and *Bacteroides* recipients, the filters were incubated anaerobically overnight at 37°C. When *E. coli* was the donor, the filters were incubated aerobically. *Bacteroides* transconjugants were selected on TYG medium containing relevant antibiotics under anaerobic conditions. *E. coli* transconjugants were incubated on LB selective plates aerobically. The antibiotics used and their concentrations were as follows: ampicillin, 100 μg/ml; erythromycin, 3 μg/ml; gentamicin, 200 μg/ml; rifampin, 10 μg/ml; streptomycin, 100 μg/ml; tetracycline, 10 μg/ml; trimethoprim, 100 or 300 μg/ml; and cefoxitin, 20 μg/ml. During experiments to test for tetracycline induction or erythromycin induction, the *Bacteroides* strains were grown in TYG medium containing 2 μg of tetracycline/ml or 3 μg of erythromycin/ml.

In experiments to determine whether natural isolates of *ermB*-carrying *Bacteroides* strains could transfer *ermB* by conjugation, thymidine-requiring spontaneous mutants of the donors were selected so that the auxotrophy could be used in the mating experiment to select against donors. These thymidine-requiring spontaneous mutants were also resistant to trimethoprim. BT4001, whose DNA does not hybridize to known *Bacteroides* CTns and which is rifampin resistant (Rif^r), was the recipient. Transconjugants were selected as Rif^r, erythromycin-resistant (Em^r) colonies. There is one report in the literature that claims that BT4001, a derivative of ATCC 29148, contains a copy of *ermB* (7). We saw no evidence of the presence of *ermB* sequences based on DNA-DNA hybridization or PCR. Moreover, the genome sequence of BT4001, which was completed recently (25), contains no *ermB* gene. The *ermB*-containing isolates were the donors in separate filter mating experiments.

Some elements that appear to be self-transmissible are actually mobilized by self-transmissible elements present in the donor strain. Accordingly, BT4001 transconjugants isolated in the original mating, designated BT4020, were then used as donors in a second mating experiment with BT4100 (trimethoprim resistant [Tp^r], thymidine deficient, and erythromycin sensitive [Em^s]) as the recipients. Transconjugants were selected as Tp^r Em^r colonies and were checked to make sure that they were Thy^- Rif^r.

Species identification of WH207. WH207, the community isolate that contained the transmissible *ermB* gene, had not been identified to the species level. To determine its species identification, a universal forward bacterial 16S rRNA primer, 5′AGAGTTTGAT(C/T)(A/C)TGGC3′ (22), and a *Bacteroides* specific reverse primer, 5′CCTTGTTACGACTTAGCCC3′ (13), were used to amplify by PCR a 1.4-kb segment of the 16S rRNA gene. The cycling conditions were as follows: 95°C for 5 min; 25 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min; followed by 72°C for 5 min. The product was gel purified and sequenced.

Southern blot, dot blot, and colony hybridization procedures. Colony hybridization, Southern hybridization, and dot blot analyses were done according to the methods of Sambrook et al. (17) and the Renaissance kit protocols (PerkinElmer Life Sciences). Probes were labeled with fluorescein-dUTP by the random primer method as outlined in the PerkinElmer Life Sciences Renaissance kit protocol. Hybridization was carried out at 42°C overnight with denatured probes. Nonspecifically hybridized probes were removed with two washes of increasing stringency. To prevent nonspecific protein binding, the membrane was treated with the blocking reagent provided in the kit. The conditions used were calculated to allow hybridization of the probe only to DNA that shared more than 85% sequence identity with it.

Isolation of DNA flanking the *ermB* gene. Genomic DNA preparations were made from WH207 (Table 1), and aliquots were digested with various restriction enzymes. The *ermB* gene was amplified by PCR as described in a prior publication (18), and a probe was constructed. Southern blot analysis, using the *ermB* probe, identified a 3-kb *ermB*-containing band in the *Hin*dIII-digested WH207 genomic DNA lane. *Hin*dIII fragments between 2 and 4 kb were isolated by extraction from an agarose gel. These fragments were then ligated with *Hin*dIII-digested pUC19 and transformed into DH5αMCR-competent cells. The ampicillin-resistant transformants were screened by colony hybridization with the *ermB* probe. Plasmids from positive colonies were analyzed by restriction digestion and Southern blotting. A plasmid, pAG1, was shown to contain a 3-kb *Hin*dIII fragment that hybridized to the *ermB* probe. Then, a probe made from the cloned fragment in pAG1 hybridized to a 3-kb *Hin*dIII fragment of WH207. The 3-kb fragment in pAG1 was sequenced.

Plasmid rescue technique to obtain DNA adjacent to the *ermB* gene. A plasmid rescue technique was employed to clone more of the sequence from the *ermB* element (Fig. 1). A 1.5-kb fragment containing N-terminal *ermB* sequence and upstream sequence was cloned into the tetracycline-resistant (Tc^r) *Bacteroides* suicide vector, pLYL001. The clone was then mobilized into BT4020, a transconjugant containing the *ermB* element, and Tc^r colonies were isolated. The Tc^r insertion mutants obtained after mating were analyzed by Southern hybridization to confirm that the recombination had occurred within the 1.5-kb region. Various restriction enzymes were then chosen to cut the genomic DNA of the insertion mutant so that the plasmid DNA remained intact. A Southern experiment with an internal-region probe helped to determine the size of the rescued piece for each enzyme. A *Pst*I fragment containing pLYL001 and 12 kb of the *ermB* element was identified. The genomic DNA from the insertion mutant was cut with *Pst*I, and ligation reactions were set up to favor monomeric circularization. This mixture was used to transform *E. coli* DH5αMCR, and cells with the intact

