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Cloning and Hybridization Analysis of *ermP*, a Macrolide-Lincosamide-Streptogramin B Resistance Determinant from *Clostridium perfringens*

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The erythromycin resistance determinant from *Clostridium perfringens* CP592 was cloned and shown to be expressed in *Escherichia coli*. The resultant plasmid, pJIR122 (7.9 kilobase pairs [kb]), was unstable since in both *recA*$^+$ and *recA E. coli* hosts spontaneous deletion of 2.7 kb, including the erythromycin resistance determinant, was observed. Subcloning, as well as deletion analysis with BAL 31, localized the erythromycin resistance gene (*ermP*) to within a 1.0-kb region of pJIR122. A 0.5-kb fragment internal to *ermP* was ^{32}P labeled and used as an *ermP*-specific probe in DNA hybridization experiments which used target DNA prepared from representatives of each of the known *erm* classes and also from erythromycin-resistant isolates of a variety of clostridial species. Hybridizing sequences were detected in DNA from several *Clostridium difficile* isolates and a *Clostridium paraputrificum* strain; however, *ermP* was not widespread in erythromycin-resistant *C. perfringens* isolates. The *ermP* determinant hybridized to, and shared significant restriction identity with, the *ermB* class gene from the streptococcal plasmid pAMβ1. No hybridization was detected with the other six hybridization classes of *erm* determinants.

Resistance to macrolides, lincosamides, and streptogramin B (MLS) antibiotics has been detected in isolates from a variety of bacterial genera, including *Staphylococcus*, *Streptococcus*, *Bacteroides*, *Bacillus*, and *Streptomyces* (8, 10, 29). In many MLS-resistant isolates, resistance is due to the dimethylation of 23S rRNA, which results in a reduced affinity between the antibiotic and the target ribosome (19, 20). The genes coding for these RNA methylases are designated *erm* genes (5). On the basis of DNA hybridization studies, seven classes of *erm* gene (*ermA* to *ermG*) have been identified (5, 11). These classes are distinct in DNA hybridization experiments, but the amino acid sequences of the RNA methylases are highly conserved (11).

The isolation of clostridia resistant to MLS antibiotics was first reported by Sebald et al. (34) when they identified a *Clostridium perfringens* isolate (CP590) resistant to erythromycin, clindamycin, and lincomycin. On the basis of further studies, it was concluded that the resistance determinant was located on the 63-kilobase-pair (kb) plasmid pIP402 (7). More recently, erythromycin-resistant isolates have been isolated from a number of *Clostridium* spp. (12, 31, 33, 36, 41). Although the response of many of these strains to the streptogramin B antibiotics has not been reported, many isolates are also resistant to the lincosamide antibiotics, clindamycin and lincomycin. The resistance profiles are therefore consistent with the *erm*-mediated MLS resistance phenotype observed in other genera.

To determine the relationship between the erythromycin resistance determinants from *C. perfringens*, other clostridial species, and MLS resistance determinants from other genera, the erythromycin resistance determinant from *C. perfringens* CP592 (7), a derivative of CP590, was cloned. In this paper, we report cloning and analysis of this gene, designated *ermP*. The *ermP* determinant was found to be common in *Clostridium difficile* and was also detected in an isolate of *Clostridium paraputrificum*. However, only 5 of 40 erythromycin-resistant *C. perfringens* isolates examined contained sequences that hybridized with *ermP*.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and culture media. All strains and plasmids used in this study are listed in Tables 1 and 2, respectively. All clostridial strains were cultured as previously described (1) by using media supplemented with erythromycin, lincomycin, or clindamycin at a concentration of 25 μg/ml. *Bacillus subtilis* and *Escherichia coli* strains were cultured by using 2×YT broth or agar (40), supplemented with ampicillin (100 μg/ml) or erythromycin (50 μg/ml) as required.

Preparation and characterization of DNA. Clostridial plasmid and chromosomal DNA was isolated from mid-log cultures by the sodium dodecyl sulfate lysis-buoyant density gradient ultracentrifugation method of Abraham and Rood (2). Plasmid isolation from large-scale cultures (100 ml) of *B. subtilis* and *E. coli* was by the Triton X-100 cleared-lysate (44) or alkaline lysis (6) methods. Plasmid DNA from small-scale (10 ml) *E. coli* cultures was prepared by the rapid-boil method (17). Target DNA for dot blot hybridization experiments was prepared, from 2-ml cultures, by lysing cells by using a scaled-down version of the clostridial lysis procedure above. The resultant crude cell lysates were used without further purification.

