ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1995, p. 1830–1834 Vol. 39, No. 8
0066-4804/95/$04.00+0
Copyright © 1995, American Society for Microbiology

The Closely Related *ermB-ermAM* Genes from *Clostridium perfringens*, *Enterococcus faecalis* (pAMβ1), and *Streptococcus agalactiae* (pIP501) Are Flanked by Variants of a Directly Repeated Sequence

DAVID I. BERRYMAN† AND JULIAN I. ROOD*

Department of Microbiology, Monash University, Clayton 3168, Australia

Received 28 December 1994/Returned for modification 10 April 1995/Accepted 5 June 1995

The *Clostridium perfringens* macrolide-lincosamide-streptogramin B resistance gene, *ermBP*, was sequenced and shown to be identical to the *ermB-ermAM* gene from the promiscuous *Enterococcus faecalis* plasmid pAMβ1 and to have at least 98% nucleotide sequence identity to other *ermB-ermAM* genes. Flanking the *ermBP* structural gene were almost identical directly repeated 1,341-bp sequences (DR1 and DR2). These repeats potentially encoded a 298 (or 284)-amino-acid protein that had sequence similarity to chromosomal and plasmid partitioning proteins. The pAMβ1 and *Streptococcus agalactiae* (pIP501) *erm* determinants appeared to have DR2 but had similar internal 973- or 956-bp deletions in DR1, respectively. Some of the other *ermB-ermAM* class determinants had small portions of DR1, but none had complete copies. It is postulated that the *C. perfringens ermBP* determinant was derived from an enterococcal or streptococcal determinant that had complete copies of both DR1 and DR2.

Resistance to macrolide-lincosamide-streptogramin B (MLS) antibiotics such as erythromycin is common in several bacterial genera. It is generally mediated by dimethylation of the ribosomal 23S rRNA target site, which reduces the affinity between the antibiotic and the ribosome. The genes that encode these 23S rRNA methylases are designated as erythromycin resistance transmethylase (*erm*) genes. At least 17 *erm*-type MLS resistance genes, from many bacterial genera, have been identified (19). These genes have been divided into seven hybridization classes, although the derived amino acid sequences of the rRNA methylases have considerable similarity (19).

Two distinct *erm* genes have been cloned from the gram-positive anaerobic bacterium *Clostridium perfringens*. The recombinant plasmid pJIR122 carries the *ermBP* gene, which belongs to the *ermB-ermAM* hybridization class (4). Genes which hybridize to *ermBP* are also present in MLS-resistant isolates of *Clostridium difficile* and *Clostridium paraputrificum* (4). The second gene, *ermQ*, has been sequenced and shown to be the most common erythromycin resistance determinant in *C. perfringens* (3).

Genes belonging to the *ermB-ermAM* class have been found in numerous bacterial genera, including *Enterococcus*, *Streptococcus*, *Lactobacillus*, and *Clostridium* and *Escherichia coli* (19). Their broad distribution indicates that they are readily transferred between different genera. Determinants from *Enterococcus faecalis* and *Streptococcus agalactiae* are located on the conjugative plasmids pAMβ1 (18) and pIP501 (16), respectively. Both plasmids have a broad host range, and pAMβ1 has been shown to transfer into the genus *Clostridium* (25, 37). Other *ermB-ermAM* class genes are located on transposable elements such as Tn*917* (32) and Tn*1545* (10). Restriction and hybridization analysis revealed that the *ermBP* gene was closely related to the determinant from pAMβ1. The similarity extended into the regions flanking the *erm* structural genes; however, its precise extent was not determined (4). To further characterize the relationship between these determinants, the sequence of the *ermBP* gene and its flanking regions was determined and comparative sequence analysis was carried out.