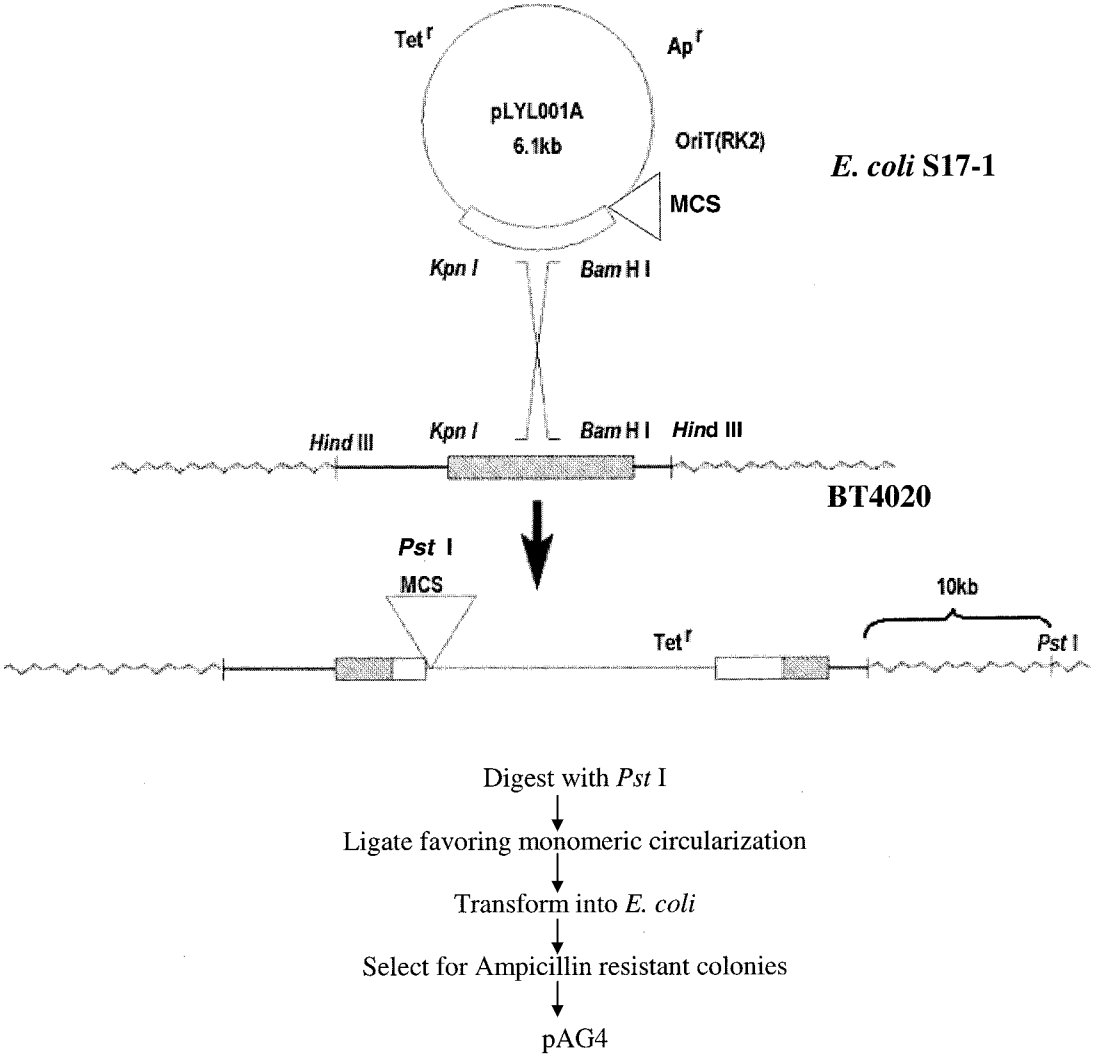

FIG. 1. Schematic representation of the plasmid rescue experiment done to obtain additional flanking sequences and thus reach an end. BT4020 is the transconjugant obtained by mating WH207 with BT4001. CTnBST is the only known mobile element in this strain.

vector were obtained by selecting for ampicillin resistance. The clone was named pAG4, and the rescued fragment was then sequenced from this clone.

Sequencing. DNA sequencing was done at the W. M. Keck Center for Comparative and Functional Genomics at the University of Illinois, Urbana, with the Applied Biosystems model 373A version 2.0.1S dye terminator sequencing system. Primer walking was done to obtain 13 kb of sequence from CTnBST.

PFGE. For pulsed-field gel electrophoresis (PFGE), DNA samples were prepared, digested with restriction enzymes in agarose plugs, and electrophoresed in 1% agarose gels as described in the Bio-Rad CHEF-DRIII PFGE system manual. The DNA was electrophoresed in 0.5× Tris-borate-EDTA (0.045 M Tris, 0.045 M borate, 0.001 M EDTA, pH 8.3) at 14°C. The voltage was 6 V/cm, and the field angle was 120°. The pulse parameters and run times were varied as needed. The Bio-Rad lambda ladder and the *Saccharomyces cerevisiae* ladder were used to estimate the sizes of restriction fragments.

Preparation of constructs for plasmid mobilization experiments. To determine whether the CTnBST could mobilize coresident plasmids, the plasmid of interest had to be mobilized into a *Bacteroides* strain containing CTnBST from *E. coli* by triparental matings. Three plasmids were tested; pLYL01 had the *mob* and *oriT* region from *Bacteroides* plasmid pBI143, pT-COW had the *mob* and *oriT* region from *Bacteroides* plasmid pB8-51, and pLYL11 contained the *mob* and *oriT* region from *Bacteroides* mobilizable transposon of NBU1.

For the triparental matings, the donors were DH5αMCR cells containing the relevant plasmid and HB101 containing the IncPα plasmid RP1. The *B. thetaiotaomicron* strain BT4020 (Em^r Rif^r), which carried a single copy of CTnBST, was the recipient. The *E. coli*-to-*Bacteroides* sp. matings were done as described earlier (20).

