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Characterization of a New Type of *Bacteroides* Conjugative Transposon, Tc^r Em^r 7853

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Results of previous investigations suggested that the conjugative transposons found in human colonic *Bacteroides* species were all members of a closely related family of elements, exemplified by Tc^r Em^r DOT. We have now found a new type of conjugative transposon, Tc^r Em^r 7853, that does not belong to this family. Tc^r Em^r 7853 has approximately the same size as the Tc^r Em^r DOT-type elements (70 to 80 kbp) and also carries genes encoding resistance to tetracycline (Tc^r) and erythromycin (Em^r); however, it differs from previously described conjugative transposons in a number of ways. Its transfer is not regulated by tetracycline and its transfer genes are not controlled by the regulatory genes *rteA* and *rteB*, which are found on Tc^r Em^r DOT and related conjugative transposons. Its ends do not cross-hybridize with the ends of Tc^r Em^r DOT-type conjugative transposons, and the Em^r gene it carries does not cross-hybridize with *ermF*, the Em^r gene found on all previously studied *Bacteroides* conjugative transposons. There is only one region with high sequence similarity between Tc^r Em^r 7853 and previously characterized elements, the region that contains the Tc^r gene, *tetQ*. This sequence similarity ends 145 bp upstream of the start codon and 288 bp downstream from the stop codon. A 2-kbp region upstream of *tetQ* on Tc^r Em^r 7853 cross-hybridized with four additional *Eco*RV fragments of *Bacteroides thetaiotaomicron* 7853 DNA other than the one that contained *tetQ*. These additional cross-hybridizing bands were not part of Tc^r Em^r 7853, but one of them cotransferred with Tc^r Em^r 7853 in some matings. Thus, at least one of the additional cross-hybridizing bands may be associated with another conjugative element or with an element that is mobilized by Tc^r Em^r 7853. DNA that cross-hybridized with the upstream region was found in one clinical isolate of *Bacteroides ovatus* and four Tc^r isolates of *Prevotella ruminicola*.

Bacteroides species harbor two types of self-transmissible elements, plasmids and conjugative transposons (6, 8, 16). The conjugative transposons not only transfer themselves but can also mobilize coresident plasmids, unlinked integrated DNA elements called NBUs (11, 15), and a mobilizeable transposon, Tn*4399* (3). *Bacteroides* spp. are opportunistic human pathogens, and multiply resistant clinical isolates are becoming more common. The spread of resistance genes within the *Bacteroides* group appears to be mediated primarily by conjugative transposons and the elements that they mobilize (6); however, information about the range of transmissible elements found in *Bacteroides* spp. is still limited. The *Bacteroides* conjugative transposons characterized to date have all been closely related. All share sequence similarity at their ends, and in most cases this similarity extends throughout the element (8, 9, 15). Most are 70 to 80 kbp in size and carry the tetracycline resistance (Tc^r) gene, *tetQ*. Some also carry an erythromycin resistance (Em^r) gene, *ermF*. Transfer of the *Bacteroides* conjugative transposons is generally enhanced 100- to 1,000-fold by preexposure of donors to tetracycline. Tetracycline regulation is mediated by a three-gene operon containing *tetQ* and two genes, *rteA* and *rteB*, which encode a two-component regulatory system (14). *rteA* and *rteB* are essential not only for self-transfer of the element itself but also for mobilization of coresident plasmids (14). This family of conjugative transposons is exemplified by Tc^r Em^r DOT, a conjugative transposon originally found in a Tc^r Em^r clinical isolate, *Bacteroides thetaiotamicron* DOT (8).

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A variant of the Tc^r Em^r DOT type of conjugative transposon is Tc^r Em^r 12256, which is approximately twice as large as Tc^r Em^r DOT and appears to be a composite element consisting of a Tc^r Em^r DOT-type element inserted into an unrelated element (1, 9). Self-transfer of Tc^r Em^r 12256 is not enhanced by tetracycline; however, plasmid mobilization by this element is tetracycline regulated (16). Tc^r Em^r 12256, like the Tc^r Em^r DOT type of conjugative transposon, carries the *tetQ-rteA-rteB* operon, and this operon is clearly functional because a disruption in the operon eliminates both self-transfer and plasmid mobilization (13). Another variant member of the Tc^r Em^r DOT family is XBU4422, a cryptic conjugative transposon (9). The ends of XBU4422 have high sequence similarity with the ends of the Tc^r Em^r DOT-type elements; however, there are only scattered regions of similarity between the interior portions of XBU4422 and Tc^r Em^r DOT. In addition, XBU4422 does not carry the *tetQ-rteA-rteB* operon (1, 9).

