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A *Bacteroides* Tetracycline Resistance Gene Represents a New Class of Ribosome Protection Tetracycline Resistance

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The ribosome protection type of tetracycline resistance (Tc^r) has been found in a variety of bacterial species, but the only two classes described previously, Tet(M) and Tet(O), shared a high degree of amino acid sequence identity (>75%). Thus, it appeared that this type of resistance emerged recently in evolution and spread among different species of bacteria by horizontal transmission. We obtained the DNA sequence of a Tc^r gene from *Bacteroides*, a genus of gram-negative, obligately anaerobic bacteria that is phylogenetically distant from the diverse species in which *tet*(M) and *tet*(O) have been found. The *Bacteroides* Tc^r gene defines a new class of ribosome protection resistance genes, Tet(Q), and has a deduced amino acid sequence that was only 40% identical to Tet(M) or Tet(O). Like *tet*(M) and *tet*(O), *tet*(Q) appears to have spread by horizontal transmission, but only within the *Bacteroides* group.

Bacteroides species are significant opportunistic pathogens of humans (8), and many clinical isolates of *Bacteroides* are now resistant to tetracycline. Recently, a 2.7-kb *Sst*I fragment carrying a tetracycline resistance (Tc^r) gene was cloned from the *Bacteroides thetaiotaomicron* clinical isolate DOT (38, 44). The cloned gene appeared to be the only Tc^r gene in the strain of origin because a gene disruption made by using the 0.9-kb *Eco*RI-*Eco*RV fragment, which is shown in the present report to be internal to the Tc^r gene, made the strain susceptible to tetracycline (44).

There are three known types of tetracycline resistance mechanisms: tetracycline efflux, ribosome protection, and tetracycline modification (21, 36). Of these, the ribosome protection type of resistance appears to be the most widespread in nature. It has been found in a variety of bacteria including mycoplasmas and gram-positive and gram-negative organisms, whereas the efflux type of resistance seems to be limited mainly to members of the family *Enterobacteriaceae* and other γ-purple bacteria and to several species of gram-positive bacteria. Tetracycline modification has been found only in two strains of the obligately anaerobic genus *Bacteroides* and is an anomaly because it does not confer tetracycline resistance in *Bacteroides* species and functions as a resistance mechanism only in *Escherichia coli* strains grown under aerobic conditions (43).

Tc^r genes are classified on the basis of DNA hybridization. Two genes fall into the same Tc^r class if they cross-hybridize by Southern blot analysis under conditions of high stringency. Many classes of tetracycline efflux genes have been found, but only two classes of ribosome protection resistance genes, *tet*(M) and *tet*(O), have been defined previously (1, 22, 42). Here we report that the Tc^r in *Bacteroides* species is of the ribosome protection type.

Several representatives of *tet*(M) and *tet*(O) have been sequenced (19, 22, 24, 26, 38, 42), and the deduced amino acid sequences share a remarkable degree of identity. Members of the Tet(M) class share 92 to 97% amino acid sequence identity, and members of the Tet(O) class share 98% identity. Amino acid sequence identity between members of Tet(M) and Tet(O) is also high (75 to 77%). These similarities seemed to indicate not only that *tet*(M) and *tet*(O) are spread by horizontal transmission but also that the ribosome protection type of resistance gene appeared relatively recently in evolution. It has been proposed that *tet*(O) originated in gram-positive bacteria and has spread horizontally to gram-negative bacteria (42).

Although *tet*(M) and *tet*(O) have been found in a variety of gram-negative and gram-positive bacteria, bacterial genera of these types do not represent the full range of phylogenetic diversity seen in the bacteria. Thus, the apparent homogeneity of ribosomal protection Tc^r genes could be due in part to an incomplete sampling of bacterial isolates. Since *Bacteroides* species are phylogenetically distant from the organisms in which *tet*(M) and *tet*(O) have been found, it was possible that other extant classes of ribosome protection Tc^r genes might be found in this genus. Here we report the DNA sequence and characteristics of a *Bacteroides* Tc^r gene. We report that although this *Bacteroides* resistance gene shares some amino acid identity with Tet(M) and Tet(O), it is much more distantly related to these proteins than the proteins are to each other. We designated this new ribosome protection tetracycline resistance gene *tet*(Q).

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. Unless otherwise indicated, all experiments involving *Bacteroides* species were done with *B. thetaiotaomicron* BT4001, a spontaneous Rif^r derivative of the *B. thetaiotaomicron* type strain 5482 (Tc^s [16]). The following strains were used in a survey in which the genomic DNAs of *Bacteroides* isolates were screened by Southern blotting for hybridization to the internal *Eco*RI-*Eco*RV fragment from the cloned *tet*(Q) gene: *B. caccae* 8608 and *B. thetaiotaomicron* 2808, Tc^r clinical isolates (16); *B. distasonis* C30-45, *B. distasonis* 6308, *B. uniformis* 3537, *B. uniformis* T1-1 (16), and *B. uniformis* C7-17 (15), Tc^r fecal isolates; *B. distasonis* 6779 (Tc^r), *B. distasonis* 6781 (Tc^r Ap^r), *B. fragilis* 8371 (Tc^r Em^r), *B. ovatus* 7991 (Tc^r Em^r), *B. thetaiotaomicron* 7853 (Tc^r Em^r), *B. thetaiotaomicron* 8702 (Tc^r), and *B. vulgatus* 8526 (Tc^r Ap^r), clinical isolates that were provided by Colin Getty, Wadsworth Veterans Administration Hospital, Los Angeles, Calif.; and *B. fragilis* AK87, a Tc^r clinical isolate