MATERIALS AND METHODS

Bacterial strains and culture conditions. All *E. coli* strains were derivatives of DH5α (Bethesda Research Laboratories). *C. perfringens* isolates were from France (6), Wisconsin (27), Germany (33), and Belgium (G. Daubé, Université de Liège). *C. difficile* isolates were from Australia (4), Europe (12, 36), and Japan (23). Streptococcal isolates were from France (P. Courvalin, Institut Pasteur). *E. coli* and clostridial strains were cultured as described before (4). Streptococcal strains were grown in nutrient broth no. 2 (Oxoid) containing 0.3% yeast extract (Oxoid). Erythromycin (20 μg/ml for clostridial and streptococcal strains; 100 μg/ml for *E. coli*) or ampicillin (100 μg/ml) was added to the medium as required.

DNA preparation and cloning. *E. coli* plasmid DNA was isolated as previously described (4, 22). DNA was isolated from 100 ml of *C. perfringens*, *C. difficile*, and streptococcal cultures by using either the Sarkosyl or sodium dodecyl sulfate lysis procedures (4). All cloning experiments used the plasmid vector pUC18 and standard methods (20). Restriction endonucleases and other enzymes were from Boehringer-Mannheim, New England Biolabs, or Promega and were used in accordance with the manufacturer's instructions.

DNA sequencing and analysis. The *ermBP* sequence was obtained from subclones and deletion derivatives of pJIR122 (4). The partial pAMβ1-derived sequence was obtained from pDL216, a derivative of pAMβ1 (18). Oligonucleotide primers were synthesized by using an Applied Biosystems DNA synthesizer (model 381A). Sequencing reactions were performed on plasmid DNA templates by using a T7 Sequencing kit (Pharmacia) with labelled ^{35}S-dATP (Amersham) according to the manufacturer's instructions. When necessary, deaza-dGTP was used in place of dGTP. Individual template sequence data were compiled into contiguous sequences by using the ESEE program (9) and were analyzed with ANGIS software operated by the University of Sydney.

PCR analysis. PCR analysis was carried out with a GeneAmp PCR kit (Perkin-Elmer Cetus) in a Hybaid Intelligent Heating Block (model IHB 2024). The reaction mixtures contained approximately 100 ng of template DNA and 1 μM each primer. The PCR consisted of 35 cycles, each of 1 min at 94°C, 1 min at 45°C, and 2 min at 72°C.

Nucleotide sequence accession number. The GenBank accession number of the DNA sequence of the 4,129-nucleotide *ermBP* gene region is U18931. The sequences of the following *ermB-ermAM* class gene regions were from sources indicated: pAMβ1 (7, 21, 24), pIP501 (5, 17, 26), Tn*917* (28), pAM77 (15), and *E. coli ermBC* (8).

RESULTS AND DISCUSSION

Sequence analysis of the *ermBP* gene region. The complete nucleotide sequence of the 4,129-bp *ermBP* gene region from the recombinant plasmid pJIR122 (4) was determined un-

* Corresponding author. Phone: 61 3 9905 4825. Fax: 61 3 9905 4811. Electronic mail address: Julian.Rood@med.monash.edu.au.

† Present address: State Agricultural Biotechnology Centre, Murdoch University, Murdoch 6150, Australia.

FIG. 1. Nucleotide sequence of the *ermBP* gene region. The region sequenced consisted of 4,129 bp located between, but not including, the *Sac*I and *Hin*dIII restriction sites in pJIR122 (4). Selected restriction sites are indicated. The direct repeats located upstream (DR1) and downstream (DR2) of the *ermBP* structural gene are marked by arrows above the sequence. The *ermBP* structural gene and the ORF3 sequence are indicated by lines above the text. The predicted translation products of *ermBP*, ORF3, and the DR1- and DR2-derived ORF298 sequences are shown below the nucleotide sequence. The start of the ORF284 amino acid sequence is indicated by the boldface M. Putative ribosome-binding site (RBS) sequences upstream of the *ermBP* gene (RBS1) and the ORF3 sequence (RBS2) are indicated by underlining. The palindromic regions, *palA* and *palB*, that flank the ORF298 sequences are indicated by the inwardly facing arrows above the text. The 6-bp repeats that are associated with the *palA* and *palB* sequences are also indicated by arrows above the sequence. The asterisk identifies the single base change within one of these repeats. The inverted repeats located at the end of the *ermBP* gene are indicated by double underlining. The IR38 sequence (28) is indicated by the sequence in boldface and italics. The divergence points between the *ermBP* sequence and the other *erm* determinants, as shown diagrammatically in Fig. 2, are indicated above the sequence as follows: pAMβ1┐, pIP501┐, pAM77┐, *ermBC*┐, and Tn*917*┐.