In all human Tc^r *Bacteroides* clinical isolates studied to date, *tetQ* has proved to be located on a conjugative transposon of the Tc^r Em^r DOT type (4). This uniformity may be misleading, however, because *tetQ* has been found on a plasmid in an isolate of *Prevotella ruminicola*, a species of gram-negative anaerobe that is distantly related to the colonic *Bacteroides* spp. (2). *P. ruminicola* is one of the numerically predominant species of bacteria in the rumina and intestines of livestock animals. The *tetQ* gene on the *P. ruminicola* plasmid pRRI4 is virtually identical to the one found on Tc^r Em^r DOT, but the region of sequence similarity between pRRI4 and Tc^r Em^r DOT ends about 2 kbp upstream and 0.2 kbp downstream of *tetQ* (4, 10). A recent comparison of Tc^r genes found in natural isolates of *P. ruminicola* and Tc^r genes found in clinical isolates of human colonic *Bacteroides* spp. suggests that horizontal

transfer of *tetQ* is occurring in nature between the animal and human strains (4). Except for the strain containing pRRI4, all of the *tetQ* genes in *P. ruminicola* strains, like the *tetQ* genes in the human clinical isolates, appear to be chromosomally located, suggesting that conjugative transposons might be responsible for horizontal transfer of *tetQ* in nature. None of the Tc^r Em^r DOT-type conjugative transposons are capable of transferring from *Bacteroides* spp. to *P. ruminicola* (10). Only the variant element Tc^r Em^r 12256, which has about 70 to 80 kbp of extra DNA, was capable of this type of transfer. In addition, DNA from the *tetQ*-carrying strains of *P. ruminicola* did not cross-hybridize with the ends of Tc^r Em^r DOT-type elements. These findings suggest that if the *tetQ* alleles in *P. ruminicola* are transferred on conjugative transposons, these conjugative transposons are not members of the Tc^r Em^r DOT family and that the diversity of conjugative transposons in the *Bacteroides* phylogenetic group is greater than previously assumed.

Recently, in a screen of 12 newly acquired Tc^r *Bacteroides* clinical isolates, we found that DNA from one of these isolates cross-hybridized with a *tetQ* probe but failed to cross-hybridize with XBU4422, indicating that this isolate did not carry a conjugative transposon with ends of the Tc^r Em^r DOT-type elements. We report here that the *tetQ* gene in this strain is located on a conjugative transposon, Tc^r Em^r 7853, which defines a new family of *Bacteroides* conjugative transposons. This new conjugative transposon, like the *P. ruminicola* plasmid pRRI4, shares high sequence similarity with the Tc^r Em^r DOT-type conjugative transposons in a small region around *tetQ* but not outside that region.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The following *Bacteroides* clinical isolates were obtained from Sidney Finegold, Wadworth VA Hospital, Los Angeles, Calif.: *B. thetaiotaomicron* 7853 and 8702, *B. distasonis* 6781 and 6778, and *B. ovatus* 7991. Clinical isolates *B. fragilis* V479, *B. fragilis* 12256, and *B. thetaiotaomicron* DOT have been described previously (16). *B. distasonis* C30-45 was obtained from the culture collection of the Virginia Polytechnic Institute Anaerobe Laboratory (Blacksburg, Va.). All of these isolates were Tc^r. *B. thetaiotaomicron* 4100 (Thy$^-$) and 4001 (Rif^r), which were used in mating experiments, were derivatives of *B. thetaiotaomicron* 5484 (8, 16). Similarly, *B. uniformis* 1100 (Thy$^-$) and 1001 (Rif^r) were derivatives of *B. uniformis* 0061 (16). Both *B. thetaiotaomicron* 5482 and *B. uniformis* 0061 are sensitive to tetracycline and erythromycin. *B. uniformis* 0061 contains the cryptic element XBU4422 (9).

Bacteroides strains were grown in prereduced Trypticase (BBL Microbiology Systems, Cockeysville, Md.)-yeast extract-glucose (TYG) (7) broth or TYG agar. To test for tetracycline-dependent enhancement of conjugal transfer, bacteria were grown in TYG broth containing tetracycline (final concentration, 1 μg/ml) prior to mating, and tetracycline (0.1 μg/ml) was included in the agar plates on which the matings were done.

The *P. ruminicola* strains used in this study ($B_1$4, JSP3, N2-2, 20-63, and 20-78) have been described previously (4, 7). Except for *P. ruminicola* 20-63 and 20-78, which were isolated from the same pig, all were isolated from different animals in different geographical locations. All but *P. ruminicola* $B_1$4 were Tc^r and carried *tetQ* (4). *P. ruminicola* $B_1$4R, a spontaneous rifampin-resistant mutant of *P. ruminicola* $B_1$4 (7, 10), was used in the mating experiments with *Bacteroides* spp. *P. ruminicola* strains were grown in modified Bryant's medium (7, 10) or on an agar-based version of this medium. Both *Bacteroides* and *Prevotella* strains were incubated under an atmosphere of nitrogen (80%)-carbon dioxide (20%). *Escherichia coli* strains were grown in Luria broth or on Luria broth agar.

DNA isolation, cloning, and analysis. Plasmid isolation, restriction digest analysis, and Southern hybridization were done as described previously (8, 16). Pulsed-field electrophoresis of *Not*I-digested DNA from *B. thetaiotaomicron* 7853 was done as described previously (1). A Southern blot of the pulsed-field gel was hybridized with the ^{32}P-labeled 0.9-kbp internal *Eco*RI-*Eco*RV fragment of *tetQ*. The *tetQ* region of Tc^r Em^r 7853 was cloned using *tetQ* from Tc^r Em^r DOT (pNFD13-2) (5) as a hybridization probe. Southern analysis of chromosomal DNA from *B. thetaiotaomicron* 7853 cut with various restriction enzymes showed that *tetQ* was on a 7.5-kbp *Pst*I fragment. *Pst*I fragments in this size range were eluted from an agarose gel of *Pst*I-digested chromosomal DNA and cloned into the *Pst*I site of pFD160 (12). *E. coli* DH5α was transformed with this mixture, and transformants were screened by colony hybridization using the 2.7-kbp *tetQ*-containing segment of pNFD13-2 (5) as a probe. The clone that cross-hybridized with *tetQ* was designated pMPN78.