Plasmid mobilization experiments. To test whether CTnBST could mobilize plasmids or NBU1, conjugation experiments in which BT4020 isolates carried each of the plasmids as a donor were done. The recipient was *E. coli* HB101 (streptomycin resistant [Sm^r]). Only if CTnBST is able to supply the transfer functions can these plasmids transfer from *Bacteroides* to *E. coli*. Transconjugants were selected on LB agar plates containing ampicillin and streptomycin. Plasmid preparations were made from transconjugants to confirm that the correct plasmid was present.

Nucleotide sequence accession numbers. The new sequences for *ermB* from WH207 and CTnBST identified as a result of the present study were submitted to the GenBank database under the accession numbers AY349154 and AY345595, respectively.

RESULTS

Characteristics of the six *ermB*-containing isolates. Five of the six isolates whose DNA hybridized with the *ermB* gene probe were phenotypically erythromycin resistant (at an erythromycin concentration of 3 to 10 μg/ml) as detected by the broth dilution technique. The exception was WH202, which contained *ermB* sequences but was sensitive to erythromycin.

TABLE 2. The six *ermB*-containing strains found in the strain collection of the Salyers laboratory

ermB strain	Origin	Antibiotic resistance	Genotype by probe hybridization[a]	Difference from *ermB* sequence in WH207 (bp)
WH207 (*B. uniformis*)	Volunteer, Woods Hole, Mass.	Tc^r Em^r	*ermB tetQ*; D	
WH202	Volunteer, Woods Hole, Mass.	Tc^r	*ermB tetQ*; D	1
WH714	Sewer, Woods Hole, Mass.	Tc^r Em^r	*ermB tetQ*; D	2
DH3760	Clinical, Loyola VA Hospital, Chicago, ILL.	Tc^r Em^r	*ermB tetQ*; D	1
Bov7991 (*B. ovatus*)	Clinical, Wadsworth Anaerobe Laboratory, Los Angeles, Calif.	Tc^r Em^r	*ermB ermG tetQ*; D	1
BF8371 (*B. fragilis*)	Clinical, Wadsworth Anaerobe Laboratory, Los Angeles, Calif.	Tc^r Em^r	*ermB ermF tetQ*; D	0

[a] Erythromycin resistance genes do not cross-hybridize under high-stringency conditions. D, CTnDoT joined ends.

Two of the six isolates had two *erm* genes, DNA from Bov7991 hybridized to *ermB* and *ermG* probes and DNA from BF8371 hybridized to *ermB* and *ermF* probes. All six isolates were resistant to tetracycline, and DNA from these strains hybridized to the *tetQ* gene, a gene that is carried by many *Bacteroides* CTns. All six isolates hybridized to probes that detected the ends of CTnDOT (Table 2).

To determine whether all of these isolates carried identical or near-identical *ermB* genes, the *ermB* gene in each isolate was amplified by PCR and sequenced. WH207 and Bov7991 contained *ermB* genes with identical sequences. The *ermB* gene in WH202 was identical to the *ermB* gene in BF8371. Since BF8371 was resistant to erythromycin, the sensitivity of WH202 was presumably due to the failure of the promoter to function in *Bacteroides* spp. The sequences of all of the other *ermB* genes were nearly identical except for single-base-pair substitutions at positions 380, 397, and 469. The species identities of two of the *ermB*-containing isolates had been determined previously; Bov7991 is a *B. ovatus* strain, and BF8371 is a *B. fragilis* strain (S. M. Finegold, Wadsworth VA Hospital Anaerobe Laboratory, Los Angeles, Calif., personal communication). Thus, at least two different *Bacteroides* species were represented in the collection of *ermB*-containing isolates. The fact that *ermB* genes found in different *Bacteroides* species are virtually identical supports the hypothesis that the *ermB* genes have been moving into *Bacteroides* species by horizontal gene transfer.

The *ermB* sequences from Bov7991 and WH202 were identical to the sequence of an *ermB*(P) gene found previously in *Clostridium perfringens* (1), and all of the *Bacteroides ermB* genes had sequences that were virtually identical to those of *ermB* genes found in gram-positive cocci such as *Streptococcus pneumoniae* (19). Our finding of *ermB* genes in *Bacteroides* was the first time that this type of *erm* gene had been found in a gram-negative anaerobe. All of the *ermB*-containing isolates had been isolated after 1990. This finding raises the possibility that *ermB* has entered *Bacteroides* strains only recently.

The *ermB* gene in strain WH207 is on a transmissible element. To test whether any of the *ermB* genes found in the *Bacteroides* strains were carried on a transmissible element that is still active in *Bacteroides*, mating experiments were done with BT4001 as the recipient. All of the *ermB*-containing strains except WH202, which was phenotypically erythromycin sensitive, were tested. Transconjugants were obtained only when WH207 was the donor, at a transfer frequency of 10^{-5} transconjugants/recipient. All transconjugants obtained (designated BT4020) hybridized with an *ermB* gene probe, confirming that erythromycin resistance cotransferred with *ermB*. None of these transconjugants contained *tetQ*, indicating that *tetQ* was not on the same transmissible element as *ermB*. Moreover, DNA from the transconjugants did not hybridize with DNA from the ends of CTnDOT, indicating that *ermB* was carried on some other transmissible element.