equivocally for both DNA strands (Fig. 1). Comparison of the 738-bp *ermBP* sequence showed that it was identical to the *erm* gene from pAMβ1 and differed from the *ermBC* gene in only two positions. The other *ermB-ermAM* genes examined (from pIP501, pAM77, and Tn*917*) all had greater than 98% nucleotide sequence identity to *ermBP*. Located 4 bp downstream of *ermBP* was a 138-bp open reading frame (ORF) that was almost identical to sequences located immediately downstream of the Tn*917*, pAM77, and pIP501 *erm* genes and was designated ORF3 (5, 14, 28).

Comparative analysis of the *ermBP* gene region. Located immediately upstream of the *erm* genes from pAM77, pIP501, and Tn*917* are leader sequences that are responsible for the inducible expression of these *erm* genes by a translation attenuation mechanism (14, 19). A similar but inactive leader se-

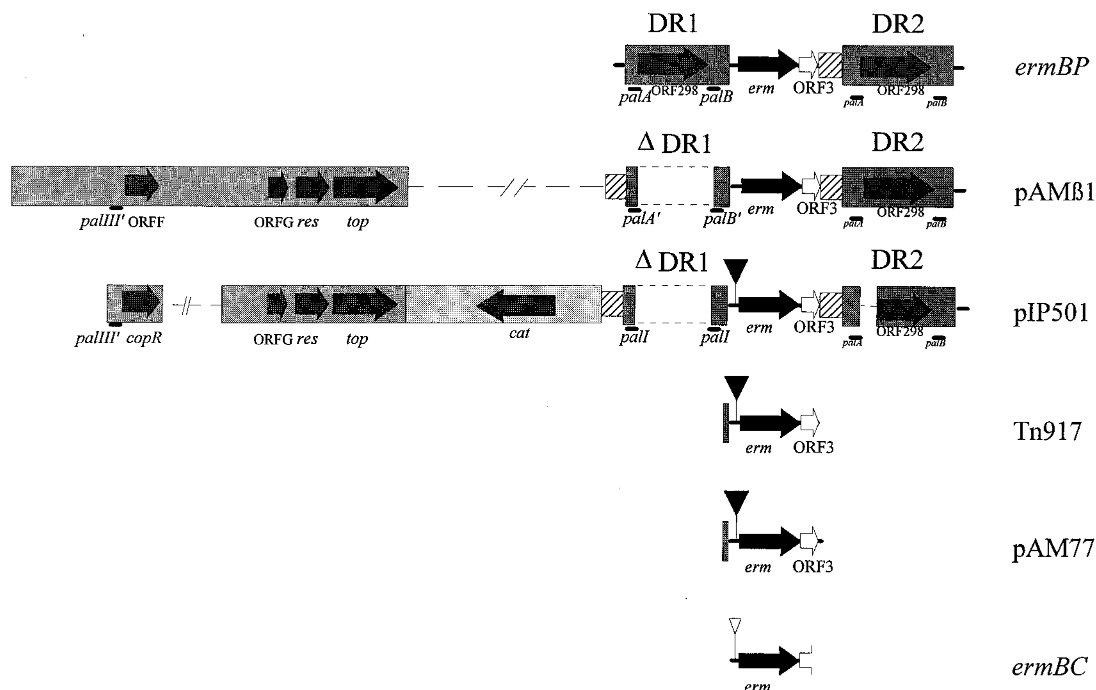

FIG. 2. Comparison of the genetic organization of the *ermB-ermAM* gene regions. The approximate extent and organization of the *ermBP*, pAMβ1, pIP501, Tn*917*, pAM77, and *ermBC* gene regions are shown schematically and not necessarily to scale. The data used to construct this figure were from sequences described in the text and from other sources (29, 30). The functions of genes not specifically referred to in the text are as follows: *cat*, chloramphenicol acetyltransferase; *top*, topoisomerase; *res*, resolvase; and *copR* and ORF F plasmid copy number. No function has yet been assigned to the ORF G sequence. Regions of nucleotide sequence similarity are indicated by the same shading. The approximate extents of the deletions in the pAMβ1 and pIP501 DR1-like sequences (ΔDR1) are indicated. The filled arrows indicate the individual ORFs and their respective directions of transcription. The approximate locations of the palindromic sequences (*palA*, *palB*, *palI*, and *palIII'*) but not their extents are indicated by the boldface lines below the filled boxes. The *palIII'* sequence adjacent to the *copR* gene has been designated as such on the basis of its similarity to the analogous pAMβ1 sequence. The *palA'* and *palB'* sequences in pAMβ1 and the *palI* sequences in pIP501 represent the portions of the *ermBP*-derived *palA* and *palB* homologs that are present at the ends of the deletion in these DR1-like sequences. Functional and nonfunctional leader peptide sequences are indicated by filled and open inverted triangles, respectively. Regions for which no sequence data was available are indicated by the broken lines.

quence is also located upstream of the *ermBC* gene (8, 19). The *erm* gene from pAMβ1 is not preceded by a leader peptide sequence, and, as a consequence, this gene is constitutively expressed (7, 21), as is the *ermBP* gene (data not shown). Analysis of the sequences immediately upstream of the *erm* gene showed that the *ermBP* and pAMβ1 sequences were identical and that both determinants were missing the leader sequences found upstream of the inducible genes.