In experiments to determine whether DNA upstream and downstream of *tetQ* on the conjugative transposon in *B. thetaiotaomicron* 7853 cross-hybridized with DNAs from other Tc^r strains of *Bacteroides* spp. and *P. ruminicola*, specific fragments of the cloned region were isolated from a low-melting-point agarose gel, labeled with [^{32}P]dCTP, and used as a probe on Southern blots. DNA sequencing was done using the dideoxy-chain termination reaction with T7 polymerase and reagents provided in the Sequenase 2.0 kit (U.S. Biochemicals, Cleveland, Ohio). DNA sequences were analyzed using Genetics Computer Group software.

Matings. Matings between different *Bacteroides* strains or between *Bacteroides* spp. and *E. coli* were done on nitrocellulose filters as described previously (16). To obtain independent transconjugants for the pulsed-field gel analysis and Southern blotting experiments, eight separate matings were done between *B. thetaiotaomicron* 7853 (Tc^r Em^r Thy$^-$ Rif^s) and *B. thetaiotaomicron* 4001 (Tc^s Em^s Rif^r). A single Rif^r Tc^r Em^r transconjugant colony from each mating was streaked to obtain a single colony, and that colony was used in the analysis. These same transconjugants were also used in Southern blotting experiments to determine whether *tetQ* colocalized with the *Not*I bands containing element insertions. To determine whether the transmissible element in *B. thetaiotaomicron* 7853 retained its integrity during successive transfers and to determine the host range of the element, we mated *B. thetaiotaomicron* 7853 with *B. thetaiotaomicron* 4001 to produce *B. thetaiotaomicron* 4009. This strain was mated with *B. thetaiotaomicron* 4100 to produce *B. thetaiotaomicron* 4109. *B. thetaiotaomicron* 4109 was mated with *P. ruminicola* $B_1$4 to produce *P. ruminicola* $B_1$4/7853. Finally, this strain was mated with *B. thetaiotaomicron* 4001 to produce *B. thetaiotaomicron* 4009-2. DNAs from these strains were probed on Southern blots with the cloned *tetQ* region of the 7853 element (pMPN78). Matings between *Bacteroides* spp. and *P. ruminicola* were done on agar slants in sealed tubes as described previously (7, 10). Mating frequencies are expressed as the number of transconjugants per recipient. To confirm that the *tetQ*-containing region cloned in pMPN78 carried a functional *tetQ* gene, the plasmid was transferred from *E. coli* to *B. thetaiotaomicron* 4001 (Tc^s), and the resulting *B. thetaiotaomicron* transconjugants were tested for resistance to tetracycline.

TABLE 1. Self-transfer of Tc^r Em^r 7853

Donor[a]	Recipient[a]	Transfer frequency[b]
BT 7853	BT 4001	2×10^{-7}–8×10^{-7}
	PR $B_1$4R	1×10^{-6}–3×10^{-6}
BT 4109[c]	BT 4001	1×10^{-6}–2×10^{-6}
	PR $B_1$4R	3×10^{-7}–4×10^{-7}
BU 1109[d]	BU 1001	2×10^{-6}–3×10^{-6}
	PR $B_1$4R	1×10^{-5}–2×10^{-5}
PR $B_1$4R (Tc^r Em^r 7853)[e]	BT 4100	1×10^{-4}–2×10^{-4}
	BU 1100	2×10^{-6}–4×10^{-6}

[a] Abbreviations: BT, *B. thetaiotaomicron*; BU, *B. uniformis*; PR, *P. ruminicola*.
[b] Number of transconjugants per recipient. Frequencies shown are for matings in which no antibiotic was included in the medium. The same ranges of frequencies were seen when the donors were exposed to tetracycline, erythromycin, or both (data not shown).
[c] BT 4109 is a transconjugant from a mating of BT 4009 (transconjugant from a mating between BT 7853 and BT 4001) with BT 4100.
[d] BU 1109 is a transconjugant from a mating of BU 1009 (transconjugant from a mating of BT 7853 and BU 1001) with BU 1100.
[e] Transconjugant from a mating of BT 4109 and PR $B_1$4R.

RESULTS AND DISCUSSION

Identification of a new type of *tetQ*-carrying conjugative transposon. Previous investigations of Tc^r strains of human colonic *Bacteroides* strains have found that the Tc^r gene in these strains, *tetQ*, is generally transmissible and is located on a conjugative transposon whose ends cross-hybridize with the ends of XBU4422 (1, 9). DNA from clinical isolate *B. thetaiotaomicron* 7853, however, did not cross-hybridize with XBU4422, although it did have a single 3.6-kbp *Eco*RV band that cross-hybridized with an internal *Eco*RI-*Eco*RV segment of *tetQ* (not shown). Conjugation experiments with *B. thetaiotaomicron* 7853 as a donor and *B. thetaiotaomicron* 4001 as a recipient demonstrated that the *tetQ* gene of *B. thetaiotaomicron* 7853 could be transferred and that a Tc^r transconjugant from this mating, designated *B. thetaiotaomicron* 4009, was capable of retransferring *tetQ* to *B. thetaiotaomicron* 4100 (to generate *B. thetaiotaomicron* 4109) or to *B. uniformis* 1100 (Table 1). Thus, *tetQ* in *B. thetaiotaomicron* 7853 appeared to be carried on a transmissible element that was not related to the Tc^r Em^r DOT-type conjugative transposon.