Although *ermB* was not carried on CTnDOT, it was possible that the element that carried *ermB* was being mobilized out of WH207 by CTnDOT, which is known to be capable of mobilizing coresident elements such as mobilizable plasmids and mobilizable transposons that are otherwise incapable of conjugal transfer. To determine whether the *ermB*-carrying element could retransfer from BT4020, a mating experiment was done in which BT4020 was used as the donor and BT4100, a Thy^- Tp^r derivative of BT4000, was used as the recipient. The transfer frequency in this mating experiment was again 10^{-5} transconjugants/recipient. The fact that the transfer frequency remained the same whether WH207 or BT4020 was the donor indicates that the *ermB*-containing element is self-transmissible and its frequency is not stimulated by some other transmissible element present in WH207.

Transfer of the CTnDOT-type elements is enhanced 1,000-fold by the exposure of the donor to tetracycline; exposure of the donor to tetracycline did not affect the transfer frequency of the *ermB* element (Table 3). The transfer frequency was also unaffected by exposure of the WH207 or BT4020 donors to erythromycin (Table 3).

WH207 was isolated in Woods Hole, Mass., from the feces of a healthy volunteer but had not been identified to the species level. To determine the species of WH207, its 16S rRNA gene was amplified and sequenced. One of the two closest matches was to a 16S rRNA gene from a rumen bacterium that had been isolated in Australia but not given a species name (99% identity; GenBank accession no. AF139524). The other sequence match (99% identity) was to the 16S rRNA gene from a *B. uniformis* strain (accession no. AB050110). Thus, WH207 appears to be a *B. uniformis* strain. With WH207, the number of different *Bacteroides* species in which *ermB* has been found amounts to three.

The *ermB* element is a CTn. Plasmid preparations were made from the six original *ermB* isolates and from the transconjugant (BT4020) to determine if the *ermB* element was a plasmid. BT4001 has a 30-kb plasmid. This plasmid was present in BT4020, but no additional plasmids were seen. No plasmids were found in BF8371 and DH3760, two of the six original *ermB*-containing strains. Plasmid preparations from all of the isolates were probed on Southern blots with the *ermB*

TABLE 3. Transfer frequencies obtained in filter mating assays.

Donor	Recipient	Induction[d]	Transfer frequency[e]	
			Tc^r element	Em^r element
WH207 Thy$^-$[a]	BT4001	None	$<10^{-8}$	10^{-5}
		TC	10^{-5}	10^{-5}
		EM	$<10^{-8}$	10^{-5}
BT4020[b]	BT4100	None	NA	10^{-5}
		EM	NA	10^{-5}
BT4021[c]	BT4001	None	NA	10^{-5}
		EM	NA	10^{-5}

[a] WH207 has a Tc^r and an Em^r element.
[b] Transconjugant of WH207 Thy$^-$-BT4001 mating.
[c] Transconjugant of BT4020-BT4100 mating.
[d] The donors were induced with either 2 μg of tetracycline (TC)/ml, 3 μg of erythromycin (EM)/ml, or not induced with any antibiotic.
[e] Transfer frequency is defined as the number of transconjugants per recipient. NA, not applicable.

probe. None of the isolates hybridized with this probe (data not shown). Thus, it seemed likely that the *ermB* element was integrated into the chromosome.

To determine whether the *ermB* element in BT4020 is integrated into the chromosome, PFGE experiments were done (2). DNA from recipient strain BT4001 and three independent isolates was digested with I-*Ceu*I. Comparison of the fragment patterns showed that a 300-kb segment of DNA from BT4001 shifted to 400 kb in DNA from the transconjugants (Fig. 2A). Thus, the element that transfers *ermB* is normally integrated into the chromosome and is about 100 kb in size (Fig. 2A). PFGE was also done with DNA restricted with *Not*I and *Sfi*I (data not shown). Several differences were observed between the BT4001 pattern and the pattern for BT4020, indicating that there are *Not*I and *Sfi*I sites within the element.

To confirm that the band shift seen in DNA digested with I-*Ceu*I was due to the *ermB* element, a Southern blot of the gel shown in Fig. 2A was probed with a 3-kb *Hin*dIII segment that contains the *ermB* gene. The probe hybridized to the shifted band, proving that the band shift observed was due to the integration of the *ermB* element (Fig. 2B). Taken together, our results support the hypothesis that the *ermB* gene from WH207 is carried on a 100-kb CTn. This CTn has been designated CTnBST.