Comparison of the sequences upstream and downstream of the *ermBP* gene confirmed the previous suggestion that there were directly repeated sequences flanking the *ermBP* structural gene (4). The repeats were 1,341-bp (DR1) and 1,340-bp (DR2) in size and had 98.9% nucleotide sequence identity. Both had almost identical ORFs which, when translated, would lead to a 298- or a 284-amino-acid polypeptide, depending on the methionine start codon (Fig. 1). Located 5 bp upstream of the ORF298 start codons in both DR1 and DR2 were 47-bp palindromic sequences, which were designated *palA* sequences (Fig. 1). These sequences consisted of three direct repeats (D) and three inverted repeats (I) of the sequence AATCAC and one TATCAC sequence (D*), in the order 5′-D*ADAAIDAD TTITI-3′. Two additional directly repeated copies of the AATCAC repeat were also present immediately upstream of the *palA* sequences (Fig. 1). Located after both ORF298 stop codons were 48-bp sequences, which were designated *palB* sequences (Fig. 1); these were almost identical to the upstream *palA* sequences but were not preceded by the two direct repeats.

Analysis of the ORF298 amino acid sequences. The DR1- and DR2-derived ORF298 amino acid sequences had similarity to proteins such as ParA from bacteriophage P1 (35), pTAR from *Agrobacterium tumefaciens* (11), RepB from the enterococcal plasmid pAD1 (34), SopA from the F plasmid (13), and IncC from plasmid RK2 (31). These proteins belong to a superfamily of ATPases and have been proposed or demonstrated to be associated with plasmid or chromosomal partitioning. Since the *ermBP* determinant is located on the nonconjugative, low-copy-number *C. perfringens* plasmid, pIP402 (6), it is possible that the putative ORF298 (or ORF284) protein may be associated with the partitioning of this plasmid in *C. perfringens*.

Comparative analysis of the *ermBP* determinant. The regions flanking the *ermBP* gene were almost identical to the equivalent sequences from pAMβ1 and pIP501 and had significant similarity to the regions flanking the other *ermB-ermAM* class genes. The pAMβ1 and pIP501 sequences located 5′ to the respective *erm* genes diverged from the *ermBP* sequence at the left end of DR1. However, for the following 220 bp, the pAMβ1 and pIP501 sequences upstream of DR1 were almost identical to the sequences located upstream of DR2 (Fig. 2). Both sequences diverged at position 2418, which was located at the end of an inverted-repeat sequence (Fig. 1). In addition, both the pAMβ1 and the pIP501 DR1 regions had internal deletions (973 and 956 bp, respectively) that resulted in the loss of the entire ORF298 sequence (Fig. 2). The deletion endpoints in both plasmids were located within the *palA* and *palB* sequences; however, they appeared to have arisen from separate deletion events, since the endpoints were different (Fig. 1).