B. thetaiotaomicron 7853 was resistant to erythromycin (Em^r) as well as to tetracycline. In matings between the *B. thetaiotaomicron* 4109 donor and either *B. thetaiotaomicron* 4001 or *B. uniformis* 1001 recipients, the Em^r gene cotransferred with *tetQ* and vice versa in all of the 840 transconjugants tested. This indicated that the Em^r gene was located on the same conjugal element as *tetQ*. In contrast to elements of the Tc^r Em^r DOT type, which carry the same Em^r gene, *ermF*, DNA from *B. thetaiotaomicron* 7853 did not cross-hybridize on Southern blots with a probe containing *ermF* (data not shown). Thus, the Em^r determinant carried on the conjugal element in strain 7853 was not *ermF*.

Transfer properties of the new conjugal element. Most of the Tc^r Em^r DOT-type elements characterized to date exhibit tetracycline-stimulated transfer (15, 16). This was not true of the conjugal element in *B. thetaiotaomicron* 7853. Transfer frequencies were unaffected by preincubation of donors with tetracycline, erythromycin, or a combination of the two (data not shown). The host range of the element in *B. thetaiotaomicron* 7853 also differed from that of the Tc^r Em^r DOT-type elements. Tc^r Em^r DOT can mobilize coresident plasmids to either *Bacteroides* or *E. coli* recipients (16). Plasmid mobilization, like element self-transfer, is stimulated by tetracycline (15, 16). *B. thetaiotaomicron* 4109 mobilized pRDB7, a chloramphenicol-resistant derivative of pRDB5 (7), to *B. thetaiotaomicron* 4001 at a frequency of 1×10^{-6} to 3×10^{-6} transconjugants per recipient; however, transfer of pRDB7 to *E. coli* HB101 or *E. coli* EM24 was not detected ($<10^{-9}$ transconjugants per recipient). This plasmid mobilization frequency between *Bacteroides* donors and *Bacteroides* recipients was lower than that seen when Tc^r Em^r DOT was the mobilizing element and the donor was stimulated with tetracycline. In the same strain background (*B. thetaiotaomicron* 4100 or 4001) and under the same conditions, Tc^r Em^r DOT mobilized pRDB7 to *E. coli* recipients at a frequency of 1×10^{-5} to 10×10^{-5} transconjugants per recipient. Even taking into account the lower mobilization frequency of the 7853 element in the matings of *Bacteroides* donors to *Bacteroides* recipients we should have been able to detect mobilization to *E. coli* recipients if it occurred.

Another indication of the difference in host range between the element in *B. thetaiotaomicron* 7853 and that of the Tc^r Em^r DOT-type elements was the ability of the 7853 element to transfer itself from *Bacteroides* spp. to *P. ruminicola* $B_1$4 (Table 1). Only one of the Tc^r Em^r DOT type of conjugative transposons is capable of transfer to *P. ruminicola* $B_1$4, the compound conjugative transposon Tc^r Em^r 12256 (10), and transfer of Tc^r Em^r 12256 to *P. ruminicola* could well be mediated by proteins encoded by the portion of the 12256 element that is unrelated to the Tc^r Em^r DOT elements. In matings of *B. thetaiotaomicron* 4109 or *B. uniformis* 1109 with *P. ruminicola* $B_1$4, Tc^r was transferred at frequencies of 10^{-7} and 10^{-5} per recipient, respectively (Table 1). The *tetQ* gene on the 7853 element conferred resistance on *P. ruminicola*, as expected from the fact that *tetQ* on Tc^r Em^r 12256 also conferred resistance on *P. ruminicola* (10). An unexpected finding was that the Em^r gene on the 7853 element also conferred resistance on *P. ruminicola* $B_1$4, although the plating efficiency on erythromycin-containing medium (20 to 30%) was lower than that normally seen with *Bacteroides* strains (100%). Previously, we had tested several different alleles of *ermF* for expression in *P. ruminicola*, and none of them was able to confer a selectable level of resistance on *P. ruminicola* $B_1$4 (7, 10).

A *P. ruminicola* transconjugant containing the 7853 element retransferred the element to *B. thetaiotaomicron* 4001 at a frequency that was 100-fold higher than the frequency of transfer from *B. thetaiotaomicron* 4109 to *P. ruminicola* $B_1$4 (Table 1). However, when *B. uniformis* 1100 was the recipient, the transfer frequency was the same as the transfer frequency from *B. uniformis* to *P. ruminicola* (Table 1). One explanation for the differences in transfer frequency to different recipients could be differences in the restriction-modification systems of donor and recipient that allow some copies of the incoming element to be degraded before they can integrate.

