ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1994, p. 1041–1046
0066-4804/94/$04.00+0

Vol. 38, No. 5

Cloning and Sequence Analysis of *ermQ*, the Predominant Macrolide-Lincosamide-Streptogramin B Resistance Gene in *Clostridium perfringens*

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Received 9 August 1993/Returned for modification 14 October 1993/Accepted 2 March 1994

The erythromycin resistance determinant from *Clostridium perfringens* JIR100 has been cloned, sequenced, and shown to be expressed in *Escherichia coli*. An open reading frame with sequence similarity to *erm* genes from other bacteria was identified and designated the *ermQ* gene. On the basis of comparative sequence analysis, it was concluded that the *ermQ* gene represented a new Erm hybridization class, designated ErmQ. Genes belonging to the ErmQ class were found to be widespread in *C. perfringens*, since 30 of 38 macrolide-lincosamide-streptogramin B-resistant *C. perfringens* strains, from diverse sources, hybridized to an *ermQ*-specific gene probe. The *ermQ* gene therefore represents the most common erythromycin resistance determinant in *C. perfringens*.

Resistance to erythromycin and macrolide-lincosamide-streptogramin B (MLS) antibiotics such as clindamycin and lincomycin has been detected in a number of gram-positive and gram-negative genera (18, 19). Resistance is usually mediated by a 23S rRNA methylase encoded by a gene designated *erm* (erythromycin resistance methylase). Hybridization analysis of *erm* genes from a range of different bacterial genera has shown that there are eight distinct hybridization classes, designated ErmA to ErmG (18). Although these *erm* genes do not hybridize with each other, sequence analysis has shown that their DNA and deduced protein sequences have from 17 to 70% sequence identity (10, 18).

Research in this laboratory has involved the cloning and molecular analysis of antibiotic resistance determinants from the gram-positive pathogen *Clostridium perfringens* (1, 2, 4, 15, 27, 30). As part of these studies, an MLS resistance determinant, *ermBP*, from *C. perfringens* CP592 was cloned and shown to belong to the ErmB-ErmAM hybridization class (5, 29). Sequences which hybridized to an *ermBP*-specific gene probe were found to be widespread in MLS-resistant isolates of *Clostridium difficile*. However, only 5 of the 40 MLS-resistant *C. perfringens* isolates that were tested hybridized to the *ermBP*-specific probe. Examination of the nonhybridizing *C. perfringens* strains with probes representing each of the Erm hybridization classes showed that none of the strains hybridized to probes from any of the recognized Erm classes (5).

To determine whether the nonhybridizing *C. perfringens* MLS resistance determinants represented one or more new Erm hybridization classes or mediated MLS resistance by another mechanism, the erythromycin resistance determinant from one of these *C. perfringens* isolates, JIR100, was cloned in *Escherichia coli*. The nucleotide sequence of the resistance gene was elucidated, and the distribution of this gene in *C. perfringens* was determined.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and culture media. All *E. coli* strains were derivatives of JM105 (35) or DH5α (Bethesda Research Laboratories, Inc.) and were grown overnight in 2× YT broth or agar (21) supplemented with ampicillin (100 μg/ml) or erythromycin (100 μg/ml); no growth of plasmid-free strains was detected under these conditions. Erythromycin-resistant *C. perfringens* isolates included CP592 from France (7), 20 isolates (including JIR100) from three pig farms in Western Australia (28), 13 isolates from seven pig farms in Wisconsin (31), three human isolates from Germany (34), and one isolate from Japan (from Y. Miyoshi, Ibaraki Prefecture Institute of Public Health). Three MLS-resistant isolates of *C. difficile* from Japan (25) were also examined. *C. perfringens* strains were cultured with nutrient agar medium (26) or Trypticase-peptone-glucose broth as previously described (31). Erythromycin (20 μg/ml), nalidixic acid (20 μg/ml), and rifampin (1 μg/ml) were added as required. The plasmids used in this study are listed in Table 1. Culture medium components were obtained from BBL Microbiology Systems, Oxoid, and Difco Laboratories.

Preparation and characterization of plasmid DNA. Small-scale (10-ml cultures) *E. coli* DNA preparations were made with an alkaline lysis method (23). Large-scale (100-ml cultures) isolation of plasmid DNA was done by the Triton X-100 or alkaline lysis procedure (20). Chromosomal DNA and plasmid DNA were isolated from *C. perfringens* cultures with the Sarkosyl and sodium dodecyl sulfate lysis procedures (3, 31).

Restriction endonucleases were obtained from Bethesda Research Laboratories, Boehringer Mannheim, New England Biolabs, and Pharmacia and were used with the buffers described by the manufacturers. Electrophoresis of DNA was performed with 0.6 to 2.0% (wt/vol) agarose gels as previously described (36). All agarose gels contained λcI857 S7 DNA (New England Biolabs) cleaved with *Hin*dIII as molecular size standards.