Recent studies have suggested that similar *pal*-like sequences may act as hot spots for plasmid cointegrate formation and resolution (17). It is likely that recombination between the *palA* and *palB* homologs in progenitor *erm* determinants resulted in the loss of the region between the repeats and created the ΔDR1-like sequences found in pAMβ1 and pIP501.

The sequence similarity between *ermBP* and the other *ermB-ermAM* determinants also continued upstream of the *erm* genes. The *ermBC* sequence diverged before the start of DR1. In contrast, the pAM77 and Tn*917* sequences diverged at an identical position 28 bp into DR1 (Fig. 1 and 2).

Analysis of the sequences downstream of these *erm* genes showed that the *ermBC*, Tn*917*, and pAM77 sequences diverged 111, 139, and 207 nucleotides past the *erm* stop codon, respectively (Fig. 1). Therefore, none of these determinants contained DR2. In contrast, the downstream similarity between *ermBP* and pAMβ1 continued through to the end of the published pAMβ1 sequence. Therefore, in an attempt to ascertain the downstream divergence point between these sequences, we obtained additional partial pAMβ1 sequence that extended for approximately 1,200 bp beyond the published sequence (data not shown). Analysis indicated that the pAMβ1 sequence was almost identical to the equivalent sequence from the *ermBP* region, to the extent that the sequence similarity extended through and beyond the right end of DR2. That is, sequence analysis did not reveal the divergence point between the two sequences.

PCR analysis carried out with DR1- or DR2-specific primers confirmed the presence of both the ΔDR1-like element and the intact DR2-like sequences in pAMβ1 (data not shown). However, similar examination of other *ermB-ermAM* isolates from *C. perfringens*, *C. difficile*, and *Streptococcus* spp. failed to detect similar DR elements in these isolates, although they were all shown to contain the *ermBP*-ORF3 region.

The downstream similarity between *ermBP* and the pIP501-derived deletion derivative pGB3631 (5) extended to within the DR2 *palA* sequence, where it diverged (Fig. 1). It is not known whether this point represents the deletion point involved in the formation of pGB3631 (1, 2). However, database searches revealed that a sequence with 95.1% nucleotide sequence identity to DR2 was present in pIP501. This similarity was in the mobilization (*mob*) region located downstream of the *erm* gene (17). The region of similarity between the *mob* and *ermBP* sequences included almost all of the DR2 sequence (Fig. 2). It is not known whether the *mob*-encoded DR2 homolog has the N-terminal-encoding region of ORF298, since the sequence of this region of pIP501 is not available.

The extent of the sequence similarity between the different *ermB-ermAM* determinants suggested that they were probably derived from a common progenitor, although they have subsequently diverged. Sequences homologous to DR1 and DR2 were detected in several of the *ermB-ermAM* gene regions, and it is likely that these sequences were also present in the progenitor *ermB-ermAM* determinant. Since the *C. perfringens ermBP* region was the only determinant found to have complete DR1 and DR2 sequences, we postulate that it is more closely related to the putative progenitor determinant than any of the other sequences examined.

It was previously proposed that the *ermBP* determinant originated in the enterococci or streptococci and was introduced into *C. perfringens* by conjugation with a bacterium carrying pAMβ1 or a related plasmid (4). The results presented in the present paper support this conclusion; however, since neither pAMβ1 nor pIP501 had complete DR1 sequences, they suggest that these plasmids were not directly responsible for the transfer of *ermBP* to *C. perfringens*. Instead, the data support the postulate that transfer to *C. perfringens* was from a progenitor conjugative plasmid that carried two copies of the entire DR sequence.

ACKNOWLEDGMENTS

We thank P. Courvalin for the provision of streptococcal isolates; G. Daubé, S. Nakamura, and W. Traub for clostridial isolates; D. LeBlanc for providing pDL216; and N. Minton and J. Oultram for generously providing their unpublished pAMβ1 sequence data.