The conjugal element in *B. thetaiotaomicron* 7853 is integrated in the chromosome. No plasmids were visible in plasmid preparations made from *B. thetaiotaomicron* 7853, indicating that the transmissible element was a conjugative transposon rather than a plasmid. To test this, we used pulsed-field electrophoresis to compare *Not*I digest patterns of DNAs from eight independent transconjugants (of matings between *B. thetaiotaomicron* 7853 and *B. thetaiotaomicron* 4001) with the *Not*I digest pattern of *B. thetaiotaomicron* 4001. If the transmissible element in *B. thetaiotaomicron* 7853 was as large as the Tc^r Em^r DOT type of conjugative transposon, a shift should have been detectable in the *Not*I band into which the element inserted. Band shifts were observed in all transconjugants (Fig. 1A). In six of the eight transconjugants (lanes 2, 3, and 6 to 8 and a transconjugant [not shown] whose DNA had the same banding pattern as that shown in lane 2), the increase in size of

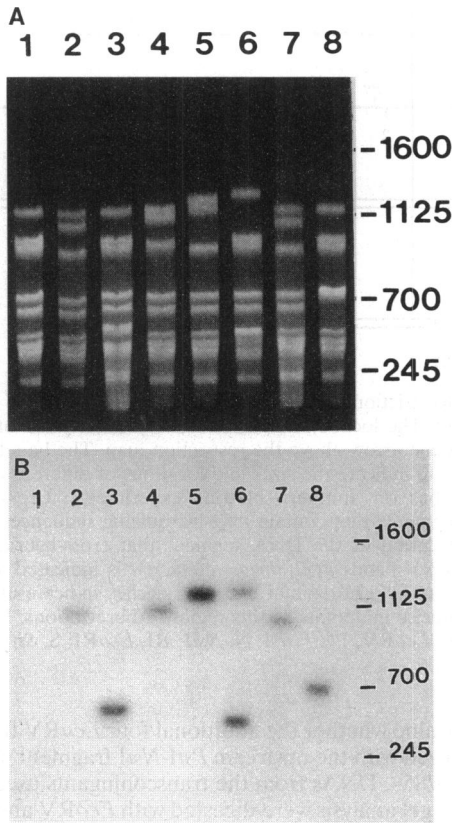


FIG. 1. (A) Ethidium-stained pulsed-field gel of *Not*I-digested DNAs from the recipient (*B. thetaiotaomicron* 4001 [lane 1]) and from seven transconjugants, each obtained from a separate mating between *B. thetaiotaomicron* 4109 and *B. thetaiotaomicron* 4001 (lanes 2 to 8). An eighth independent transconjugant (not shown) had the same pattern as that seen in lane 2. (B) Southern blot of the gel shown in panel A, probed with an internal 0.9-kbp *Eco*RI-*Eco*RV fragment from *tetQ*. In all cases, the *tetQ*-hybridizing bands were the ones that were shifted in molecular weight (A).

the shifted band indicated that the integrating element was 70 to 80 kbp in size. Two transconjugants exhibited band shifts that indicated that a larger insertion had occurred. In one case, the increase in band size was about 150 kbp (lane 4), and in another case it was about 220 kbp (lane 5). These larger band shifts could have resulted from duplication of a 70-kbp conjugative transposon or from tandem insertions in the same site. One transconjugant (lane 6) had 70- to 80-kbp shifts in two different *Not*I bands. This could have resulted either from two separate transfer and insertion events or from a second transposition event after the conjugative transposon had entered in the recipient. In previous studies of the Tc^r Em^r DOT-type conjugative transposons, no multiple insertions or duplications were observed (1); thus, this property of the 7853 element may reflect another difference between this element and the Tc^r Em^r DOT type of element. Southern hybridization analysis, with *tetQ* as the probe, demonstrated that all of the shifted *Not*I bands seen in Fig. 1 cross-hybridized with *tetQ* (Fig. 1B). Pulsed-field gel analysis and Southern blot analysis of transconjugants from different matings between different donor and recipient sets indicated that the 7853 element retained its integrity through sequential matings.

Results of the pulsed-field gel analysis suggest that the transmissible *tetQ* gene in *B. thetaiotaomicron* 7853 is carried on a conjugative transposon similar in size to the Tc^r Em^r DOT type of conjugative transposons (70 to 80 kbp). We have designated this conjugative transposon Tc^r Em^r 7853. Since the conjugative transposon integrated in the same *Not*I band (the band that is approximately 900 kbp in size in Fig. 1A) in four of the eight transconjugants, there may be a preferred integration site for Tc^r Em^r 7853 in this band. Nonetheless, the fact that insertions occurred in several other *Not*I bands raises the possibility that there is more than one integration site in the chromosome. The Tc^r Em^r DOT-type conjugative transposons also have a limited number of sites in the chromosome but are not absolutely site specific (1).

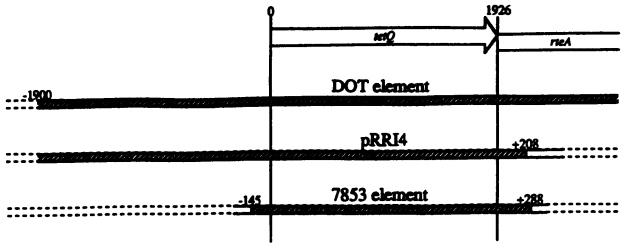
The *tetQ* region of Tc^r Em^r 7853. The *tetQ* gene of the Tc^r Em^r DOT-type conjugative transposons is the first gene in a three-gene operon that also contains two regulatory genes, *rteA* and *rteB*, which are essential for transfer (14). Southern hybridization experiments, using a hybridization probe (p6T3) that contained the entire *tetQ-rteA-rteB* operon (14, 15), showed that Tc^r Em^r 7853 lacked at least part of the DNA in this region. That is, only a single 7.5-kbp *Pst*I fragment of Tc^r Em^r 7853 DNA cross-hybridized with the 13-kbp segment of Tc^r Em^r DOT cloned in p6T3. We cloned the cross-hybridizing 7.5-kbp *Pst*I fragment of Tc^r Em^r 7853. Transfer of this cloned region (on pMPN78) into *B. thetaiotaomicron* 4001 rendered the transconjugants Tc^r, confirming that the *tetQ* cross-hybridizing DNA in Tc^r Em^r 7853 actually encoded a functional Tc^r gene.