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which was provided by A. Kuritza, Yale University Medical School, New Haven, Conn. Also included in the survey were the following *B. thetaiotaomicron* BT4001 transconjugants from different Tc^r clinical isolates carrying self-transmissible chromosomal elements: *B. thetaiotaomicron* BT4002 (Tc^r transconjugant from *B. fragilis* V479 [40]), *B. thetaiotaomicron* BT4003 (Tc^r Em^r transconjugant from *B. fragilis* CEST), *B. thetaiotaomicron* BT4004 (Tc^r Em^r transconjugant from *B. fragilis* ERL [40]), *B. thetaiotaomicron* BT4005 (Tc^r transconjugant from *B. fragilis* Tc^r Em^r ERL), *B. thetaiotaomicron* BT4007 (Tc^r Em^r transconjugant from *B. thetaiotaomicron* DOT2 [40]), and *B. thetaiotaomicron* BT4008 (Tc^r Em^r transconjugant from *B. fragilis* 12256 [40]). *B. caccae* 3452A, *B. distasonis* 4243, and *B. thetaiotaomicron* 5482 are Tc^s Em^s type strains (16) that were used as controls. *B. thetaiotaomicron* BT4001 and *B. uniformis* BU1001 (Tc^s Em^s; Rif^r derivative of *B. uniformis* 0061 [39]) were also used as controls.

The plasmids used and constructed in this study were based on pFD160, a pUC19-pBI143 chimeric shuttle vector (41). *Bacteroides* strains were grown either in prereduced Trypticase (BBL Microbiology Systems)-yeast extract-glucose (TYG [15]) under an 80% N_2–20% CO_2 atmosphere or on TYG agar plates in a GasPak jar. For most experiments involving *E. coli*, the strain used was *E. coli* DH5α (Tc^s [13]). For maxicell experiments, *E. coli* LCD44 (RecA MetE Tc^s) was used (5). *E. coli* strains were grown in Luria broth (LB) or on LB agar plates unless otherwise indicated.

Plasmids were introduced into *E. coli* by the method of Lederberg and Cohen (20). To test plasmids for Tc^r expression in *B. thetaiotaomicron*, constructs were mobilized from *E. coli* as described previously (39). Since transfer frequencies were several log units above the background, failure to attain a Tc^r transconjugant was a reasonable indication that a construct failed to express Tc^r.

DNA isolation and analysis. Plasmids were isolated from *E. coli* or *Bacteroides* species by the Ish-Horowitz modification of the procedure of Birnboim and Doly (23). Chromosomal DNAs from *Bacteroides* species were isolated by the method of Saito and Miura (34). Restriction digests and ligation with T4 DNA ligase were done by standard procedures (23). Electrophoretic resolution of restriction digests was done in 0.8 to 1.0% agarose slab gels in 1× or 4× GGB (1× GGB is 0.04 M Tris, 0.02 M sodium acetate, and 0.002 M EDTA). For Southern hybridization experiments, *Bacteroides* chromosomal DNA was digested with both *Eco*RI and *Eco*RV, electrophoresed on a 1% agarose gel, and capillary blotted (23) onto a polyester-reinforced nitrocellulose membrane (Schleicher & Schuell, Keene, N.H.). The probe, the internal 0.9-kb *Eco*RI-*Eco*RV fragment from *tet*(Q), was labeled by nick translation with [α-^{32}P]dCTP (800 Ci/mmol [29]). The high-stringency hybridization and wash conditions have been described previously (39). Low-stringency hybridization conditions were the same as those for high-stringency hybridization, except that the hybridization solution did not contain formamide and blots were washed at 25°C. Blots of genomic digests of strains containing the cloned *Bacteroides* Tc^r gene were probed under low-stringency conditions with ^{32}P-labeled pAM120 (9) to test the similarity of the Tc^r gene to *tet*(M) gene from the streptococcal Tn*916*.

MIC of tetracycline. In most experiments in which *E. coli* was used as the host, MICs were attained by LB broth dilution to serial increments of 5 μg of tetracycline per ml by using a final inoculum of 10^7 CFU/ml (subculture volume, 0.1 ml). Visible growth was scored after 12 and 24 h of incubation at 37°C. In one experiment, the tetracycline MIC was determined by streaking the *E. coli* host onto LB agar plates containing 5-μg/ml increments of tetracycline. Growth was scored after 24 and 48 h of incubation at 37°C. When a *Bacteroides* species was the host, MICs were determined by broth dilution in TYG broth with serial increments of 5 μg of tetracycline per ml by using a final inoculum of 10^7 CFU/ml. Growth was scored after 24 h of incubation at 37°C.