Restriction endonucleases were purchased from Pharmacia, Boehringer-Mannheim Biochemicals, or Bethesda Research Laboratories, Inc. Restriction digestions were performed as previously described (23). Agarose gel electrophoresis of DNA and the determination of DNA fragment sizes were as previously described (2) with *Hin*dIII- or *Bst*EII-cleaved lambda DNA or *Cfo*I-cleaved pUC18 DNA as molecular size standards. DNA fragments were isolated and purified from low-melting-temperature agarose (SeaPlaque; FMC Corp., Marine Colloids Div.) by using a phenol extraction procedure (23).

Cloning methods. All cloning experiments used the *E. coli*

TABLE 1. Origin and relevant characteristics of bacterial strains

Strain	Characteristics[a]	Source or reference
E. coli		
JM105	*thi rpsL endA sbcB15 hspR4* Δ(*lac-proAB*) (F′ *traD36 proAB lacI*^{q}*Z* ΔM15)	42
DH5α	F$^-$ *endA hsdR17 supE44 thi*-1 λ$^-$ *recA1 gyrA96 relA1* φ80*dlacZ* ΔM15	Bethesda Research Laboratories
JIR917	JM105(pJIR122)	This study
JFM65	C600 *hsdR hsdM rpsL srl1300*::Tn*10 recA56*	32
IY38	C600 *hsdR hsdM rspL*	32
C. perfringens		
CP592	Em^r Cc^r Ln^r (pIP402)	7
JIR100	Em^r Cc^r Ln^r	1
JIR149	Em^r Cc^r Ln^r	31
JIR190	Em^r Cc^r Ln^r	1
CW469, CW471, CW472, CW631	Em^r Cc^r Ln^r	33
C. difficile		
CD18	Em^s Cc^s Ln^s	United States (36)
L253	Em^r Cc^s Ln^s	United Kingdom (16)
L289	Em^r Cc^r Ln^r	United Kingdom (16)
630	Em^r Cc^r Ln^r	Switzerland (41)
662	Em^r Cc^r Ln^r	Switzerland (41)
AM480	Em^r Cc^s Ln^s	I.M.V.S., Adelaide, via R. G. Wilkinson
AM1180	Em^r Cc^r Ln^r	Latrobe Valley Hospital, via R. G. Wilkinson
AM1182	Em^r Cc^s Ln^s	Royal Melbourne Hospital, via R. G. Wilkinson
AM1185	Em^r Cc^r Ln^r	Royal Melbourne Hospital, via R. G. Wilkinson
C. sporogenes CW486	Em^r Cc^s Ln^r	United States, porcine feces (33)
C. paraputrificum CW498	Em^r Cc^r Ln^r	United States, porcine feces (33)

[a] Em^r and Em^s, Erythromycin resistant and susceptible, respectively; Cc^r and Cc^s, clindamycin resistant and susceptible, respectively; Ln^r and Ln^s, lincomycin resistant and susceptible, respectively.

vector pUC18. The DNA to be cloned was cleaved with the appropriate restriction enzyme and ligated to similarly cleaved vector DNA as previously described (3). Transformation of *E. coli* strains was as previously described (23). Transformants were generally selected on media containing ampicillin and erythromycin. When this was not possible, transformants were plated on media containing ampicillin, and transformants harboring recombinant plasmids were identified by their white color in the presence of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and isopropyl-β-D-thiogalactopyranoside (IPTG) (40). The identity of recombinant molecules was determined by restriction analysis.

Construction of deletion derivatives. Subclone pJIR208 was constructed by the deletion of the 2.75-kb *Ava*I fragment of pJIR122 as follows. Plasmid pJIR122 was cleaved with *Ava*I, and the resultant restriction fragment ends were filled with deoxynucleotides by using the Klenow fragment of DNA polymerase I (23). The fragments were then ligated and used to transform *E. coli* DH5α to erythromycin resistance. Plasmids pJIR211 and pJIR212 were derived by digestion of pJIR208 with either *Acc*I or *Hin*cII and deletion of the 0.15-kb *Acc*I fragment and the 0.35-kb *Hin*cII fragment, respectively. Since digestion with *Hin*cII generated fragments with blunt ends, only the *Acc*I fragments were filled with deoxynucleotides prior to ligation and transformation. Additional deletion derivatives (e.g., pJIR229 and pJIR231) were generated by limited BAL 31 nuclease digestion from the *Eco*RI site of pJIR208 as follows. Approximately 5 μg of pJIR208 DNA was cleaved with *Eco*RI, phenol extracted, and ethanol precipitated. The DNA pellet was suspended in 100 μl of BAL 31 reaction buffer (23) and equilibrated at 37°C. Two units of BAL 31 nuclease (Bethesda Research Laboratories) was added, and 10-μl samples were removed at 1-min intervals to tubes containing 10 μl of ethylene glycol-bis(β-aminoethyl ether)-*N*, *N*, *N*′, *N*′-tetraacetic acid (EGTA) (23). The digested DNA was examined by gel electrophoresis, and samples containing appropriately sized fragments were cleaved with *Pst*I and ligated to *Pst*I-*Sma*I-cleaved pUC18. The resultant DNA was used to transform *E. coli* DH5α to ampicillin or ampicillin and erythromycin resistance. Plasmid DNA was prepared from selected transformants, and the size of the cloned insert was determined from *Hin*dIII-*Eco*RI digests.