Isolated fragments of the cloned region were used as DNA hybridization probes to locate approximately where the high similarity between Tc^r Em^r 7853 and Tc^r Em^r DOT ended on either side of *tetQ*. The DNA sequences of these regions of Tc^r Em^r 7853 were then obtained and compared with the DNA sequences of the corresponding regions on Tc^r Em^r DOT (Fig. 2). The DNA sequences of Tc^r Em^r DOT and Tc^r Em^r 7853 were virtually identical in the region immediately upstream and downstream of *tetQ*; however, this sequence identity ended abruptly 288 bp downstream of the stop codon of *tetQ* and 145 bp upstream of the start codon.

The *tetQ*-carrying *P. ruminicola* plasmid, pRRI4, also has high sequence similarity with Tc^r Em^r DOT in its *tetQ* region but has little similarity with Tc^r Em^r DOT outside this region (10). To determine whether the endpoints of similarity between Tc^r Em^r DOT and pRRI4 were the same as those between Tc^r Em^r DOT and Tc^r Em^r 7853, as would be expected if the same *tetQ* cassette had inserted in both cases, we obtained the DNA sequence of the regions upstream and downstream of *tetQ* in pRRI4. Downstream of the stop codon of *tetQ* on pRRI4, there was an abrupt endpoint of sequence identity with Tc^r Em^r DOT; however, the location of the endpoint (208 bp from the stop codon of *tetQ*) was different from that of the endpoint downstream of *tetQ* on Tc^r Em^r 7853 (Fig. 2). The upstream endpoint of the region of identity between pRRI4 and Tc^r Em^r DOT was also different from that of Tc^r Em^r 7853 and was at least 1,900 bp upstream of the start codon of *tetQ*. Thus, *tetQ* appears not to be carried on a mobile cassette of constant size. There were no obvious sequence similarities between the DNAs adjacent to the upstream and downstream similarity endpoints on the two elements (Fig. 2B). Both Tc^r Em^r 7853 and pRRI4 had a short segment of *rteA*, which was virtually identical in sequence to *rteA* of Tc^r Em^r DOT; however, the rest of *rteA* and all of *rteB* were missing.

We tested segments of pRRI4 outside the *tetQ* region for homology to Tc^r Em^r 7853. Three *Eco*RI fragments of pRRI4 (1, 4.5, and 10.5 kbp), which cover virtually all of this 19.5-kbp plasmid that lies outside the 3.5-kbp *tetQ* region, were used as