Analysis of the proteins expressed from the clone. The maxicell procedure was executed as described by Sancar et al. (37), with *E. coli* LCD44 used as the host. Maxicell fractionation was performed by an adaptation of the method of Tai and Kaplan (45). In some experiments, proteins expressed from the cloned segment were detected by using an *E. coli*-derived in vitro transcription-translation system (7) in kit form (Amersham, Arlington Heights, Ill.). In both cases, proteins were solubilized in sodium dodecyl sulfate (SDS) at 37°C and were then resolved on highly cross-linked 11% SDS-polyacrylamide gels.

DNA sequencing and analysis. Progressive unidirectional deletions were introduced into the cloned 2.7-kb *Sst*I fragment by an adaptation of the exonuclease III procedure of Henikoff (14) in kit form (Erase-a-Base system; Promega, Madison, Wis.). Both strands were sequenced by the dideoxy-chain termination reaction with the T7 DNA polymerase variant and reagents provided in the Sequenase 2.0 kit (U.S. Biochemicals, Cleveland, Ohio). Computer analysis of nucleotide and amino acid sequences was performed by using Genetics Computer Group software (6) on a MicroVAX system. Pairwise comparisons of sequences were made by using the GAP program. The GAP program uses the algorithm of Needleman and Wunsch to align sequences (25). Sequence similarity searches were done by using the FASTA program (28). The sequences that were compared and used to construct the amino acid alignment for this study were *tet*(M) from *Staphylococcus aureus* (GenBank accession number M21136), *Streptococcus faecalis* (GenBank accession number X04388), and *Ureaplasma urealyticum* (GenBank accession number X06901) and *tet*(O) from *Campylobacter jejuni* (GenBank accession number M18896) and *Streptococcus mutans* (GenBank accession number M20925).

Nucleotide sequence accession number. The full DNA sequence of the 2.7-kb *Sst*I fragment has been deposited in the EMBL Data Library (accession number X58717).

RESULTS

Characteristics of the *Bacteroides* Tc^r gene product. Both orientations of the cloned *Sst*I fragment (pNFD13-2 and pNFD13-6; Fig. 1) conferred resistance in *Bacteroides* species. The MIC of tetracycline for *B. thetaiotaomicron* bearing pNFD13-2 in broth cultures was 80 μg/ml. *Bacteroides* strains lacking Tc^r did not grow in 2 μg of tetracycline per ml.

Previous experience with *Bacteroides* antibiotic resistance genes indicated that these genes are not expressed in *E. coli* (11, 36, 50). Nevertheless, both clones of the *B. thetaiotaomicron* DOT Tc^r gene, pNFD13-2 and pNFD13-6, conferred Tc^r in *E. coli* DH5α. The MIC was 40 to 50 μg/ml when the MIC was determined with broth cultures. If susceptibility was determined by using LB agar plates, the MIC was much lower (10 μg/ml). The fact that the *Bacteroides* gene was expressed in *E. coli* allowed us to localize the gene and characterize the gene product using techniques developed for use in *E. coli*.

Deletions into the leftward *Eco*RV site (pNFD13-

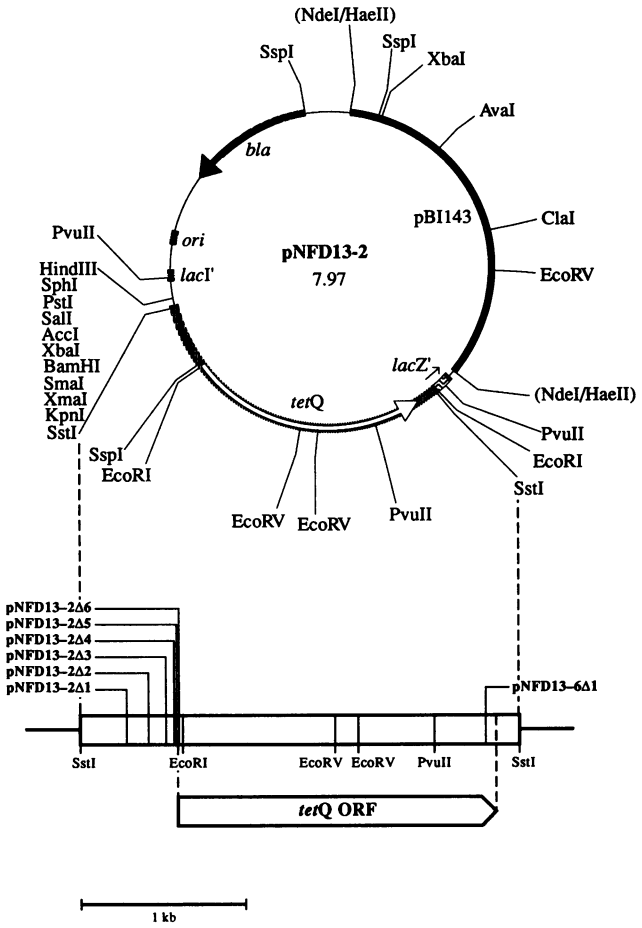

FIG. 1. Partial restriction map of pNFD13-2 showing the 2.7-kb *Sst*I insertion into the *Sst*I site of pFD160. pNFD13-6 (not shown) has the same insert but in the opposite orientation. The endpoints of important deletion derivatives are indicated by the labeled brackets. The orientation and extent of the large open reading frame (ORF) encoding Tet(Q) are indicated below by the arrow.