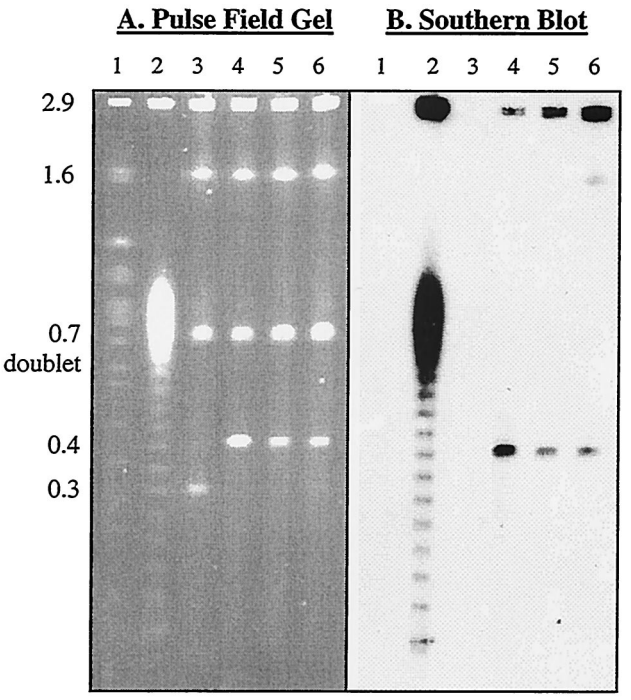

FIG. 2. (A) PFGE of I-*Ceu*I-digested recipient (lanes 3) and transconjugant (lanes 4 to 6) DNA was carried out in a 1% agarose–0.5× Tris-borate-EDTA gel run at 6 V/cm, with an initial pulse of 10 s and a final pulse of 120 s, for 30 h with a Bio-Rad CHEF-DR III PFGE apparatus. A Bio-Rad lambda ladder PFG marker (lanes 2) was used along with an *S. cerevisiae* chromosomal PFG marker (lanes 1). The approximate sizes of the bands of interest are indicated to the left of the figure in megabases. BT4001 is the recipient. All other strains shown are transconjugants containing the *ermB* element. A 300-kb band in the recipient shifted to 400 kb in the transconjugants, indicating that the element is integrated into the chromosome and is at least 100 kb in size. The 2.9-Mb band is in the well. (B) Southern blot of the PFG with I-*Ceu*I-digested recipient and transconjugant DNA. The probe was the 3-kb *Hin*dIII region containing *ermB* plus flanking DNA. A λ *Hin*dIII probe was also used to detect the lambda PFG marker. No bands are visible in the lane for strain BT4001 because it does not have the *ermB* element. All other lanes contain strains that are transconjugants containing the *ermB* element. A 400-kb band hybridizes to the 3-kb *Hin*dIII probe, proving that the band shift seen in the PFG is due to integration of the *ermB* element. An additional band is seen in one lane, which might be due to partial digests or the presence of another copy of CTnBST.

Analysis of the region around the *ermB* gene. Using the plasmid rescue approach, we cloned a total of 13 kb of DNA from the *ermB* region. A map of this cloned region is shown in Fig. 3. The *ermB* gene from *Bacteroides* was virtually identical to genes from gram-positive bacteria such as *C. perfringens* (GenBank accession no. X58285 and U18931) (1), *Enterococcus faecalis* (accession no. Y00116, AE016833, M11180, M36722, X92945, and U86375) (4), *Streptococcus pyogenes* (accession no. X64695 and X66468) (5, 6), *Streptococcus pneumoniae* (accession no. X52632) (19), *Clostridium difficile* (accession no. AJ294529), *Staphylococcus aureus* (accession no. Y13600), *Bacillus cereus* (accession no. AF480455 to AF480460), *Staphylococcus intermedius* (accession no. AF239773 and AF299292) (3), *Streptococcus agalactiae* (accession no. X72021), *Enterococcus faecium* (accession no. AF507977, AF516335, AF229200, and AF242872), *Enterococcus hirae* (accession no. AF406971 and X81655), *Lactobacillus reuteri* (accession no. AF080450, AF205068, and AY082384), *Staphylococcus lentus* (accession no. U35228) (23), and *Lactobacillus fermentum* (accession no. U48430). The region downstream of *ermB* in CTnBST was also 99% identical to a sequence that is located next to an *ermB* gene found in *C. perfringens*, although some DNA seems to have been deleted in CTnBST in this region. No more sequence from the *C. perfringens* strain was available in the databases, so we were unable to determine if the identity extended beyond this point (1).

Figure 3 shows the results of BLAST searches of the rest of the cloned 13-kb region. Six low-similarity matches were found to be putative genes by BLASTX. Only two of these are complete open reading frames (ORFs), the *ssr* (site-specific recombinase) and *tpn* (transposase) genes. The remaining four, des-

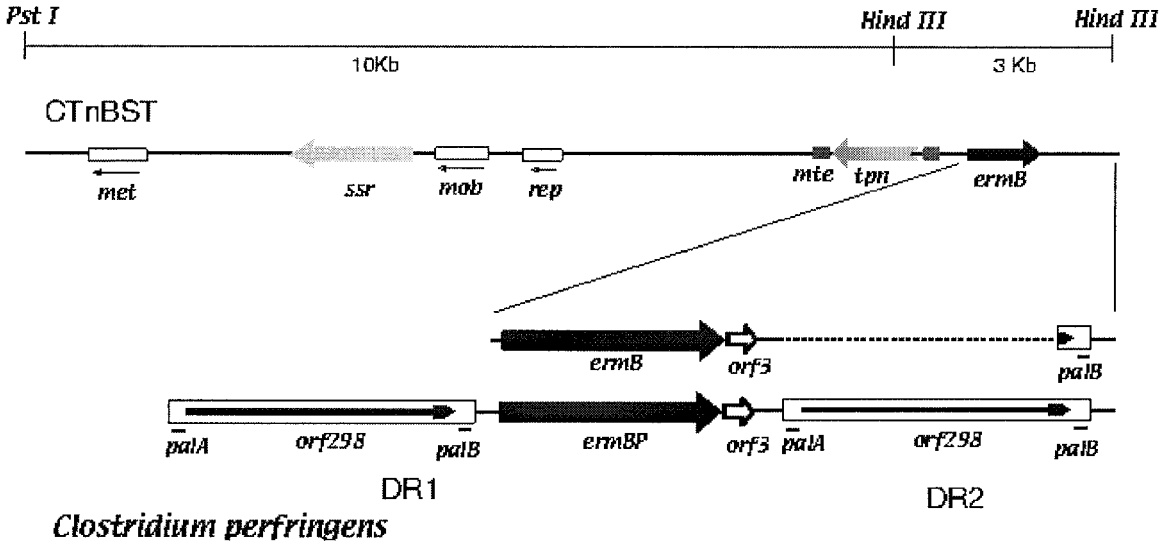

FIG. 3. Matches obtained when a BLAST search was performed with the 13 kb of DNA sequence obtained from CTnBST. The 1.7-kb sequence from the right end is 99% identical to a sequence found for *C. perfringens* (accession no. U18931.1) (1). The *ermB* and *ermB*(P) genes are nearly identical, as is *orf3*. Only part of direct repeat 2 (DR2) is present in CTnBST; *orf298* is truncated, and only a copy of *palB* is present. Sections of DR2 consisting of *palA* and most of *orf298* are truncated and are indicated by broken lines. A Tn*10*-like transposase gene (*tpn*) was found inserted into a methyltransferase-like (*mte*) gene. Truncated ORFs for *rep*, *mob*, and *met* were found. An ORF which had low identity to site-specific recombinases (ssr) was also found.