A



B

UPSTREAM SIMILARITY ENDPOINTS

```
     -195                                                        -136
7853   TGCTCAGGAA CTTATAAATA AAGTTAAATC AAAGTGTTGC GGAATTAAGG GTGTAATTTT
DOT    GTTAAAAAAT CCT..... ....CCTACT TTTGTTAGAT ATATTTTTTT GTGTAATTTT
pRRI4  GTTAAAAAAT CCTTAAAAAA TCCTCCCACT TTTGTTAGAT ATATTTTTTT GTGTAATTTT
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DOWNSTREAM SIMILARITY ENDPOINTS

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          +198                                                  +247
pRRI4      GGTGAAACAA TGACAAGAAT TTCCGCATCA TCAATAATAG TATTGCTGCC
DOT, 7853  GGTGAAACAA TACTGGAATG GAACGATAAA GATATCGAGC ATTACCATGC

          +278                                                  +327
7853       CCGTTTCAAG GATTACAAGG AAATATATAT CGAACCGAAC CAATTATCAT
DOT        CCGTTTCAAG GCCACCGAGC CAGCAGAGCG CATCGATAGT GTGCGCAGTC
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FIG. 2. (A) Comparison of the *tetQ* regions of Tc^r Em^r DOT, pRRI4, and Tc^r Em^r 7853. The nucleotide corresponding to the start codon of *tetQ* is designated by 0, and the nucleotide corresponding to the stop codon is designated by 1926. The cross-hatched areas indicate where the elements have nearly identical DNA sequences. The endpoints of identity are indicated by the numbers of bases upstream of the start codon of *tetQ* or downstream of the stop codon. (B) DNA sequences of the regions around the upstream and downstream similarity endpoints. The vertical arrows indicate the end of identity between Tc^r Em^r 7853 and Tc^r Em^r DOT or (in the case of the downstream endpoints) between pRRI4 and Tc^r Em^r DOT.

probes on Southern blots containing *Eco*RV digests of DNA from *B. thetaiotaomicron* 7853 or 4109. The 10.5-kbp fragment was the only one that cross-hybridized with DNAs from these strains. The hybridization was weak and was to the 3.6-kbp *Eco*RV fragment of band that contains the amino-terminal portion of *tetQ*. The observed cross-hybridization probably represents the 200 bp of sequence identity between pRRI4 and Tc^r Em^r 7853 that lies immediately upstream of the start codon of *tetQ* (Fig. 2) and is located at one end of the 10.5-kbp *Eco*RI fragment of pRRI4. This result suggests that Tc^r Em^r 7853 does not share regions of sequence similarity to pRRI4 outside the *tetQ* region.

Repetitive DNA segment. An interesting feature of the *tetQ* region of Tc^r Em^r 7853 was revealed when a 2.7-kbp *Pst*I-*Nsi*I fragment containing the region upstream of *tetQ* (1 in Fig. 3) was used to probe Southern blots of *Eco*RV-digested DNA from *B. thetaiotaomicron* 7853. Five bands cross-hybridized with this fragment (Fig. 4), whereas only two cross-hybridizing bands were expected on the basis of the restriction map of the cloned region (Fig. 3). By contrast, an internal 0.9-kbp *Eco*RI-*Eco*RV fragment of *tetQ* and a 1.7-kbp *Sst*I-*Pst*I fragment downstream of *tetQ* (5 and 6, respectively, in Fig. 3) hybridized to only one band of the size expected from the restriction map of the region.

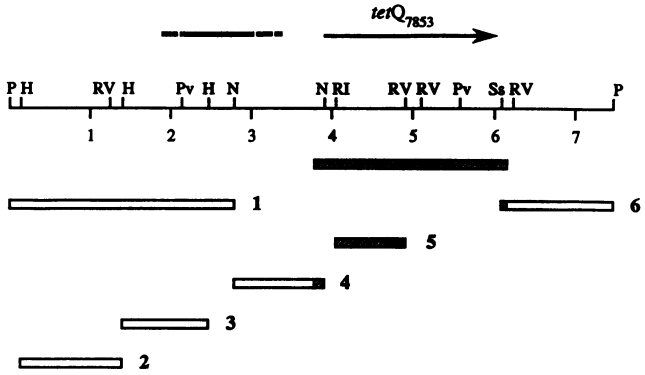

FIG. 3. Restriction map of the 7.5-kbp region of Tc^r Em^r 7853 that contains *tetQ*. The location and direction of transcription of *tetQ* are indicated by the arrow above the restriction map. The bars below the restriction map indicate the sizes and locations of different fragments used as probes on Southern blotting experiments. Cross-hatching indicates fragments that contain *tetQ*-hybridizing sequences. The approximate location of the DNA segment that cross-hybridizes with multiple *Eco*RV bands in *B. thetaiotaomicron* is indicated above the restriction map by a horizontal line. The dashes indicate uncertainty about the precise endpoints of this region. Abbreviations: P, *Pst*I; H, *Hin*dIII; RV, *Eco*RV; Pv, *Pvu*II; N, *Nsi*I; RI, *Eco*RI; S, *Sst*I.

To determine whether the additional four *Eco*RV fragments that hybridized with the upstream *Pst*I-*Nsi*I fragment were part of Tc^r Em^r 7853, DNAs from the transconjugants used for the pulsed-field gel analysis were digested with *Eco*RV and probed with the *Pst*I-*Nsi*I fragment. In most transconjugants, only the two *tetQ*-linked bands were seen, but in three transconjugants there was one extra band (Fig. 4). In two cases, the extra band comigrated with one of the bands in *B. thetaiotaomicron* 7853. In the third case, the extra band had a size different from any of those seen in *B. thetaiotaomicron* 7853. These results suggest that, whatever the identity of the DNA fragments that hybridize with the upstream fragment, they are not part of Tc^r Em^r 7853. However, at least one of them appears to be associated with an element that is cotransferred with Tc^r Em^r 7853 at a fairly high frequency. Since no plasmids were visible in *B. thetaiotaomicron* 7853 and no new plasmids appeared in the transconjugants, the mobilized element is probably another

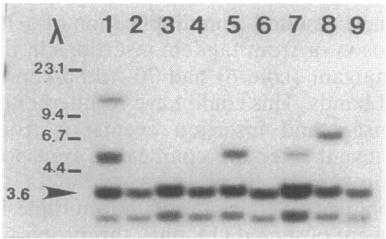

