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The Erythromycin Resistance Gene from the *Bacteroides* Conjugal Transposon Tc^r Em^r 7853 Is Nearly Identical to *ermG* from *Bacillus sphaericus*

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Tc^r Em^r 7853, from *Bacteroides thetaiotaomicron* 7853, is a large chromosomal conjugative transposon which encodes resistance to both tetracycline (Tc^r) and erythromycin (Em^r). The erythromycin resistance gene of Tc^r Em^r 7853 did not cross-hybridize with *ermF*, the Em^r gene found on previously studied *Bacteroides* regular and conjugative transposons. We have cloned and sequenced the erythromycin resistance gene from Tc^r Em^r 7853. The DNA sequence of this gene was 99.6% identical to that of *ermG* from *Bacillus sphaericus*.

Many strains of *Bacteroides* carry large (>70 kb) chromosomal conjugative transposons (10, 12). These elements are capable of self-transfer from the chromosome of the donor to the chromosome of the recipient. They can also mobilize coresident plasmids, and they can excise, circularize, and mobilize 10- to 12-kb discrete, unlinked chromosomal elements called nonreplicating *Bacteroides* units (21). A growing body of research suggests that the dissemination of antibiotic resistance genes among the *Bacteroides* group is mediated primarily by conjugative transposons and the elements that they mobilize (13). One such conjugative transposon, Tc^r Em^r 7853, has been shown to differ from other *Bacteroides* conjugative transposons in several ways. It lacks homology with other characterized *Bacteroides* elements outside the region that contains the Tc^r gene, *tetQ*. It has a host range which includes *Prevotella ruminicola* as well as *Bacteroides* species. Finally, it carries an Em^r determinant which is capable of expression in *P. ruminicola* and does not cross-hybridize with *ermF* (10).

So far, *ermF* is the only Em^r gene reported for *Bacteroides* clinical isolates. The sequences of several *Bacteroides ermF* alleles have been determined (5, 11, 23). All of these were nearly identical at the nucleotide level and were closely related to previously characterized rRNA *N*-methyltransferases. Previously, we have demonstrated that *ermF* does not confer resistance on *P. ruminicola*, even when placed downstream of different promoters (16, 20). There have been reports of Em^r *Bacteroides* clinical isolates that did not cross-hybridize with *ermF* probes (2, 4, 11), but none of these genes have ever been cloned and characterized. We report here the cloning and sequencing of the Tc^r Em^r 7853 Em^r gene. Other studies have demonstrated natural transfer of *ermB* and *ermC* from gram-positive cocci to *Escherichia coli* (3). So far, there have been no reports of gene transfer between *Bacteroides*, the numerically predominant genus of the human intestine, and the gram-positive bacteria. This report provides the first evidence that such transfers may occur.

Bacterial strains, plasmids, and growth conditions. *Bacteroides uniformis* 0061 and *Bacteroides thetaiotaomicron* 5482 were obtained from the culture collection of the Virginia Polytechnic Institute Anaerobe Laboratory. *B. uniformis* 1100 is a spontaneous Thy$^-$ mutant of *B. uniformis* 0061. *B. thetaiotaomicron* 4001 is a spontaneous Rif^r mutant of *B. thetaiotaomicron* 5482. *B. thetaiotaomicron* 7853 was obtained from Sidney Finegold, Wadworth VA Hospital, Los Angeles, Calif. *B. uniformis* 1109 is a Tc^r Em^r 7853 transconjugant of *B. uniformis* 1100 (10). *Bacteroides* strains were grown in prereduced Trypticase-yeast extract-glucose broth or on Trypticase-yeast extract-glucose agar medium in a BBL Microbiology Systems GasPak jar system (6, 19).

E. coli DH5α MCR (GIBCO/BRL) was used for cloning. *E. coli* J53, used as a helper in matings, contains the self-transmissible IncP plasmid R751 (8, 18). *E. coli* strains were grown in Luria-Bertani broth. The *E. coli-Bacteroides* mobilizable shuttle vector pFD160 is a pUC19-pBI143 chimera (22).

Cloning and sequence analysis of the Tc^r Em^r 7853 *erm* gene. All DNA isolations and manipulations were performed as described previously (14, 17). *B. uniformis* 1109 (a Tc^r Em^r 7853 transconjugant) chromosomal DNA was partially digested with *Sau*3AI and ligated to pFD160 cut with *Bam*HI. *E. coli* DH5α MCR was transformed and selected on Luria-Bertani broth plates containing ampicillin (100 μg/ml). Resulting colonies were resuspended and pooled in Luria-Bertani broth containing ampicillin (100 μg/ml) for use as an inoculum for donor cultures in matings with *B. thetaiotaomicron* 4001 (Em^s). Triparental nitrocellulose filter matings of recombinant *E. coli* DH5α MCR containing the possible clones and the helper *E. coli* J53(R751) with *B. thetaiotaomicron* 4001 were performed as described previously (17). Mating mixtures were allowed to incubate on the filters overnight and were then plated on Trypticase-yeast extract-glucose plates containing erythromycin (10 μg/ml) and gentamicin (200 μg/ml) to isolate *B. thetaiotaomicron* transconjugants containing a Tc^r Em^r 7853 erythromycin resistance gene clone.