ignated *met* (methylase), *rep* (replication protein), *mob* (mobilization protein), and *mte* (methyltransferase) in Fig. 3, were truncated in CTnBST. The deduced amino acid sequence of the protein encoded by the CTnBST homolog of *ssr* had 27% identity to a site-specific recombinase, the DNA invertase Pin homolog of *Clostridium acetobutylicum* (GenBank accession no. AAK79913). The closest match to the protein encoded by *tpn*, with 29% amino acid identity, was a putative transposase in *Deinococcus radiodurans* (accession no. NP_051548), which is similar to IS*10*-like transposases based on homology searches. The DDE motif was found in the sequence of the *tpn* gene product. The transposase gene seems to have inserted into a truncated *mte* gene. The closest match to the protein encoded by *mte*, with 67% amino acid identity, is a hypothetical protein from *Clostridium thermocellum* ATCC 27405 (accession no. ZP_00061314). Also, the protein encoded by the *mob* gene shown in Fig. 3 was similar to recombination and mobilization proteins, with 31% identity at the amino acid level to a recombination protein in *Staphylococcus aureus* (accession no. NP_052168). The region indicated as *rep* in Fig. 3 had a corresponding amino acid sequence that is 37% identical to replication proteins in *Staphylococcus haemolyticus* (accession no. CAC16673).

Comparison of the *ermB* regions from the six original *ermB* isolates. We used a 3-kb fragment from WH207 which contained the 738-bp *ermB* gene, 1,292 bp of upstream flanking DNA, 961 bp of downstream flanking DNA, and a 10-kb fragment that contained the rest of the cloned DNA (Fig. 3) to probe DNA from the *ermB* isolates that did not transfer the gene under the conditions we used. As expected, the hybridization pattern seen with WH207 DNA was the same as that seen with DNA from two transconjugants (BT4020 and BT4021), except that there was a doublet in the WH207 DNA probed with the 3-kb *ermB*-containing probe that was not present in the transconjugants (Fig. 4A). Thus, there could be a second copy of *ermB* in WH207. Both probes hybridized to different-size bands in the six isolates, and the intensity was different even though approximately the same amounts of DNA were loaded in all the lanes (Fig. 4). These results indicate that although the regions surrounding the *ermB* gene in all six strains were similar enough to cross-hybridize on a high-stringency Southern blot under conditions that should detect only DNA segments that were more than 85% identical to the probe, the regions were not identical.

Dot blot analyses of strains in our collection that did not hybridize with the *ermB* probe identified 10 additional isolates that hybridized to the 10-kb probe; 3 of these were clinical isolates and 7 were community isolates. All of these isolates were isolated after 1980. DNA from five of these isolates was probed on a Southern blot with the 10-kb probe. Although three of the five isolates had similarly sized *Hin*dIII fragments, the other two had weakly hybridizing fragments of different sizes.

The ends of the CTn are more than 16 kb from the right end and 20 kb from the left end of the cloned region. Although there were sequences within the cloned region that might have encoded an integrase, which would be expected to be located near one end of the CTn, the fact that the Southern hybridization patterns were the same in the original strain and in the transconjugants (Fig. 5) indicated that the cloned region was internal to the CTn. To estimate the distance between the ends of the cloned region and the ends of the element, we digested DNA from WH207 and two transconjugants, BT4020 and BT4001, with *Nsi*I, *Kpn*I, and *Sac*II and probed these digests on Southern blots with the 3- and 10-kb cloned fragments (Fig. 5A and B). The positions of these sites within the cloned DNA are indicated in Fig. 5C. By analyzing the resulting Southern hybridization patterns, we deduced that the cloned region was