FIG. 4. Southern blot of *Eco*RV-digested DNA from *B. thetaiotaomicron* 7853 (lane 1) and seven independent transconjugants from a mating between *B. thetaiotaomicron* 7853 and *B. thetaiotaomicron* 4001 (lanes 2 to 9). These are the same transconjugants, arranged in the same order, as those shown on the pulsed-field gel in Fig. 1. The probe was fragment 1 in Fig. 3. The location of the 3.6-kbp *Eco*RV band that cross-hybridized with *tetQ* (fragment 5 in Fig. 3) is indicated by a horizontal arrow. What appears in this exposure to be a single thick band of *B. thetaiotaomicron* 7853 DNA that falls between the 4.4- and 6.7-kbp λ standards was revealed to be a doublet on other blots or with a lighter exposure.

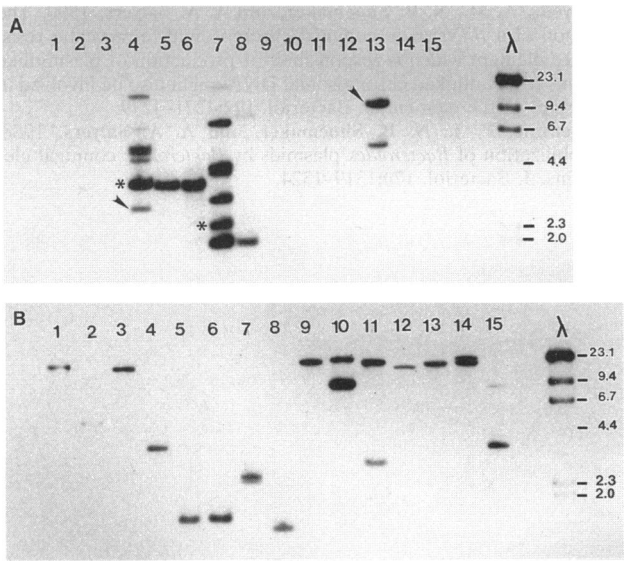

FIG. 5. (A) *Eco*RV-digested DNAs from *Bacteroides* clinical isolates known to contain a Tc^r Em^r DOT-like element (*B. fragilis* 12256 [lane 1], *B. fragilis* V479 [lane 2], and *B. thetaiotaomicron* DOT [lane 3]), *B. thetaiotaomicron* 7853 (lane 4), *P. ruminicola* strains (JSP3 [lane 5], N2-2 [lane 6], 20-63 [lane 7], and 20-79 [lane 8]), and previously unstudied *Bacteroides* Tc^r clinical isolates (*B. distasonis* 6781 [lane 9], *B. distasonis* 6779 [lane 10], *B. distasonis* 30-45 [lane 11] *B. thetaiotaomicron* 8702 [lane 12], *B. ovatus* 7991 [lane 13], *B. vulgatus* 8526 [lane 14], and *B. fragilis* 8371 [lane 15]). The blot was probed sequentially, first with fragment 2 and then with fragment 3 (Fig. 3). The blot was then stripped and reprobed with fragment 5. Arrows indicate bands that hybridized with fragment 2. Other bands appeared only after the blot was probed with fragment 3. Asterisks indicate bands that cross-hybridized with fragment 5 (see panel B). (B) The same *Eco*RV digests as those shown in panel A, probed with the *tetQ* probe (fragment 5 in Fig. 3). λ, *Hin*dIII lambda standards.

integrating element. If so, this would explain why one of the bands (lane 8 of Fig. 4) does not comigrate with any of the bands in *B. thetaiotaomicron* 7853. We also probed a Southern blot of the pulsed-field gel shown in Fig. 1 with the *Pst*I-*Nsi*I fragment. In one of the three transconjugants with extra *Eco*RV bands (lane 8 of Fig. 4), this probe detected a second *Not*I fragment, in addition to the one containing Tc^r Em^r 7853; however, this was not the case for the other two transconjugants with extra *Eco*RV bands (lanes 5 and 7 of Fig. 4). Thus, in at least one case, the element that was cotransferred with Tc^r Em^r 7853 inserted in a place different from that of Tc^r Em^r 7853.

By using smaller fragments of the *tetQ* upstream region as probes (fragments 3 and 4 in Fig. 3), we were able to localize the DNA that was hybridizing with the extra bands in *B. thetaiotaomicron* 7853 to within a 2-kb region immediately upstream of *tetQ*. Fragments marked 3 and 4 (Fig. 3) cross-hybridized with multiple bands, whereas the other fragments hybridized only with a single band in *Eco*RV-digested DNA from *B. thetaiotaomicron* 7853 and the transconjugants.

To determine if the region upstream of *tetQ* in Tc^r Em^r 7853 is commonly found in clinical *Bacteroides* isolates, we probed *Eco*RV-digested DNA from various Tc^r clinical isolates with fragments 1, 2, and 3 (Fig. 3). There was no cross-hybridizing DNA in any of the clinical *Bacteroides* isolates tested except for one strain of *B. ovatus*, strain 7991 (Fig. 5). DNA from this strain hybridized with fragments 1, 2, and 3. Although regions homologous to the *tetQ* upstream region of Tc^r Em^r 7853 were not commonly found in clinical *Bacteroides* isolates, four Tc^r strains of *P. ruminicola* had at least one *Eco*RV fragment that cross-hybridized with all of the probes except fragment 2. In two cases, one of the bands that cross-hybridized with the *tetQ* upstream region also hybridized with an internal fragment of *tetQ* (Fig. 5B), indicating that *tetQ* is probably linked to the upstream DNA segment in these strains. Other restriction digest data (not shown) supported the hypothesis that *tetQ* was linked with the same upstream region in the four *P. ruminicola* strains. DNAs from these strains did not cross-hybridize with the downstream fragment from Tc^r Em^r 7853, fragment 6 in Fig. 3, so the cross-hybridizing DNAs in the *P. ruminicola* strains were not part of a Tc^r Em^r 7853 type of element. The results of these experiments suggest that the DNA segment upstream of *tetQ* in Tc^r Em^r 7853 is widespread in *P. ruminicola* strains. The multiple bands seen in some strains raise the possibility that the hybridizing region contains an insertion sequence; however, we do not yet have evidence that the region contains a transposable element. Although cross-hybridizing DNA was found in many *P. ruminicola* strains, there was no cross-hybridizing DNA on the *tetQ*-carrying *P. ruminicola* plasmid, pRRI4, outside *tetQ* (not shown).

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