Cloning methods. Recombinant plasmids were constructed by cleaving the DNA to be cloned with the appropriate restriction endonuclease and ligating it to similarly cleaved pUC18 DNA (Table 1). DNA was isolated from agarose gels with the GeneClean Kit (Bio 101) in accordance with the

TABLE 1. Relevant characteristics of plasmids

Plasmid designation	Characteristics	Source or reference
Escherichia coli		
pUC18	Ap^r *lacZ'*	35
pJIR122	Ap^r Em^r (pUC18Ω5.2-kb *Sac*I, *C. perfringens* CP592) (*ermBP*)	5
pEM9592	MLS^r (*ermA*)	24
pIJ43	MLS^r (*ermE*)	33
pFD214	MLS^r (*ermF*)	32
pJIR432	Ap^r Em^r (pUC18Ω10.3-kb *Eco*RI, *C. perfringens* JIR100) (*ermQ*)	This study
pJIR444	Ap^r Em^r (pUC18Ω10.3-kb *Eco*RI, pJIR432 [opposite orientation])	This study
pJIR450	Ap^r Em^s (pJIR432Δ3.8-kb *Xba*I)	This study
pJIR459	Ap^r Em^r (pUC18Ω3.8-kb *Xba*I-*Eco*RI, pJIR432)	This study
pJIR460	Ap^r Em^r (pUC18Ω4.7-kb *Eco*RI-*Pst*I, pJIR432)	This study
pJIR478	Ap^r Em^r (pJIR459Δ2.6-kb *Hin*dIII-*Hpa*I)	This study
pJIR479	Ap^r Em^s (pJIR478Δ0.42-kb *Hin*dIII)	This study
pJIR745	Ap^r Em^s (pUC18*Sma*IΩ0.38-kb *Ssp*I, pJIR460)	This study
Enterococcus faecalis pAMβ1	MLS^r (*ermB-ermAM*)	17
Staphylococcus aureus pE194	MLS^r (*ermC*)	14
Bacillus subtilis		
pBD90	MLS^r (*ermD*)	9
pBD364	MLS^r (*ermG*)	22

manufacturer's instructions. *E. coli* cells were transformed as previously described (20). Transformants were selected on 2× YT agar containing ampicillin (100 μg/ml) and, when required, erythromycin (100 μg/ml). When necessary, recombinant molecules were screened with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and isopropyl-β-D-thiogalactopyranoside (IPTG), and their identities were confirmed by restriction analysis. T4 DNA ligase, calf intestine alkaline phosphatase, ATP, dithiothreitol, X-Gal, and IPTG were obtained from Boehringer Mannheim.

Dot blot hybridization analysis. Hybridization experiments were done under high-stringency conditions as previously described (5). Purified plasmids (Table 1) which represented the ErmA to ErmG hybridization classes were used as controls.