One erythromycin-resistant clone was obtained from 40 filter matings. This construct, designated pEMR78, contained a 3.3-kb *Sau*3AI partial digestion fragment with an internal *Hin*dIII site which cut the 3.3-kb cloned fragment into 2.3- and 1.0-kb fragments. The 1.0-kb fragment was cloned into pUC19 and sequenced by the method of Sanger et al. (15) as provided by the Sequenase 2.0 kit (U.S. Biochemical) from the *Hin*dIII site by using pUC19 reverse primer. Single-stranded sequence from pEMR78 of the entire Tc^r Em^r 7853 Em^r gene and adjacent DNA was then obtained by the University of Illinois Biotechnology Facility using synthesized primers and Applied Biosystems model 373A version 2.0.1S dye terminator se-

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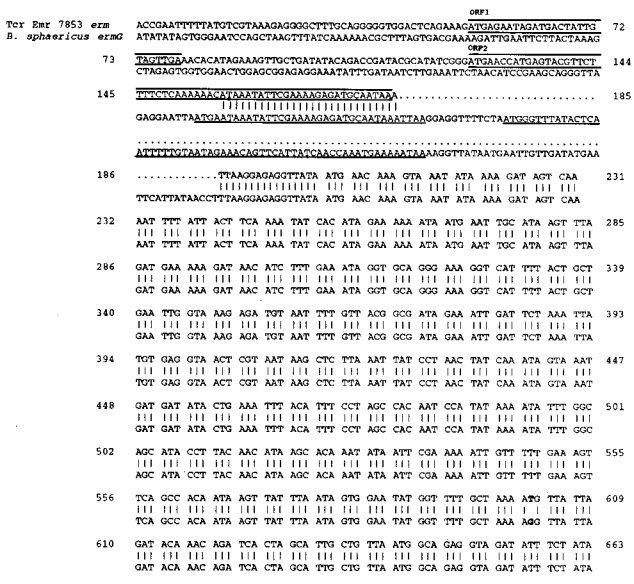

FIG. 1. Nucleotide sequence alignment of the Tc^r Em^r 7853 and *B. sphaericus* erythromycin resistance genes. Numbers correspond to the Tc^r Em^r 7853 nucleotide sequence with the methyltransferase coding region beginning at base 202. Nucleotide differences within the two methyltransferase ORFs are at bases 602, 715, and 810. The *Hin*dIII site used for subcloning is indicated. ORFs upstream of the Tc^r Em^r 7853 Em^r gene are underlined and overlined. *B. sphaericus ermG* upstream ORFs are underlined.

quencing. The Tc^r Em^r 7853 Em^r gene region sequence, aligned with the *Bacillus sphaericus ermG* gene region, is shown in Fig. 1.

Three potential open reading frames (ORFs) have been detected within the region around the *Hin*dIII site. The largest ORF encoded a protein of 244 amino acids, and its DNA sequence was virtually identical to that of *ermG* found in *B. sphaericus* (9). There were only three nucleotide changes between this gene and *ermG*. Additional nucleotide identity ended abruptly 44 bp upstream and 96 bp downstream of the methyltransferase ORFs (Fig. 1). This suggested that a foreign DNA segment was integrated into the 7853 element. Our observation of nearly identical nucleotide sequences upstream, within, and downstream of the erythromycin resistance gene in genera as phylogenetically distant as *Bacteroides* and *Bacillus* suggests that the presence of this antibiotic resistance gene in both genera is a result of horizontal transfer.

Comparison of upstream sequences. In general, *erm* gene expression is regulated by erythromycin (25). A model for the regulation of *ermG* in which stalling of a ribosome under the influence of erythromycin during translation of either one of two short leader sequence ORFs (11 and 19 amino acids, respectively) frees the methyltransferase Shine-Dalgarno sequence and start codon and allows translation of *ermG* has been proposed (9). The Tc^r Em^r 7853 *erm* upstream region contains two potential ORFs encoding peptides of 8 and 19 amino acids (Fig. 1). The second of these upstream ORFs has high-level sequence identity at its carboxy terminus with ORF1 of the *B. sphaericus ermG* leader sequence. We found no detectable differences in the lag times or growth rates of cultures of *B. thetaiotaomicron* 7853 pregrown with and without erythromycin (data not shown). Thus, the *ermG* allele in *B. thetaiotaomicron* may be expressed constitutively.

Potential origin of the Tc^r Em^r 7853 Em^r gene. The G+C content of the Tc^r Em^r 7853 erythromycin resistance gene was 27%, whereas the G+C content of the *B. thetaiotaomicron* chromosome is 42% (7). The G+C contents of DNA upstream and downstream of the sequence identity endpoints were 40 and 48%, respectively. The G+C content of the *B. sphaericus* chromosome is 47%, suggesting that *ermG* may also be a recent immigrant in *B. sphaericus* (9). Of the eight generally accepted *erm* hybridization classes, the ErmG class has the highest level of amino acid identity (71%) to ErmC from *Staphylococcus aureus* (1). The G+C content of *S. aureus* is 32 to 36% (24). Thus, the Em^r gene found in the *Bacteroides* element Tc^r Em^r 7853 may have been transferred not directly from *B. sphaericus* but from some as yet uncharacterized *ermG* source, possibly one of the gram-positive cocci.

The possibility that the resident microflora of the human colon can act as a reservoir for antibiotic resistance genes has been raised by many investigators, but virtually all previous studies of possible gene transfer events between different members of the microflora or between members of the microflora and transient colonizers of the intestinal tract have focused on the numerically minor species of gram-negative and gram-positive bacteria. Our findings provide additional evidence that *Bacteroides* is a major player in the transfer of antibiotic resistance genes, including those found in gram-positive bacteria.

Nucleotide sequence accession number. The *erm* region sequence of Tc^r Em^r 7853 has been assigned the GenBank accession number L42817.

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