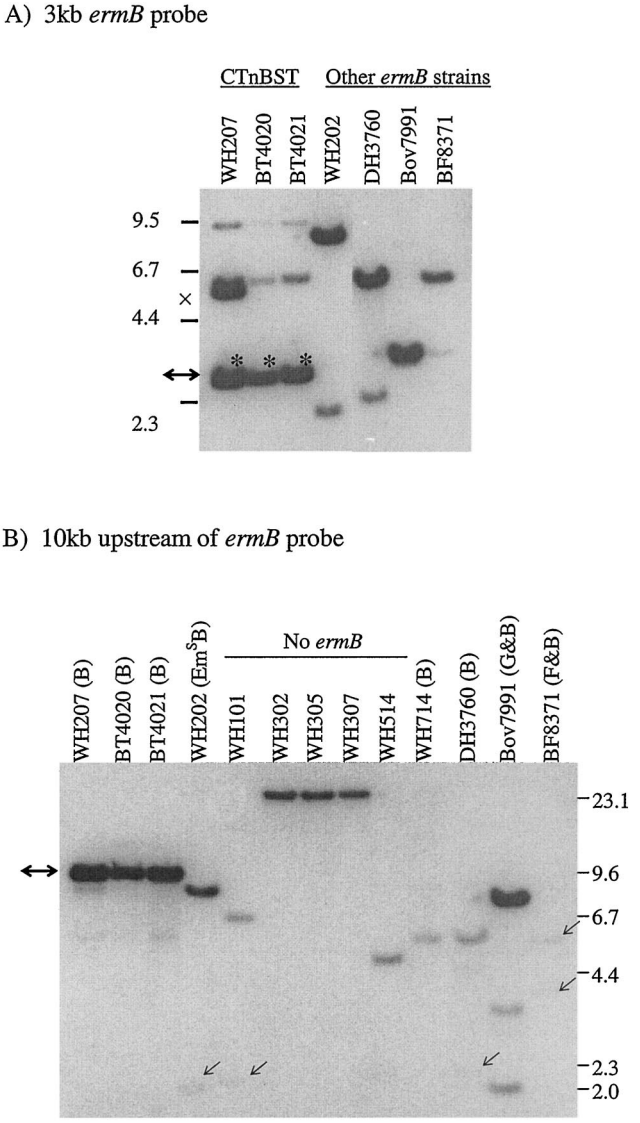

FIG. 4. Results of Southern hybridization with probes from CTnBST. The strain from which the DNA was obtained is indicated above each lane. The DNA was digested with *Hin*dIII and *Pst*I in all the lanes. The probes used were a 3-kb *Hin*dIII region from CTnBST (A) and a 10-kb *Hin*dIII-*Pst*I region upstream of the 3-kb probe (B). The asterisk indicates the *ermB*-containing band. The band containing the probe is also indicated (↔). Weakly hybridizing bands are indicated by arrows. The positions of the lambda *Hin*dIII size standards are indicated in kilobases. The multiplier indicates a doublet seen in WH207 that was not seen BT4020 and BT4100. B, *ermB*; G, *ermG*; F, *ermF*.

more than 16 kb from the right end and at least 20 kb from the left end of the available sequence (Fig. 5C). Since the sizes of these sequences do not add up to 100 kb, the size of the CTn estimated from PFGE analysis, it is clear that there is a large segment of the CTn that is not detected by probes made from the cloned DNA.

CTnBST can mobilize coresident elements. We wanted to determine whether CTnBST, like CTnDOT, could mobilize *Bacteroides* plasmids and a *Bacteroides* mobilizable transposon (NBU1). pLYL01 contains the *oriT* and the mobilization gene of pBI143, a naturally occurring *Bacteroides* plasmid that is found in many strains. pT-COW carries the *oriT* and the mobilization gene of another widely dispersed *Bacteroides* plasmid, pB8-51. pLYL11 carries the *oriT* and the mobilization gene of the mobilizable transposon, NBU1. These plasmids cannot transfer themselves from *Bacteroides* strain BT4001 to *E. coli*. All three constructs were transferred from a derivative of BT4001 that contained CTnBST and the plasmid to *E. coli* recipients at a frequency of 10^{-6} to 10^{-7} transconjugants per recipient.

DISCUSSION

In this study, we demonstrated that an *ermB* gene that has only recently entered *Bacteroides* strains is carried on a 100-kb self-transmissible integrated element designated CTnBST. CTnBST is a new CTn in *Bacteroides* strains, and it does not belong to the predominant CTnDOT family of *Bacteroides* CTns. This element is also distinct from two *ermG*-containing CTns that have been found in *Bacteroides* species (8, 21). The initial picture of CTn diversity in *Bacteroides* species was that the CTns were all related to CTnDOT. Results of this study and other recent studies show that this is not the case and that there is a widely diverse set of CTns in *Bacteroides* species. It is noteworthy that most of the matches in the databases with the 13-kb cloned region from CTnBST are to genes from gram-positive bacteria. In particular, the *ermB* gene and a segment of downstream sequence were identical to sequences found previously in *C. perfringens.* In the human colon, the two types of bacteria that predominate numerically are the gram-negative *Bacteroides* species and a group of gram-positive anaerobes about which little is known (10, 11, 24). *C. perfringens* is not one of the numerically predominant species, yet a segment of DNA found in a clinical isolate is identical to that in CTnBST. This is the strongest evidence yet that there may be horizontal transfer events occurring between gram-positive and gram-negative bacteria in the human colon, although the direction of transfer is not known. The fact that only very recently isolated strains of *Bacteroides* carry *ermB* suggests that this gene has entered *Bacteroides* species from some other source.

The hypothesis that human colonic bacteria could be serving as reservoirs of antibiotic resistance genes, constantly acquiring and transmitting these genes and possibly passing them on to pathogenic bacteria, is an old one. Only recently, however, has evidence started to accumulate that this may be the case, especially for the species of bacteria that predominate numerically. It is important to know whether colonic bacteria serve such a function because if so, the effect on the colonic microflora of antibiotic treatment for other diseases or for prophylactic uses of antibiotics needs to be taken more seriously. It is now possible to begin to explore the ecology of antibiotic resistance genes in intestinal bacterial populations, although more will have to be learned about transmissible elements and genes in the gram-positive anaerobes to obtain a complete picture. Such information is important not only for antibiotic resistance concerns but also because the extent to which these genes are transferred is likely to be a good indicator of how readily other types of gene transfers occur in the same environment.

