Nucleotide sequence of *ermFU*, a macrolide-lincosamide-streptogramin (MLS) resistance gene encoding an RNA methylase from the conjugal element of *Bacteroides fragilis* V503

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Coincident resistance to macrolide, lincosamide, and streptogramin B (MLS^r) antibiotics is widespread among procaryotic organisms and is usually due to the posttranscriptional methylation of 23S ribosomal RNA. Genes encoding such rRNA methyltransferases have been collectively designated *erm*.

Transfer of non-plasmid MLS^r by *B. fragilis* V503 was followed at the molecular level using an *ermF* gene probe (1, 2). The homology to *ermF* was used to identify and clone a 4.2 kb *HincII* fragment which contained sequences corresponding to the V503 *erm* gene (hereafter called *ermFU*). We report here the nucleotide sequence containing the *ermFU* gene and its upstream flanking sequences.

Comparison of this 1073 base pairs of nucleotide sequence with the previously described *ermF* gene (3) showed an open reading frame of 801 nucleotides and a putative translational start site at position 272. The open reading frame, the *ermFU* structural gene, displayed 98.6% identity to the *ermF* gene. Possible -35 (GAGCAA) and -10 (TATAAT) promoter sequences were observed at position 186 and at position 200 respectively, their role in expression is unknown as the sequence and structure of *Bacteroides* promoters remain uncharacterized.

The 26 base pairs upstream of the putative translational start site of *ermFU* (position 245 to 271) were identical to the 26 bp found immediately upstream of the two other *Bacteroides* *erm* genes sequenced (*ermFS* (4) and *ermF* (3, 5)) including their putative ribosome binding sites (5'AAGAAG).

The similarity between the nucleotide sequences of *ermFU* and *ermF* ended abruptly beginning 26 bases upstream of *ermFU* structural gene. Sequences further upstream (5') of position 245 showed less than 23.8% nucleotide identity to either *ermF* or *ermFS* genes. When these upstream sequences were aligned to maximize homology using the 'gap' algorithms in the GCG DNA sequence analysis software, the maximum relatedness was 44%.

Previous studies of *Bacteroides* *erm* genes had demonstrated that expression of both *ermF* (Tn4351) and *ermFS* (Tn4551) was dependent on the presence of an IS4351 promoter (3-5). There was no evidence of any large repeated sequence associated with *ermFU*. This suggested that alternative genetic events have occurred which permitted expression in *Bacteroides*. No inverted or direct repeats associated with class I transposons and insertion sequences or Tn3-like transposons were apparent. Further inspection of the sequence for patterns associated with transferable antibiotic resistance determinants yielded a surprising result. The sequence CAAAGTT, which has been identified as a probable recombinational hot-spot (RHS) within Tn21-related transposons (6) was found 78 nt 5' of the putative transcriptional start site for

ermFU (nt 186-192). Analysis of sequence 5' of the RHS in a variety of Tn21-related transposons (7, 8) had revealed a 15 bp sequence associated with the RHS. Examination of the *ermFU* sequence demonstrated that a very similar sequence was present 5' of both the RHS and the transcriptional start site of *ermFU* (nt 134 to 148). This fits very well with the comparison of G+C content. The G+C content of both the *ermFU* gene and 120 bases immediately upstream was 33%. The G+C content of *Bacteroides* species is about 46%. Similar G+C content was found in sequences located further upstream of the gene (from 1 to 150) and downstream of the gene (1072 to 1177); 46% and 42% respectively. One copy of an inverted repeat was noted in the region where the G+C content changed. The 9 bp sequence TTTTTCCTT was seen at position 156. The second inverted copy of this sequence appeared at position 277, 6 bp into the *ermFU* structural gene. Inspection of the previously characterized *ermF* and *ermFS* upstream regions and structural genes failed to find any conserved sequences similar to those associated with the Tn21-like elements.

Based on these observations we suggest that the Tn21 integron mechanism of dissemination of antibiotic resistance recently appreciated in enteric bacteria (8) may be operative in *Bacteroides*. This is also the first report of nucleotide sequence data from one of the apparently widespread *Bacteroides* chromosomal conjugal genetic elements.

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