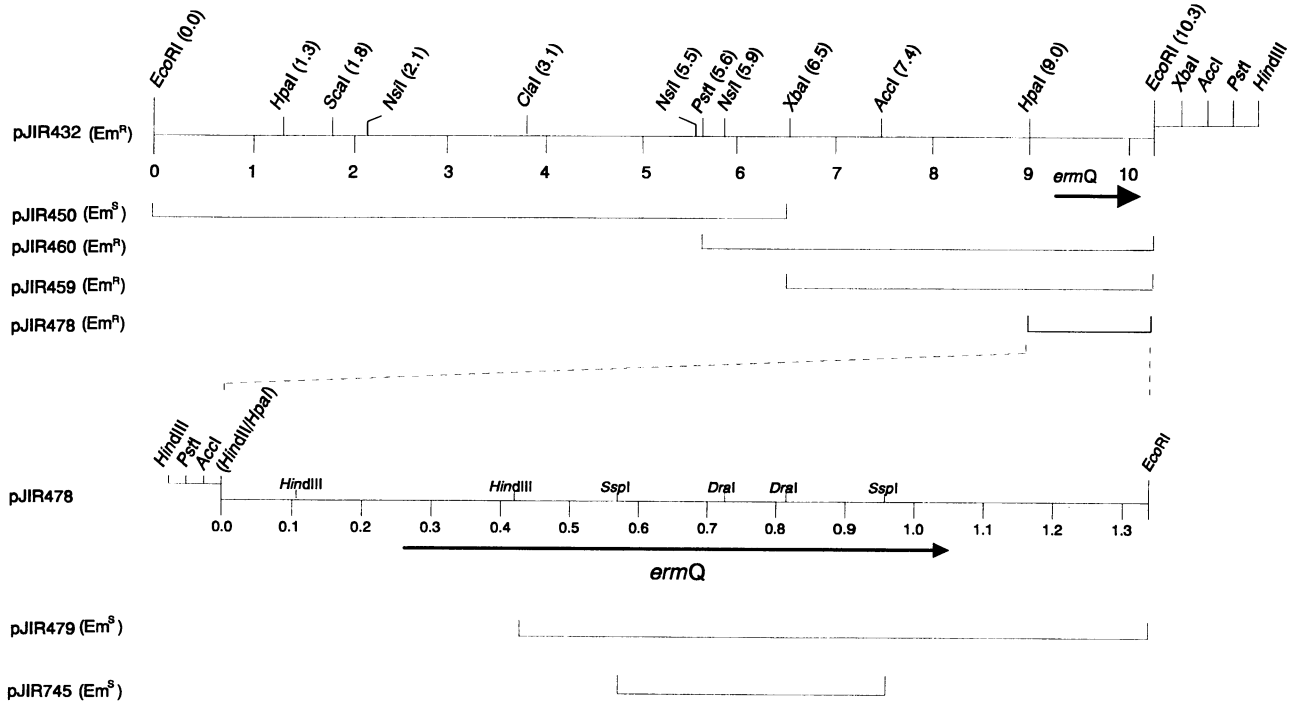

FIG. 1. Physical and genetic maps of recombinant plasmids. Restriction maps of pJIR432 and its derivatives are shown. The location and direction of transcription of the *ermQ* gene are indicated by the arrow. Only relevant restriction sites are shown. Size markers are indicated in kilobases. Em^R and Em^S signify erythromycin resistance and susceptibility, respectively.

TABLE 2. Relationship between Erm proteins[a]

Protein	% Amino acid sequence identity							
	ErmQ	ErmBP	ErmA	ErmC	ErmD	ErmE	ErmF	ErmG
ErmQ	100	41	41	43	35	20	32	41
ErmBP		100	53	51	32	22	29	53
ErmA			100	57	32	22	31	59
ErmC				100	31	23	30	71
ErmD					100	19	36	32
ErmE						100	22	23
ErmF							100	29
ErmG								100

[a] Sequence identities were calculated by comparison of the TREEALIGN sequence alignments presented in Fig. 3.

The *ermQ*-specific probe from pJIR745 was labelled with [α-^{32}P]dATP by random priming with a Bresatec Oligo Labelling kit. High-stringency washes were performed at 65°C in 0.16× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Hybridized probe was detected by autoradiography at −70°C.