2Δ*Eco*RV) or to the *Eco*RI site (pNFD13-2Δ*Eco*RI) abolished resistance, indicating that the Tc^r gene spans the 0.9-kb *Eco*RV-*Eco*RI segment (Fig. 1). The gene was further localized by making unidirectional exonuclease III deletions in the insert DNA. When the deletions were tested in *E. coli*, pNFD13-2Δ1, pNFD13-2Δ2, and pNFD13-2Δ3, which were deletions ending 0.3, 0.2, and 0.1 kb, respectively, from the left of the *Eco*RI site, did not affect the level of resistance. pNFD13-2Δ4, which extended to within 0.1 kb of the *Eco*RI site, reduced the level of resistance but did not eliminate it completely. pNFD13-2Δ5, which also extended to within 0.1 kb of the *Eco*RI site and beyond the endpoint of pNFD13-2Δ4, abolished resistance. Deletions beyond the *Eco*RI site, such as pNFD13-2Δ6, also eliminated resistance. The smallest deletion from the right, pNFD13-6Δ1, extended 0.2 kb from the right end of the cloned fragment and eliminated resistance. Thus, approximately 2.1 kb of DNA was required in order to express Tc^r in *E. coli*. A slightly larger segment was needed to confer resistance in *Bacteroides* species. The largest deletion that conferred resistance in *Bacteroides* species was pNFD13-2Δ2.

In *E. coli* maxicells, two proteins which were not expressed from the base vector pFD160 were strongly expressed from pNFD13-2. These were estimated to have molecular masses of 76 and 25.5 kDa (data not shown). Both of these proteins, along with additional proteins not seen in the maxicells, were seen when pNFD13-2 or its deletion derivatives were used as templates in an in vitro transcription-translation system (Fig. 2A). The presence of the 76-kDa protein coincided with resistance. In contrast, the 25.5-kDa protein and the other proteins were still produced from the Tc^s deletion template, pNFD13-2Δ5 (Fig. 2A, lane 3). Also, although the 76-kDa protein was produced from pNFD13-6, the reverse orientation clone, the 25.5-kDa protein was not apparent (data not shown). In *E. coli* maxicells, the 76-kDa protein partitioned predominantly with the soluble fraction (Fig. 2B).

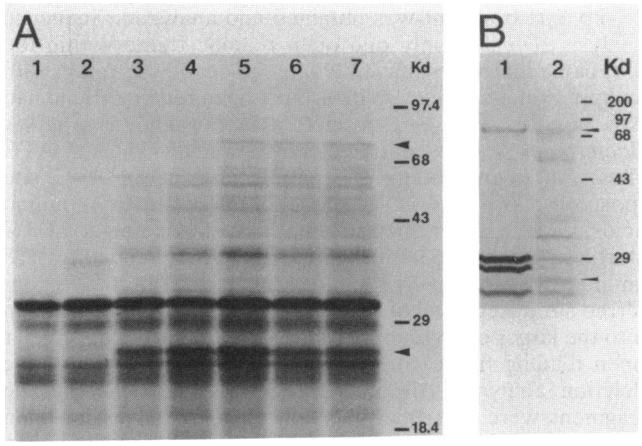

FIG. 2. Products of the 2.7-kb *Sst*I clone in *E. coli*. (A) Autoradiogram of an SDS-polyacrylamide gel of in vitro transcription-translation products. Lane 1, products from the vector control pFD160. Shown also are products from Tc^s deletion derivatives pNFD13-2Δ*Eco*RV (lane 2) and pNFD13-2Δ5 (lane 3), reduced Tc^r deletion derivative pNFD13-2Δ4 (lane 4), Tc^r deletion derivatives pNFD13-2Δ3 (lane 5) and pNFD13-2Δ1 (lane 6), and intact pNFD13-2 (lane 7). The arrowheads in the right margin mark the two bands that were consistently unique to the *Sst*I clone in maxicells. (B) Autoradiogram of soluble and membrane fractions from maxicells containing pNFD13-2. Lane 1, soluble fraction; lane 2, membrane fraction. Kd, kilodaltons.

Distribution of the cloned Tc^r gene among *Bacteroides* species. Resistance to tetracycline has been reported to be widespread in human colonic *Bacteroides* species (46). To determine whether the Tc^r gene cloned in pNFD13-2 was the predominant type of resistance in the colonic *Bacteroides* species, 21 Tc^r clinical isolates and 5 Tc^s strains were screened for hybridization with the *Eco*RV-*Eco*RI internal segment on Southern blots of genomic DNA digested with *Eco*RV and *Eco*RI. These strains represented a variety of *Bacteroides* species from a variety of sources and geographic locations. All of the Tc^r strains but none of the Tc^s strains tested hybridized with the internal fragment from the Tc^r gene on pNFD13-2. All but one Tc^r strain had a hybridizing band that was the same size as the probe fragment (0.9 kb). Of these, the *Eco*RI-*Eco*RV chromosomal band from *B. thetaiotaomicron* BT4002 exhibited weaker hybridization to the probe than was seen with DNA from the other Tc^r strains. The one strain which did not yield a 0.9-kb hybridizing band was *B. distasonis* C30-45; instead, a 19-kb *Eco*RI-*Eco*RV genomic band hybridized with the probe.