This research was supported by a grant from the Australian National Health and Medical Research Council. D.B. was the recipient of an Australian Postgraduate Research award.

REFERENCES

1. **Behnke, D., and D. Gerlach.** 1987. Cloning and expression in *Escherichia coli*, *Bacillus subtilis*, and *Streptococcus sanguis* of a gene for staphylokinase, a bacterial plasminogen activator. Mol. Gen. Genet. **210:**528–534.
2. **Behnke, D., and M. S. Gilmore.** 1981. Location of antibiotic resistance determinants, copy number control, and replication functions on the double-selective streptococcal cloning vector pGB301. Mol. Gen. Genet. **184:**115–120.
3. **Berryman, D. I., M. Lyristis, and J. I. Rood.** 1994. Cloning and sequence analysis of *ermQ*, the predominant macrolide-lincosamide-streptogramin B resistance gene in *Clostridium perfringens*. Antimicrob. Agents Chemother. **38:**1041–1046.
4. **Berryman, D. I., and J. I. Rood.** 1989. Cloning and hybridization analysis of *ermP*, a macrolide-lincosamide-streptogramin B resistance determinant from *Clostridium perfringens*. Antimicrob. Agents Chemother. **33:**1346–1353.
5. **Brantl, S., C. Kummer, and D. Behnke.** 1994. Complete nucleotide sequence of the plasmid pGB3631, a derivative of the *Streptococcus agalactiae* plasmid pIP501. Gene **142:**155–156.
6. **Bréfort, G., M. Magot, H. Ionesco, and M. Sebald.** 1977. Characterization and transferability of *Clostridium perfringens* plasmids. Plasmid **1:**52–66.
7. **Brehm, J., G. Salmond, and N. Minton.** 1987. Sequence of the adenine methylase gene of the *Streptococcus faecalis* plasmid pAMβ1. Nucleic Acids Res. **15:**3177.
8. **Brisson-Noël, A., M. Arthur, and P. Courvalin.** 1988. Evidence for natural gene transfer from gram-positive cocci to *Escherichia coli*. J. Bacteriol. **170:** 1739–1745.
9. **Cabot, E. L., and A. T. Beckenbach.** 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. Comp. Appl. Biosci. **5:**233–234.
10. **Courvalin, P., and C. Carlier.** 1986. Transposable multiple antibiotic resistance in *Streptococcus pneumoniae*. Mol. Gen. Genet. **205:**291–297.
11. **Gallie, D. R., and C. I. Kado.** 1987. *Agrobacterium tumefaciens* pTAR *parA* promoter region involved in autoregulation, incompatiability, and plasmid partitioning. J. Mol. Biol. **193:**465–478.
12. **Hayter, P. M., and J. W. Dale.** 1984. Detection of plasmids in clinical isolates of *Clostridium difficile*. Microbios Lett. **27:**151–156.
13. **Hiraga, S.** 1992. Chromosome and plasmid partitioning in *Escherichia coli*. Annu. Rev. Biochem. **61:**283–306.
14. **Horinouchi, S., W.-H. Byeon, and B. Weisblum.** 1983. A complex attenuator regulates inducible resistance to macrolides, lincosamides, and streptogramin type B antibiotics in *Streptococcus sanguis*. J. Bacteriol. **154:**1252–1262.
15. **Horinouchi, S., and B. Weisblum.** 1982. Nucleotide sequence and functional map of pE194, a plasmid that specifies resistance to macrolide, lincosamide and streptogramin type B antibiotics. J. Bacteriol. **150:**804–814.
16. **Horodniceanu, T., D. H. Bouanchaud, G. Bieth, and Y. A. Chabbert.** 1976. R-plasmids in *Streptococcus agalactiae* (group B). Antimicrob. Agents Chemother. **10:**795–801.
17. **Langella, P., Y. Le Loir, S. D. Ehrlich, and A. Gruss.** 1993. Efficient plasmid mobilization by pIP501 in *Lactococcus lactis* subsp. *lactis*. J. Bacteriol. **175:** 5806–5813.
18. **LeBlanc, D. J., and L. N. Lee.** 1984. Physical and genetic analysis of streptococcal plasmid pAMβ1 and cloning of its replication region. J. Bacteriol. **157:**445–453.
19. **Leclercq, R., and P. Courvalin.** 1991. Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification. Antimicrob. Agents Chemother. **35:**1267–1272.
20. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Martin, B., G. Alloing, V. Méjean, and J.-P. Claverys.** 1987. Constitutive expression of erythromycin resistance mediated by the *ermAM* determinant of plasmid pAMβ1 results from deletion of 5′ leader peptide sequences. Plasmid **18:**250–253.
22. **Morelle, G.** 1989. A plasmid extraction procedure on a miniprep scale. Focus **11:**7–8.
23. **Nakamura, S., K. Yamakawa, S. Nakashio, S. Kamiya, and S. Nishida.** 1987.