Nucleotide sequence analysis. Sequence analysis of supercoiled plasmid DNA was carried out with Pharmacia T7 Sequencing kits and template DNA from small-scale plasmid preparations. When required, plasmid DNA was further purified with GeneClean or phenol extraction followed by ethanol precipitation. The complete sequence of the *ermQ* gene region was determined on both strands in an overlapping manner. Sequences were analyzed with the Australian National Genomic Information Service suite of software operated by the University of Sydney. Protein and DNA sequence alignments were carried out with the CLUSTAL V (13) and TREEALIGN (12) programs.

```
CCTGCAGGTCACATAGAGAGTATAAATAATGAAAAAATTTAAAAATATCTTCGTTTGAAAATAACTACTTAATAAAGATGCAAAAATTATAAACAAATAT 100

TTAAAGCTTATTATATGAAAAATAGGAGGAAATAAAATGATTATGAATGGTGGAATAGCGTCAATAAGATTAAGAAGATAGCGCACGAGTTGTGGCTATC 200
                                                                                          -35

GTTAAACAAATTTAATATAATGTCATAAAAGAGTAAAAATAGTGAATTAAGGAGATTATGAATGAAAGCTAAAAGTAATAATTATAGAGGAAAAGTTGAT 300
              -10                                                  M  K  A  K  S  N  N  Y  R  G  K  V  D  13

ATTAGTGTATCGCAAAATTTTATTACTAGTAAAAATACTATATATAAATTAATAAAAAAAACAAATATATCCAAAAATGATTTTGTTATTGAAATTGGAC 400
 I  S  V  S  Q  N  F  I  T  S  K  N  T  I  Y  K  L  I  K  K  T  N  I  S  K  N  D  F  V  I  E  I  G   46

CAGGAAAAGGTCATATAACAGAAGCTTTATGTGAAAAAAGTTATTGGGTTACAGCTATAGAACTAGATAGAAGTTTATATGGAAATTTAATAAATAAATT 500
P  G  K  G  H  I  T  E  A  L  C  E  K  S  Y  W  V  T  A  I  E  L  D  R  S  L  Y  G  N  L  I  N  K  F 80

TAAAAGTAAAAATAATGTTACTCTTATTAATAAAGATTTTTTAAATTGGAAATTACCTAAAAAAAGAGAATATAAGGTATTTTCTAATATTCCTTTTTAT 600
  K  S  K  N  N  V  T  L  I  N  K  D  F  L  N  W  K  L  P  K  K  R  E  Y  K  V  F  S  N  I  P  F  Y  113

ATAACAACAAAGATTATTAAGAAATTATTATTAGAAGAGTTAAATTCACCAACTGATATGTGGCTAGTTATGGAGAAAGGTTCCGCAAAAAGATTTATGG 700
 I  T  T  K  I  I  K  K  L  L  L  E  E  L  N  S  P  T  D  M  W  L  V  M  E  K  G  S  A  K  R  F  M   146

GAATACCTAGAGAGAGTAAATTATCATTACTTTTAAAAACTAAATTTGATATTAAGATAGTGCACTATTTTAATAGAGAAGACTTCCATCCCATGCCTAG 800
G  I  P  R  E  S  K  L  S  L  L  L  K  T  K  F  D  I  K  I  V  H  Y  F  N  R  E  D  F  H  P  M  P  S 180

TGTAGATTGCGTCTTAGTATATTTTAAAAGAAAATATAAATATGATATATCTAAAGATGAATGGAATGAATATACAAGTTTTATATCTAAGTCTATTAAT 900
  V  D  C  V  L  V  Y  F  K  R  K  Y  K  Y  D  I  S  K  D  E  W  N  E  Y  T  S  F  I  S  K  S  I  N  213

AACTTAAGAGATGTATTTACAAAAAATCAAATTCATGCAGTAATTAAATACCTAGGTATAAATCTTAATAATATTAGTGAAGTTTCTTATAATGATTGGA 1000
 N  L  R  D  V  F  T  K  N  Q  I  H  A  V  I  K  Y  L  G  I  N  L  N  N  I  S  E  V  S  Y  N  D  W   246

TACAGTTATTTAGATATAAACAAAAGATAGATTAGTAATAGTAGATTTAAATTAGGAGGTGTTAAAATGATTATGAATGGTGGAATAGCGTCAATAAGAT 1100
I  Q  L  F  R  Y  K  Q  K  I  D  *                                                                      257

TAAGAGGATAAGTCACGGGTTGTGGCTATTATCTTAATTTAATAAAGAGTCATAATCCCGTGATATTTAAAGTTAATATTTAAATATAAAATTACAGAGA 1200

CTTATGAAGATGATAAAAAAAAaGGAATAATTGTAGGAATTAATATAAATAATAAAAATAATTTTGATGAATCTATGGAAGAATTAAAAAATTTATGCTTA 1300

GCTTGCAAAATAGAAACTGTTGGTA                                                                              1325
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FIG. 2. Nucleotide sequence of the pJIR478 insert. The sequence of the insert present in pJIR478 is shown. Note that this sequence did not quite extend back to the *Hpa*I and *Eco*RI sites at the ends of the insert. The locations of potential −35 and −10 regions are indicated. The consensus ribosome binding site located upstream of the putative *ermQ* start codon is shown in boldface. The deduced ErmQ amino sequence is shown underneath the *ermQ* sequence. The stop codon is indicated by the asterisk.

```
ErmQ   MKAKSNNYRG-KVDISV--------------------S-QNFITSKNTIYKLIKKTNISKNDFVIEIGP 47
ErmBP  MN-K-NI----KY------------------------S-QNFLTSEKVLNQIIKQLNLKETDTVYEIGT 38
ErmA   MNQK-NP----KD------------------------T-QNFITSKKHVKEILNHTNISKQDNVIEIGS 39
ErmC   MNEK-NI----KH------------------------S-QNFITSKHNIDKIMTNIRLNEHDNIFEIGS 39
ErmD   MKKK-NHKYRGKKLNRGESPNF---------------SGQHLMHNKKLIEEIVDRANISIDDTVLELGA 53
ErmE   MSSSDEQPRPRRRNQDRQHPNQNRPVLGRTERDRNRRQFGQNFLRDRKTIARIAETAELRPDLPVLEAGP 70
ErmF   MTKK-------KLPVRF--------------------TGQHFTIDKVLIKDAIRQANISNQDTVLDIGA 42
ErmG   MN-KVNI----KD------------------------S-QNFITSKYHIEKIMNCISLDEKDNIFEIGA 39
       .  . .     .                          :..   .  .  .         . . ..:

ErmQ   GKGHITEALCEKSYWVTAIELDRSLYGNLINKFKSKNNVTLINKDFLNWKLPKKREYKVFSNIPFYITTK 117
ErmBP  GKGHLTTKLAKISKQVTSIELDSHLFNLSSEKLKLNTRVTLIHQDILQFQFPNKQRYKIVGSIPYHLSTQ 108
ErmA   GKGHFTKELVKMSRSVTAIEIDGGLCQVTKEAVNPSENIKVIQTDILKFSFPKHINYKIYGNIPYNISTD 109
ErmC   GKGHFTLELVQRCNFVTAIEIDHKLCKTTENKLVDHDNFQVLNKDILQFKFPKNQSYKIFGNIPYNISTD 109
ErmD   GKGALTTVLSQKAGKVLAVENDSKFVDILTRKTAQHSNTKIIHQDIMKIHLP-KEKFVVVSNIPYAITTP 122
ErmE   VEGLLTRELADRARQVTSYEIDPRLAKSLREKLSGHPNIEVVNADFLTAEPP-PEPFAFVGAIPYGITSA 139
ErmF   GKGFLTVHLLKIANNVVAIENDTALVEHLRKLFSDARNVQVVGCDFRNFAVP-KFPFKVVSNIPYGITSD 111
ErmG   GKGHFTAELVKRCNFVTAIEIDSKLCEVTRNKLLNYPNYQIVNDDILKFTFPSHNPYKIFGSIPYNISTN 109
       ..:  :  :      :...: :  .      .     .      : .    :     .    ::. . .

ErmQ   IIKKLLLEELNSPTDMWLVM--EKGSAKRFMGIPRESKLSLLLKTKFDIKIVHYFNREDFHPMPSVDCVL 185
ErmBP  IIKKVVFESHASDIYL-IV---EEGFYKRTLDIHRTLGLLLHTQV--SIQQLLKLPAECFHPKPKVNSVL 172
ErmA   IVKRITFESQAKYSYL-IV---EKGFAKRLQNLQRALGLLLMVEM--DIKMLKKVPPLYFHPKPSVDSVL 173
ErmC   IIRKIVFDSIADEIYL-IV---EYGFAKRLLNTKRSLALFLMAEV--DISILSMVPREYFHPKPRVNSSL 173
ErmD   IMKMLLNNPASGFQKGIIVM--EKGAAKRFTSKFIKNSYVLAWRMWFDIGIVREISKEHFSPPPKVDSAM 190
ErmE   IVDWCL-EAPTIETATM-VTQLEF-ARKRTGDYGRWSRLTVMTWPLFEWEFVEKVDSAIMRLRRRAEPLL 206
ErmF   IFKILMFESLGNFLGGSIVLQLEPTQ-KLFSRKLYNPYTVFYHTF-FDLKLVYEVGPESFLPPPTVKSAL 179
ErmG   IIRKIVFESSATISYL-IV---EYGFAKRLLDTNRSLALLLMAEV--DISILAKIPRYYFHPKPKVDSAL 173
       :      .         .:   : .  :.         . .      ..          . . . . . .

ErmQ   VY--FKRK----------YKYDISKDEWNEYTSFISKSINNL----------------RDVFTKN-QI 224
ErmBP  IK--LTRHTTDVPDKYWKLYTYFVSKWVNREY---------------------------RQLFTKN-QF 211
ErmA   IV--LERHQPLISKKDYKKYRSFVYKWVNREY---------------------------RVLFTKN-QF 212
ErmC   IR--LNRKKSRISHKDKQKYNYFVMKWVNKEY---------------------------KKIFTKN-QF 212
ErmD   VR--ITRKKDAPLSH--KHYIAFRG---LAEYALKEPNIPLCVAL--------------RGIFTPR-QM 237
ErmE   EGAALERYESMVELCFTGVGGNIQASLLRK-YPRRRVEAAFDHAGVGGGAVVAYVRPEQWLRLFERLDQK 275
ErmF   LN--IKRKHLFFDFKFKAKYLAFISYLL--EKPDLSVKTAL----------------KSIFRKS-QV 225
ErmG   IV--LKRKPAKMAFKERKKYETFVMKWVNKEY---------------------------EKLFTKN-QF 212
             :            .  .       ..                            :..  :

ErmQ   HAVIKYLGI-NLNNISEVSYNDWIQ---LFR-YKQKID------------------------------ 257
ErmBP  HQAMKHAKV---NNLSTVTY---EQVLSIFNSYLLFNGRK---------------------------- 245
ErmA   RQALKHANVTNINKLSK------EQFLSIFNSYKLFH------------------------------- 243
ErmC   NNSLKHAGIDDLNNISF------EQFLSLFNSYKLFNK------------------------------ 244
ErmD   KHLRKSLKI---NNEKTVGTLTENQWAVIFNTMTQYVMHHKWPRANKRKPGEI--------------- 287
ErmE   NEPRGGQPQRGRRTGGRDHGDRRTGGQDRGDRRTGGRDHRDRQASGHGDRRSSGRNRDDGRTGEREQGDQ 345
ErmF   RSISEKFGL---NLNAQIVCLSPSQWLNCFLEMLEVVPEKFHPS------------------------- 266
ErmG   NKALKHARIYDINNISF------EQFVSLFNSYKIFNG------------------------------ 244
           .       .          .    .