DNA sequence of the Tc^r gene. The DNA sequence of the

2.7-kb *Sst*I fragment was obtained and analyzed. Sequence analysis revealed only one open reading frame within the *Sst*I clone that was sufficiently large to encode a protein with an approximate size of 76 kDa. This open reading frame had two possible start sites, since it is not known whether *Bacteroides* species initiates translation at GTG or ATG sites. An open reading frame beginning at an ATG site (position 586 of the DNA sequence) coded for 642 amino acids, while a larger open reading frame beginning at a GTG start site (position 538 of the DNA sequence) coded for 658 amino acids. However, pNFD13-2Δ4, a deletion which conferred a reduced level of resistance in *E. coli*, extended 26 bp into the larger open reading frame. The extent of the smaller open reading frame correlated well with the results of the deletion analysis. All other open reading frames in the fragment were less than 400 bp in length. A partial open reading frame started within the stop codon of the large open reading frame and extended to the end of the cloned segment. This open reading frame may have created a fusion that was responsible for the 25.5-kDa protein seen in the maxicells and the in vitro system.

The deduced amino acid sequence of the large open reading is shown in Fig. 3. The open reading frame encoded a protein of 642 amino acids (deduced molecular mass, 72,100 Da). The DNA sequence had a 40.1 mol% G+C content, in comparison with a 42 mol% G+C content for chromosomal DNA from *B. thetaiotaomicron*, the species of origin. Values for colonic *Bacteroides* species vary from 39 to 46 mol% G+C (16).

Similarity to previously studied Tc^r genes. FASTA searches of the GenBank and EMBL data bases chose representatives of *tet*(M) and *tet*(O) as the most similar relatives to the *Bacteroides* Tc^r gene. There was no significant similarity between the *Bacteroides* Tc^r gene and any of the efflux classes of resistance genes or the tetracycline modification type resistance gene *tet*(X).

On Southern blots under conditions of low stringency, a *tet*(M) probe from Tn*916* did not hybridize with genomic DNA from *Bacteroides* strains harboring *tet*(Q). These results indicated that the *Bacteroides* Tc^r is not highly similar to *tet*(M) or *tet*(O), since genes from these classes have been reported to cross-hybridize under conditions of low stringency (42, 47, 49). DNA sequence comparisons of *tet*(Q) and examples of *tet*(M) and *tet*(O) yielded identity values of 51.5 to 52.6%. When they were compared by using the same program, *tet*(M) and *tet*(O) were 75.2 to 75.7% identical. Restriction maps of the sequenced *tet*(M)- and *tet*(O)-coding sequences were generated. Only *tet*(M) of *S. aureus* contained either an *Eco*RI or an *Eco*RV site. This sequence contained a single *Eco*RV site near its downstream end.

The size of the deduced *Bacteroides* Tc^r protein (642 amino acids) was similar to the sizes of proteins encoded by *tet*(M) and *tet*(O), which ranged from 638 to 640 amino acids in length. When the amino acid sequence of Tet(Q) was aligned with Tet(M) and Tet(O), seven short regions of length variation were evident (Fig. 3). One of these regions was the carboxy-terminal part of the alignment, which also varied in Tet(O) of *C. jejuni* in comparison with other Tet(M) and Tet(O) sequences. Comparison of the deduced amino acid sequence of the *Bacteroides* Tc^r gene with those of the *tet*(M) and *tet*(O) genes demonstrated that the *Bacteroides* Tc^r gene was related to, but distinct from, those genes (Table 1). Since the *Bacteroides* Tc^r appeared to be a ribosome protection type of resistance, we designated the gene *tet*(Q), because the designations *tet*(N) and *tet*(P) have already been used (36).

DISCUSSION

In human colonic *Bacteroides* species, Tc^r genes are generally found in the chromosome on large (65- to 75-kb), self-transmissible elements rather than on plasmids (27, 35). It is likely that these elements are responsible for the spread of tetracycline resistance within the genus *Bacteroides*. The Tc^r gene described in this report was cloned from a strain containing one of the chromosomal Tc^r elements.

An internal *Eco*RI-*Eco*RV fragment from the cloned *tet*(Q) gene cross-hybridized under conditions of high stringency in Southern blots with DNA fragments of the same size from other human colonic *Bacteroides* Tc^r isolates. Since the different *Bacteroides* species in which this Tc^r gene was found share total DNA-DNA homologies of less than 45%, and in some cases less than 7% (16), results of the Southern analysis indicate that the *Bacteroides* Tc^r gene, *tet*(Q), has spread by horizontal transmission among the *Bacteroides* species, just as *tet*(M) and *tet*(O) have spread among diverse gram-positive and gram-negative bacteria. This evidence also suggests that *tet*(Q) is the predominant Tc^r determinant in the colonic *Bacteroides* species.