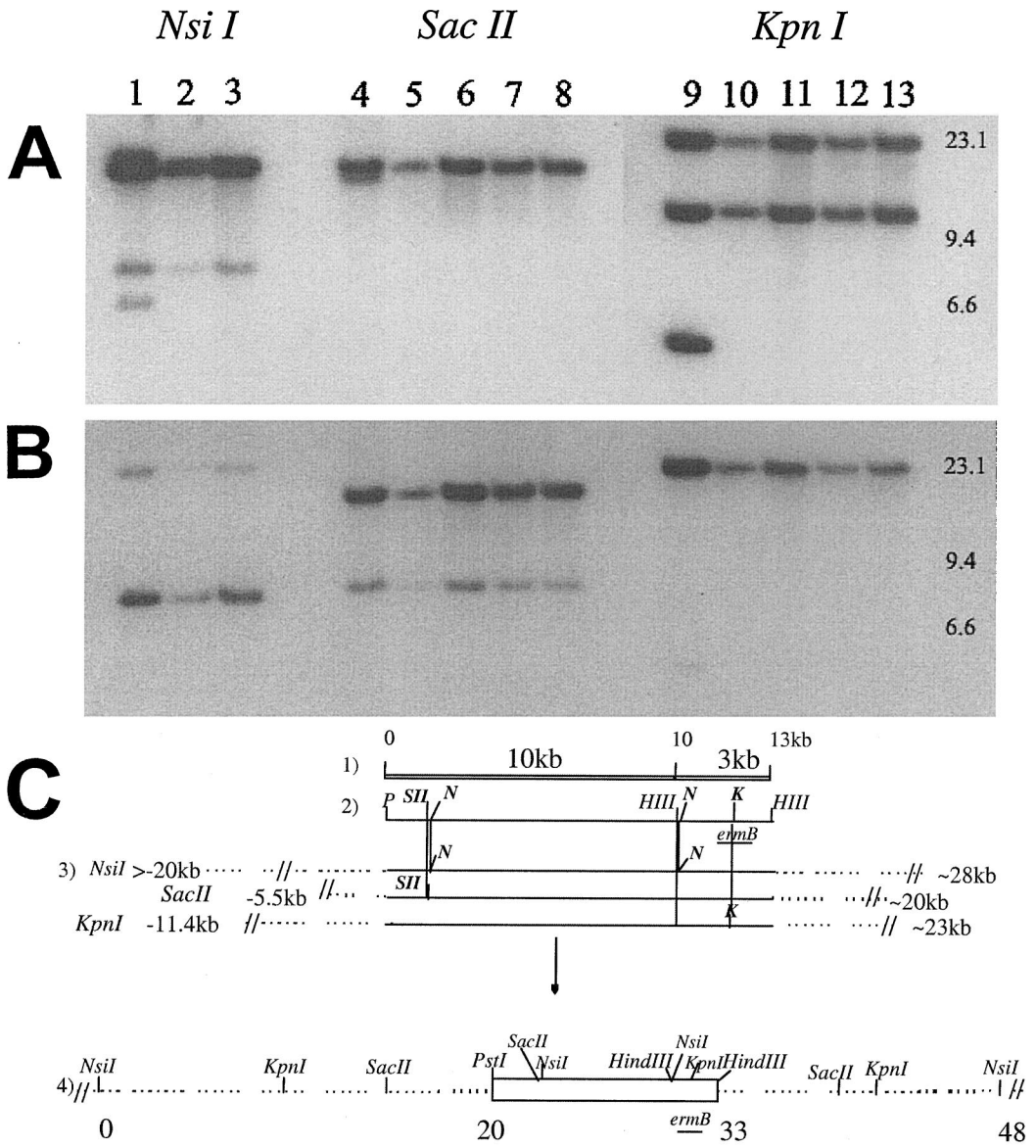

FIG. 5. (A and B) Southern blot analysis to estimate the distance to the ends from the available sequence. Lanes 1, 4, and 9, WH207 DNA; lanes 2, 3, 5 to 8, and 9 to 13, transconjugant DNA digested with *Nsi*I, *Sac*II, or *Kpn*I as indicated in the figure. The 3-kb *Hin*dIII fragment was used as the probe in panel A, and the 10-kb *Pst*I-*Hin*dIII fragment was the probe in panel B. (C) Schematic diagram indicating how the distances from the ends were estimated. Diagram 1, positions and sizes of the probes used. Diagram 2, positions of the restriction sites. P, *Pst*I; SII, *Sac*II; HIII, *Hin*dIII; N, *Nsi*I; K, *Kpn*I. The position of *ermB* is also indicated. Diagram 3, method for estimations of the distance to the ends made for each restriction enzyme with the data from the Southern blot in Fig. 4A and B. Diagram 4, consolidated figure schematic with a rectangle indicating the position of the known sequence used as the probe in the Southern hybridization. The left end is at least 20 kb from an available sequence, while the right end is at least 15 kb from an available sequence.

We were able to obtain a 13-kb section of CTnBST, and we used this segment as a probe to evaluate the distribution and diversity of related elements. Two results were notable. First, we found examples of strains whose DNA cross-hybridized with the probe and yet did not carry the *ermB* gene. This result indicates that *ermB* is entering these elements and perhaps, in some cases, being lost by them. That is, there is a fluid situation in the evolution of this group of CTns. Second, it is obvious from the variety of restriction patterns seen on Southern blots that this type of CTn has more than one form. It is not clear whether this diversity reflects rapid evolution of this type of element or repeated separate entries of such CTns into *Bacteroides* species.

Many of the *ermB* strains that contained CTnBST also contained a CTnDOT-type element. Thus, unlike plasmids, the CTns seem not to exclude each other. There was no evidence found that they are stimulating transfer of one another, however. Another activity that both types of CTns seem to be capable of is the mobilization of coresident plasmids and NBU1, which is a mobilizable transposon (12, 16). This type of activity significantly increases the potential for CTns to participate in the transfer of genes that they themselves do not carry.

A trait not shared between CTnBST and CTnDOT is the stimulation of transfer by tetracycline. CTnBST transferred itself and coresident elements at the same frequency under all the conditions tested. This could mean that in the future CTnBST-type elements might one day come to rival the CTnDOT-type elements in distribution. A recent survey found that more than 80% of recently isolated strains from more than 10 different species carried a CTnDOT-type element (18). It remains to be seen whether CTnBST-type elements possess not only the transfer capacity under conditions within the colon but also the stability in a new host that causes them to be maintained in the absence of antibiotic selection. The fact that five of the six *ermB*-containing strains were not capable of transferring the resistance under laboratory conditions despite the fact that they contained at least a portion of CTnBST DNA might mean that these CTns, in contrast to the CTnDOT elements, readily lose their transfer capacity in *Bacteroides* strains. Alternatively, CTnBST may have acquired DNA that made it transmissible.

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