ErmQ   -------------------------
ErmBP  -------------------------
ErmA   -------------------------
ErmC   -------------------------
ErmD   -------------------------
ErmE   GGRRGPSGGGRTGGRPGRRGGPGQR  370
ErmF   -------------------------
ErmG   -------------------------
```

FIG. 3. Alignment of Erm protein sequences. The amino acid sequences of the Erm proteins were deduced from the respective nucleotide sequences and aligned with the TREEALIGN program (12). Conserved amino acids are indicated by the double dots; single dots are used to denote highly conserved amino acids. The sources of the respective Erm proteins and the relevant accession numbers are as follows: ErmQ, *C. perfringens*, L22689; ErmBP, *C. perfringens*, X58285; ErmA, *Staphylococcus aureus*, K02987; ErmC, *S. aureus*, J01755; ErmG, *Bacillus sphaericus*, M15332; ErmF, *Bacteroides fragilis*, M14730; ErmD, *Bacillus licheniformis*, M29832; and ErmE, *Saccharopolyspora erythraeus*, X51891.

Nucleotide sequence accession number. The GenBank accession number for the sequence of the *ermQ* gene region is L22689.

RESULTS AND DISCUSSION

Cloning and characterization of the MLS resistance determinant from the *C. perfringens* strain JIR100. Previous results showed that the erythromycin resistance determinant from the *C. perfringens* isolate JIR100 did not hybridize to the *ermBP* gene from *C. perfringens* or to probes representing any of the identified Erm hybridization classes under high-stringency conditions (5). To clone this resistance determinant, a total DNA preparation from strain JIR100 was digested with *Eco*RI and ligated to *Eco*RI-digested, alkaline phosphatase-treated pUC18 DNA. Competent *E. coli* JM105 cells were transformed with the ligation mixture, and recombinants were selected by plating onto media containing ampicillin and erythromycin. Three erythromycin-resistant transformants were obtained. Plasmid DNA was prepared from these transformants and digested with *Eco*RI. Restriction fragments of 2.7 and 10.3 kb were observed in all three plasmids. Digestion with *Pst*I, *Hin*dIII, or *Xba*I showed that these plasmids were indistinguishable. Therefore, subsequent studies involved only

one of these plasmids, pJIR432. To determine if the erythromycin resistance determinant in pJIR432 was expressed from the pUC18-derived *lac* promoter, a plasmid with the 10.3-kb *Eco*RI insert cloned in the reverse orientation, pJIR444, was constructed. This plasmid also conferred erythromycin resistance, which suggested that the erythromycin resistance gene was expressed from its own promoter.

A detailed restriction map of pJIR432 was subsequently constructed (Fig. 1). Subcloning localized the erythromycin resistance determinant to the 4.7-kb *Eco*RI-*Pst*I fragment present in pJIR460. The deletion derivative, pJIR450, did not confer erythromycin resistance (Fig. 1). Further deletion analysis showed that the resistance gene was located on the 1.3-kb *Eco*RI-*Hpa*I fragment of pJIR478 and spanned the innermost *Hin*dIII restriction site in pJIR478 (Fig. 1).

Nucleotide sequence analysis of pJIR478. The complete sequence of the erythromycin resistance gene was determined by subcloning appropriate restriction fragments from pJIR478 into pUC18 and sequencing the resultant subclones with the universal or reverse sequencing primers. Analysis of the sequence revealed the presence of an open reading frame of 771 bp which was preceded by sequences with similarity to the consensus *C. perfringens* ribosome binding site and promoter sequences (29) and which was capable of encoding a 257-amino-acid protein (Fig. 2). Comparison of the nucleotide sequence with equivalent sequences from each of the Erm classes (ErmA to ErmG) showed that the open reading frame had between 31 and 57% DNA sequence identity to these *erm* genes. On the basis of this result and the previous hybridization data (5), it was concluded that the cloned erythromycin resistance determinant represented a new class of *erm* gene, designated *ermQ*, which was the prototype of the new ErmQ hybridization class. It also was concluded that the *ermQ* gene from JIR100 most likely mediates erythromycin resistance by the production of a 23S rRNA methylase.