Recently, Guiney and Bouic (10) demonstrated that Tc^r strains of oral *Bacteroides* species have DNAs which cross-hybridize on Southern blots with DNAs of colonic strains containing Tc^r elements. Thus, *tet*(Q) appears to be widespread within diverse groups of the genus *Bacteroides*.

Most of the bacteria in which *tet*(M), *tet*(O), and *tet*(Q) have been found are human clinical isolates. Thus, it seems likely that horizontal transfer of these genes occurs in human hosts. Nevertheless, the *tet*(Q) class seems to have remained in the *Bacteroides* group and the *tet*(M) and *tet*(O) classes have remained in a separate group of bacteria which includes some gram-positive and some gram-negative species (Fig. 4). Recently, the first possible exception to this segregation has been reported. Chromosomal DNA from two isolates of *B. ureolyticus* was reported to hybridize with a *tet*(M) probe under conditions in which high salt concentrations were used (4). Given the degree of dissimilarity of the DNA sequences of *tet*(Q) and *tet*(M), it is unlikely that *tet*(Q) would hybridize with the *tet*(M) probe under the conditions used in that study. However, *tet*(O) might hybridize with *tet*(M) under those conditions. These findings could mean that the *tet*(M) and *tet*(O) resistances have entered the *Bacteroides* group. However, superficially similar groups have often been misclassified as *Bacteroides* species in the past. The precise taxonomy of *B. ureolyticus* remains unclear.

Bacteroides chromosomal elements can mobilize coresident plasmids into *E. coli* (51), but transfer of the elements themselves to species outside of the *Bacteroides* group has not been reported. An effective barrier to horizontal transfer may exist between the *Bacteroides* group and the group of species in which *tet*(M) and *tet*(O) resistances are spread. This barrier could be due either to failure of the conjugal elements to insert in the recipient chromosome or to failure of the Tc^r gene on the element to be expressed effectively. However, further screening of clinical isolates may provide examples of *tet*(M) and *tet*(O) resistances in the *Bacteroides* group or of *tet*(Q) in species outside the *Bacteroides* group.

The degree of dissimilarity between the amino acid sequence of Tet(Q) and those of Tet(M) and Tet(O) illustrated in Table 1 provides new information about regions that may be important for ribosome protection Tc^r function. The regions with the highest amino acid identity were amino acids 1 to 134 (57% identity) and 434 to 546 (55% identity) of the alignment shown in Fig. 3. The conservation at the amino

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          0                                                                  69
Bat-TetQ  MNIINLGILA HIDAGKTSVT ENLLFASGAT EKCGCVDNGD TITDSMDIEK RRGITVRAST TSIIWNGVKC
Caj-TetO  MKIINLGILA HVDAGKTTLT ESLLYTSGAI AELGSVDEGT TRTDTMNLER QRGITIQTAV TSFQWEDVKV
Stp-TetM  MKIINIGVLA HVDAGKTTLT ESLLYNSGAI TELGSVDRGT TKTDNTLLER QRGITIQTAI TSFQWKNTKV
Consensus MkIINlGiLA HvDAGKTtlT EsLLy.SGAi .elGsVD.Gt T.TD.m.lEr qRGITiqta. TSfqW..vKv

          80                                                                 139
Bat-TetQ  NIIDTPGHMD FIAEVERTFK MLDGAVLILS AKEGIQAQTK LLFNTLQKLQ IPTIIFINKI DRAGVNLERL
Caj-TetO  NIIDTPGHMD FLAEVYRSLS VLDGAVLLVS AKDGIQAQTR ILFHALQIMK IPTIFFINKI DQEGIDLPMV
Stp-TetM  NIIDTPGHMD FLAEVYRSLS VLDGAILLIS AKDGVQAQTR ILFHALRKIG IPTIFFINKI DQNGIDLSTV
Consensus NIIDTPGHMD FlAEVyRsls vLDGAvLl.S AKdGiQAQTr iLFhaLqk.. IPTIfFINKI Dq.GidL..v

         140                                                                 209
Bat-TetQ  YLDIKANLSQ DVLFMQNVVD GSVYPVCSQT YIKEEYKEFV CNHDDNILER YLADSEISPA DYWNTIIALV
Caj-TetO  YREMKAKLSS EIIVKQKVGQ HPHINVTDND DMEQ..WDAV IMGNDELLEK YMSGKPFKMS ELEQEENRRF
Stp-TetM  YREMKAKLSS EIIVKQKVGQ HPNMRVMNFT ESEQ..WDMV IEGNDYLLEK YTSGKLLEAL ELEQEESIRF
Consensus Y.diKakLS. ei..kQkV.. hp...V...t ..eq..wd.V i.gnD.lLEk Y.sgk..... eleqee..rf