Correlation between susceptibility to chloramphenicol, tetracycline and clindamycin, and serogroups of *Clostridium difficile*. Med. Microbiol. Immunol. **176:**79–82.
24. **Oultram, J., and N. Minton.** Unpublished results.
25. **Oultram, J. D., and M. Young.** 1985. Conjugal transfer of plasmid pAMβ1 from *Streptococcus lactis* and *Bacillus subtilis* to *Clostridium acetobutylicum*. FEMS Microbiol. Lett. **27:**129–134.
26. **Pujol, C., S. D. Erlich, and L. Jannière.** 1993. GenBank accession no. U00453.
27. **Rood, J. I., E. A. Maher, E. B. Somers, E. Campos, and C. L. Duncan.** 1978. Isolation and characterization of multiply antibiotic-resistant *Clostridium perfringens* strains from porcine feces. Antimicrob. Agents Chemother. **13:**871–880.
28. **Shaw, J. H., and D. B. Clewell.** 1985. Complete nucleotide sequence of macrolide-lincosamide-streptogramin B-resistance transposon Tn*917* in *Streptococcus faecalis*. J. Bacteriol. **164:**782–796.
29. **Swinfield, T.-J., L. Jannière, S. D. Ehrlich, and N. P. Minton.** 1991. Characterisation of a region of the *Enterococcus faecalis* plasmid pAMβ1 which enhances the segregational stability of pAMβ1-derived cloning vectors in *Bacillus subtilis*. Plasmid **26:**209–221.
30. **Swinfield, T.-J., J. D. Oultram, D. E. Thompson, J. K. Brehm, and N. P. Minton.** 1990. Physical characterisation of the replication region of the *Streptococcus faecalis* plasmid pAMβ1. Gene **87:**79–90.
31. **Thomas, C. M., and C. A. Smith.** 1986. The *trfB* region of broad host range plasmid RK2: the nucleotide sequence reveals *incC* and key regulatory gene *trfB/korA/korD* as overlapping genes. Nucleic Acids Res. **14:**4453–4470.
32. **Tomich, P. K., F. Y. An, and D. B. Clewell.** 1980. Properties of erythromycin-inducible transposon Tn*917*. J. Bacteriol. **141:**1366–1374.
33. **Traub, W. H.** 1990. Comparative in vitro bactericidal activity of 24 antimicrobial drugs against *Clostridium perfringens*. Chemother. **36:**127–135.
34. **Weaver, K. E., D. B. Clewell, and F. An.** 1993. Identification, characterization, and nucleotide sequence of a region of *Enterococcus fecalis* pheromone-responsive plasmid pAD1 capable of autonomous replication. J. Bacteriol. **175:**1900–1909.
35. **Williams, D. R., and C. M. Thomas.** 1992. Active partitioning of bacterial plasmids. J. Gen. Microbiol. **138:**1–16.
36. **Wust, J., and U. Hardegger.** 1983. Transferable resistance to clindamycin, erythromycin, and tetracycline in *Clostridium difficile*. Antimicrob. Agents Chemother. **23:**784–786.
37. **Yu, P.-L., and L. E. Pearce.** 1986. Conjugal transfer of steptococcal antibiotic resistance plasmids into *Clostridium acetobutylicum*. Biotech. Lett. **8:**469–474.