The G+C content of the *ermQ* gene was 22.6 mol%, which compared favorably with the 24 to 27 mol% G+C content of chromosomal *C. perfringens* DNA (16) and suggested either that *ermQ* was a native *C. perfringens* gene or that it was transferred to *C. perfringens* and has been resident in this organism for a considerable period of time. By comparison, the G+C content of the *C. perfringens ermBP* gene, which is identical to the *erm* gene from the promiscuous enterococcal plasmid pAMβ1, is 33 mol% (6).

Comparative analysis of putative Erm proteins. The amino acid sequence of the putative ErmQ protein was deduced from the sequence of the *ermQ* gene and aligned with similar Erm sequences. The results (Fig. 3) showed that, although the *ermQ* gene did not hybridize to the other *erm* genes, the ErmQ protein had 32 to 43% identity with these proteins, except for ErmE, with which it had 20% identity (Table 2). These data confirm the conclusion that ErmQ represents a distinct class of MLS resistance determinants.

Hybridization analysis of erythromycin-resistant *C. perfringens* isolates. To determine the distribution of the *ermQ* gene in *C. perfringens*, an internal *ermQ*-specific DNA probe was prepared by isolating the insert from pJIR745 (Fig. 1). The target DNA was isolated from 32 erythromycin-resistant *C. perfringens* isolates which did not hybridize to an *ermBP* probe (5). These isolates were from a wide geographic range. Controls included plasmids representing each of the seven previously identified Erm classes (Table 1), six *C. perfringens* isolates which hybridized to *ermBP* (6), three erythromycin-resistant *C. difficile* isolates, and an erythromycin-susceptible isolate. As expected, none of the control DNA preparations hybridized to the probe. However, the results showed that DNA from 30 of the 32 non-*ermBP*-hybridizing erythromycin-resistant *C. perfringens* isolates hybridized to the *ermQ*-specific probe (data not shown). No hybridization was detected between the *ermQ* probe and genes from any other Erm hybridization class. Two isolates, JIR87 and JIR149, which did not hybridize to either the *ermQ* or *ermBP* probe were detected. These isolates were obtained from two pig farms in different regions of Western Australia. Resistance in these two isolates appears to be mediated either by an *erm* gene of a new hybridization class or by another mechanism.

The *ermQ* gene was more common in erythromycin-resistant isolates of *C. perfringens* than the *ermBP* gene, which may reflect differences in the mechanisms by which these determinants are disseminated within the *C. perfringens* population. The *ermBP* gene is a member of the ErmB-ErmAM class which is widespread among gram-positive organisms (5, 8, 11, 18, 37). The limited distribution of *ermBP* suggests that there may be barriers to its spread in *C. perfringens* even though it can be carried on a nonconjugative plasmid capable of being mobilized (7). Independent transfer of erythromycin resistance from wild-type *C. perfringens* isolates has not been reported (26, 28), although on the basis of the widespread distribution of the *ermQ* determinant, conjugative transfer of this determinant would be predicted to occur.

ACKNOWLEDGMENTS

We thank Pauline Howarth and Tracy Warner for most capable technical assistance. Y. Miyoshi, S. Nakamura, and W. Traub are thanked for the provision of strains.

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

REFERENCES

1. **Abraham, L. J., D. I. Berryman, and J. I. Rood.** 1988. Hybridization analysis of the class P tetracycline resistance determinant from the *Clostridium perfringens* R-plasmid, pCW3. Plasmid **19:** 113–120.
2. **Abraham, L. J., and J. I. Rood.** 1985. Cloning and analysis of the *Clostridium perfringens* tetracycline resistance plasmid, pCW3. Plasmid **13:**155–162.
3. **Abraham, L. J., and J. I. Rood.** 1985. Molecular analysis of transferable tetracycline resistance plasmids from *Clostridium perfringens*. J. Bacteriol. **161:**636–640.
4. **Bannam, T. L., and J. I. Rood.** 1991. Relationship between the *Clostridium perfringens catQ* gene product and chloramphenicol acetyltransferases from other bacteria. Antimicrob. Agents Chemother. **35:**471–476.
5. **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.
6. **Berryman, D. I., and J. I. Rood.** Unpublished results.
7. **Bréfort, G., M. Magot, H. Ionesco, and M. Sebald.** 1977. Characterization and transferability of *Clostridium perfringens* plasmids. Plasmid **1:**52–66.
8. **Courvalin, P., and C. Carlier.** 1986. Transposable multiple antibiotic resistance in *Streptococcus pneumoniae*. Mol. Gen. Genet. **205:**292–297.