         210                                                                 279
Bat-TetQ  AKAKVYPVLH GSAMFNIGIN ELLDAITS.F ILPPASVSNR LSSYLYKIEH DPKGHKRSFL KIIDGSLRLR
Caj-TetO  QNGTLFPVYH GSAKNNLGTR QLIEVIASKF YSSTPEGQSE LCGQVFKIEY SEKRRRFVYV RIYSGTLHLR
Stp-TetM  HNCSLFPVYH GSAKNNIGID NLIEVITNKF YSSTHRGQSE LCGKVFKIEY SEKRQRLAYI RLYSGVLHLR
Consensus .n..lfPVyH GSAknNiGi. .LievItskF ysst..gqse Lcg.vfKIEy seKr.r..y. riysG.LhLR

         280                                                                 349
Bat-TetQ  DVVRINDSEK FIKIKNLKTI NQGREINVDE VGANDIAIVE DMDDFRIGNY LGAEPCLIQG ..LSHQHPAL
Caj-TetO  DVIRISEKEK .IKITEMYVP TNGELYSSDT ACSGDIVILP N.DVLQLNSI LGNEILLPQR KFIENPLPMI
Stp-TetM  DPVRISEKEK .IKITEMYTS INGELCKIDK AYSGEIVILQ N.EFLKLNSV LGDTKLLPQR ERIENPLPLL
Consensus DvvRIsekEK .IKItemyt. .nGel...D. a.sgdIvIl. n.d.l.lns. LG.e.lLpQr ..ienplP.l

         350                                                                 419
Bat-TetQ  KSSVRPDRPE ERSKVISALN TLWIEDPSLS FSINSYSDEL EISLYGLTQK EIIQTLLEER FSVKVHFDEI
Caj-TetO  QTTIAVKKSE QREILLGALT EISDCDPLLK YYVDTTTHEI ILSFLGNVQM EVICAILEEK YHVEAEIKEP
Stp-TetM  QTTVEPSKPQ QREMLLDALL EISDSDPLLR YYVDSATHEI ILSFLGKVQM EVTCALLQEK YHVEIEIKEP
Consensus qttv.p.kpe qRe.ll.AL. eisd.DPlL. yyvds.thEi ilSflG.vQm EvicalLeEk yhVe.eikEp

         420                                                                 489
Bat-TetQ  KTIYKERPVK KVNKIIQIEV PPNPYWATIG LTLEPLPLGT GLQIESDISY GYLNHSFQNA VFEGIRMSCQ
Caj-TetO  TVIYMERPLR KAEYTIHIEV PPNPFWASVG LSIEPLPIGS GVQYESRVSL GYLNQSFQNA VMEGVLYGCE
Stp-TetM  TVIYMERPLK KAEYTIHIEV PPNPFWASIG LSVAPLPLGS GVQYESSVSL GYLNQSFQNA VMEGIRYGCE
Consensus tvIYmERPlk KaeytIhIEV PPNPfWAsiG Ls.ePLPlGs GvQyES.vSl GYLNqSFQNA VmEGirygCe

         490                                                                 559
Bat-TetQ  SGLHGWEVTD LKVTFTQAEY YSPVSTPADF RQLTPYVFRL ALQQSGVDIL EPMLYFELQI PQAASSKAIT
Caj-TetO  QGLYGWKVTD CKICFEYGLY YSPVSTPADF RLLSPIVLEQ ALKKAGTELL EPYLHFEIYA PQEYLSRAYH
Stp-TetM  QGLYGWNVTD CKICFKYGLY YSPVSTPADF RMLAPIVLEQ VLKKAGTELL EPYLSFKIYA PQEYLSRAYN
Consensus qGLyGW.VTD cKicF.yglY YSPVSTPADF R.L.PiVleq aLkkaGtelL EPyL.Feiya PQeylSrAy.

         560                                                                 629
Bat-TetQ  DLQKMMSEIE DISCNNEWCH IKGKVPLNTS KDYASEVSSY TKGLGIFMVK PCGYQITKGG YSDNIRMNEK
Caj-TetO  DAPRYCADIV STQIKNDEVI LKGEIPARCI QEYRTDLTYF TNGQGVCLTE LKGYQPAIGK FICQPRRPNS
Stp-TetM  DAPKYCANIV DTQLKNNEVI LSGEIPARCI QEYRSDLTFF TNGRSVCLTE LKGYHVTTGE PVCQPRRPNS
Consensus Dapkyca.Iv dtq.kN.evi lkGeiParci qeYrsdlt.f TnG.gvclte lkGYq.t.G. ..cqpRrpns