9. **Docherty, A., G. Grandi, R. Grandi, T. J. Gryczan, A. G. Shivakumar, and D. Dubnau.** 1981. Naturally occurring macrolide-lincosamide-streptogramin B resistance in *Bacillus licheniformis*. J. Bacteriol. **145:**129–137.
10. **Dubnau, D., and M. Monod.** 1986. The regulation and evolution of MLS resistance. Banbury Rep. **24:**369–387.
11. **Hächler, H., B. Berger-Bächi, and F. H. Kayser.** 1987. Genetic characterization of a *Clostridium difficile* erythromycin-clindamycin resistance determinant that is transferable to *Staphylococcus aureus*. Antimicrob. Agents Chemother. **31:**1039–1045.

12. **Hein, J.** 1990. Unified approach to alignment and phylogenies. Methods Enzymol. **183:**626–645.
13. **Higgins, D. G., A. J. Bleasby, and R. Fuchs.** 1992. Clustal V: improved software for multiple sequence alignment. Comput. Appl. Biosci. **8:**189–191.
14. **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.
15. **Huggins, A. S., T. L. Bannam, and J. I. Rood.** 1992. Comparative sequence analysis of the *catB* gene from *Clostridium butyricum*. Antimicrob. Agents Chemother. **36:**2548–2551.
16. **Johnson, J. L., and B. S. Francis.** 1975. Taxonomy of the clostridia: ribosomal acid homologies among the species. J. Gen. Microbiol. **88:**229–244.
17. **LeBlanc, D. J., and L. N. Lee.** 1984. Physical and genetic analyses of streptococcal plasmid pAMβ1 and cloning of its replication region. J. Bacteriol. **157:**445–453.
18. **Leclercq, R., and P. Courvalin.** 1991. Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification. Antimicrob. Agents Chemother. **35:**1267–1272.
19. **Leclercq, R., and P. Courvalin.** 1991. Intrinsic and unusual resistance to macrolide, lincosamide, and streptogramin antibiotics in bacteria. Antimicrob. Agents Chemother. **35:**1273–1276.
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. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Monod, M., S. Mohan, and D. Dubnau.** 1987. Cloning and analysis of *ermG*, a new macrolide-lincosamide-streptogramin B resistance element from *Bacillus sphaericus*. J. Bacteriol. **169:**340–350.
23. **Morelle, G.** 1989. A plasmid extraction procedure on a miniprep scale. Focus **11:**7–8.
24. **Murphy, E., and S. Löfdahl.** 1984. Transposition of Tn*554* does not generate a target duplication. Nature (London) **307:**292–294.
25. **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.
26. **Rood, J. I.** 1983. Transferable tetracycline resistance in *Clostridium perfringens* strains of porcine origin. Can. J. Microbiol. **29:**1241–1246.
27. **Rood, J. I.** 1993. Antibiotic resistance determinants of *Clostridium perfringens*, p. 141–155. *In* M. Sebald (ed.), Genetics and molecular biology of anaerobic bacteria. Springer-Verlag, New York.
28. **Rood, J. I., J. R. Buddle, A. J. Wales, and R. Sidhu.** 1985. The occurrence of antibiotic resistance in *Clostridium perfringens* from pigs. Aust. Vet. J. **62:**276–279.
29. **Rood, J. I., and S. T. Cole.** 1991. Molecular genetics and pathogenesis of *Clostridium perfringens*. Microbiol. Rev. **55:**621–648.
30. **Rood, J. I., S. Jefferson, T. L. Bannam, J. M. Wilkie, P. Mullany, and B. W. Wren.** 1989. Hybridization analysis of three chloramphenicol resistance determinants from *Clostridium perfringens* and *Clostridium difficile*. Antimicrob. Agents Chemother. **33:**1569–1574.
31. **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.
32. **Smith, C. J.** 1985. Development and use of cloning systems for *Bacteroides fragilis*: cloning of a plasmid-encoded clindamycin resistance determinant. J. Bacteriol. **164:**294–301.
33. **Thompson, C. J., T. Keiser, J. M. Ward, and D. A. Hopwood.** 1982. Physical analysis of antibiotic resistance genes from *Streptomyces* and their use in vector construction. Gene **20:**51–62.
34. **Traub, W. H.** 1990. Comparative in vitro bactericidal activity of 24 antimicrobial drugs against *Clostridium perfringens*. Chemotherapy **36:**127–135.
35. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.
36. **Young, I. G., A. Jaworowski, and M. I. Poulis.** 1978. Amplification of the respiratory NADH dehydrogenase of *Escherichia coli* by gene cloning. Gene **4:**25–36.
37. **Youngman, P. J., J. B. Perkins, and R. Losick.** 1983. Genetic transposition and insertional mutagenesis in *Bacillus subtilis* with *Streptococcus faecalis* transposon Tn*917*. Proc. Natl. Acad. Sci. USA **80:**2305–2309.