         630              646
Bat-TetQ  ..DKLLFMFQ KSMSSK
Caj-TetO  RIDKVRHMFT S
Stp-TetM  RIDKVRYMFN KIT
Consensus riDKvr.MF. k
```

FIG. 3. Deduced amino acid sequence of Tet(Q) aligned with representatives of Tet(O) (*C. jejuni* [Caj] [22]) and Tet(M) (*S. faecalis* [Stp] [24]) (Bat, *B. thetaiotaomicron*). The consensus of the sequenced ribosomal protection Tc^r genes is displayed below these sequences. Capital letters denote conservation among the ribosome protection Tc^r proteins. The four regions of conservation in GTP-binding proteins are indicated by overbars (12). Positions marked with an asterisk were found to be involved directly in GTP binding and are invariant in all GTP-binding proteins (17).

TABLE 1. Similarity matrix for the deduced amino acid sequences of sequenced ribosome protection tetracycline resistance genes

Organism	Percent similarity or identity[a]					
	S. aureus Tet(M)	*S. faecalis* Tet(M)	*U. urealyticum* Tet(M)	*C. jejuni* Tet(O)	*S. mutans* Tet(O)	*B. thetaiotaomicron* Tet(Q)
S. aureus Tet(M)		95.3	98.1	85.3	85.9	62.8
S. faecalis Tet(M)	92.2		96.7	85.7	86.1	61.1
U. urealyticum Tet(M)	96.6	95.0		85.7	86.3	62.5
C. jejuni Tet(O)	75.1	76.8	76.0		98.4	60.5
S. mutans Tet(O)	75.6	76.9	76.4	98.1		60.8
B. thetaiotaomicron Tet(Q)	41.2	41.0	41.2	41.0	41.0	

[a] Percentage of similar amino acids in the generated sequence alignment are given above the blank space, and percentage of matching amino acids in the generated sequence alignment are given below the blank space.

terminus was particularly evident in the regions thought to be important for GTP binding. It seems likely that Tet(Q) possesses ribosome-dependent GTPase activity similar to those exhibited by Tet(M) and EF-G (2). The conservation in amino acids 434 to 546 indicates that this region probably has some important function as well.

Although *tet*(Q) is similar enough to the *tet*(M) and *tet*(O) group of ribosome protection Tc^r genes to be considered a member of this gene family, it is different enough from *tet*(M) and *tet*(O) to form a new Tc^r class. The existence of *tet*(Q) as the outlying class of the ribosome protection type Tc^r genes indicates that this family of genes contains more divergence than appeared to be the case when only *tet*(M) and *tet*(O) were known. This divergence implies that the ribosome protection type of Tc^r may be more ancient than was previously thought. It also raises the question of whether other classes of ribosome protection Tc^r genes remain to be found.

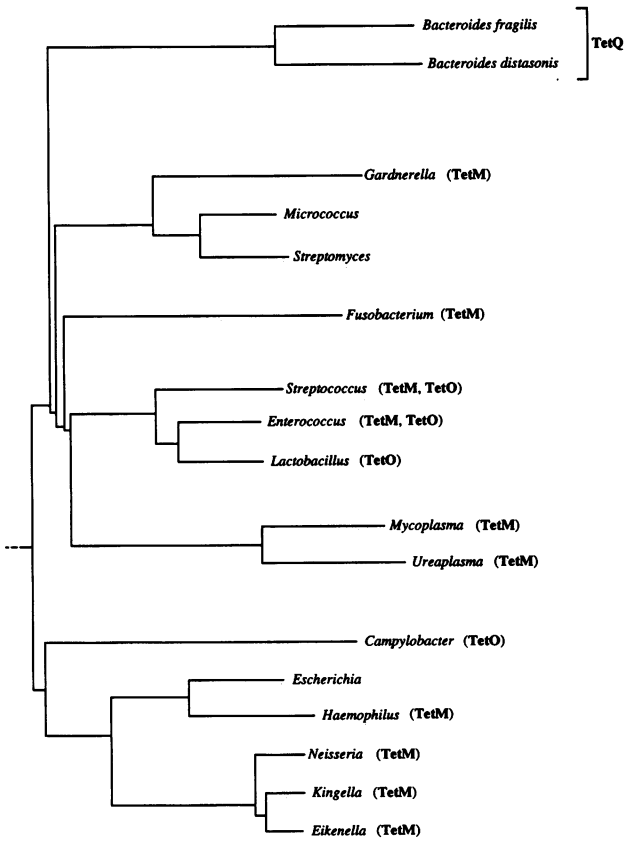

FIG. 4. Distance tree based on 16S rRNA sequences of bacteria that are known to carry ribosome protection Tc^r determinants. The determinant(s) known to be carried by representatives of a genus are indicated in bold (3, 18, 30–33, 38, 48, 52). Some bacteria that carry ribosome protection type resistances were omitted because their 16S rRNA sequences are not available.

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ADDENDUM

While the manuscript was in review, a new ribosome protection type determinant for oxytetracycline resistance from *Streptomyces rimosus*, *otr*(A), was described (D. Doyle, K. J. McDowall, M. J. Butler, and I. S. Hunter, Mol. Microbiol. **5**:2923–2933, 1991). The *otr*(A) DNA sequence (EMBL/GenBank accession X53401) was 40.6 to 41.2% identical to *tet*(M) sequences, 43.3% identical to *tet*(O) sequences, and 44.0% identical to the *tet*(Q) sequence when they were compared as described above. By deduced amino acid sequence comparison, Otr(A) was 32.5 to 33.0% identical to Tet(M) sequences, 34.5% identical to Tet(O) sequences, and 32.3% identical to Tet(Q) sequences. The fact that *otr*(A) is so different from the other ribosome protection type resistances, including *tet*(Q), further demonstrates the diversity of this group of genes and indicates that the group may have a relatively early evolutionary origin.

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