DNA hybridization studies. Probe DNA was labeled with [^{32}P]dTTP by using a nick translation kit (Amersham Corp.). For Southern hybridization experiments, target DNA was cleaved with the appropriate restriction enzyme and the resultant fragments were fractionated by electrophoresis. The DNA was transferred bidirectionally (23) to nitrocellulose filters (BA-85; Schleicher & Schuell, Inc.). For dot blot hybridization experiments, 5-μl samples of the crude cell lysates were placed onto nitrocellulose filters and the DNA was bound as previously described (43).

TABLE 2. Relevant characteristics of bacterial plasmids

Plasmid[a]	Characteristics[b]	Source or reference
E. coli		
pUC18	Ap^r *lacZ'*	42
pJIR122	Ap^r Em^r; pUC18Ω(*Sac*I: 5.2-kb insert from *C. perfringens* CP592)	Recombinant
pJIR190	Ap^r Em^r; pUC18Ω(*Sac*I; 5.2 kb; orientation opposite to that of pJIR122)	Recombinant
pJIR208	Ap^r Em^r; pJIR122Δ(*Ava*I, 2.75 kb)	Recombinant
pJIR211	Ap^r Em^r; pJIR208Δ(*Acc*I, 2.9 kb)	Recombinant
pJIR212	Ap^r Em^s; pJIR208Δ(*Hin*cII, 3.1 kb)	Recombinant
pJIR229	Ap^r Em^r; pUC18Ω(*Pst*I-BAL31: pJIR208, 1.0 kb)	BAL 31 derivative
pJIR231	Ap^r Em^s; pUC18Ω(*Pst*I-BAL31: pJIR208, 0.85 kb)	BAL 31 derivative
pJIR233	Ap^r Em^s; pJIR231Δ(*Hin*cII, 0.35 kb)	Recombinant
pJIR155	Ap^r Em^s; pJIR122Δ2.7 kb	Spontaneous deletion derivative of pJIR122
pEM9592	Em^r (*ermA*)	27
pIJ43	Em^r (*ermE*)	37
pFD214	Em^r (*ermF*)	35
Streptococcus faecalis pAMβI	Em^r (*ermB*)	22
Staphylococcus aureus pE194	Em^r (*ermC*)	18
B. subtilis		
pBD90	Em^r (*ermD*)	9
pBD364	Em^r (*ermG*)	25

[a] Recombinant plasmid pFD214 contains the cloned erythromycin resistance determinant from the *Bacteroides ovatus* plasmid, pBI136. Plasmids pEM9592, pIJ43, pBD90, and pBD364 contain the chromosomally encoded erythromycin resistance determinants from *S. aureus*, *Streptomyces erythraeus*, *Bacillus subtilis*, and *Bacillus sphaericus*, respectively.

[b] Ap^r, Ampicillin resistant; Em^r and Em^s, erythromycin resistant and susceptible, respectively.

Hybridization of labeled probe DNA to filter-bound target DNA was as previously described (23). Two stringency washes (30 min each) were performed in 0.16× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at 65°C, and hybridized probe DNA was detected by autoradiography at −70°C.

RESULTS

Cloning and characterization of the erythromycin resistance determinant from CP592. *C. perfringens* CP592 was selected as the source of the clostridial erythromycin resistance determinant because the determinant is reported to be located on the plasmid pIP402 (7). However, initial cloning attempts using plasmid DNA prepared from CP592 were unsuccessful. Therefore, it was decided to clone the determinant by using a shotgun strategy.

Total cellular DNA was isolated from CP592, cleaved with *Sac*I, and ligated to pUC18. Recombinant plasmids containing the resistance determinant from CP592 were identified by their ability to transform *E. coli* JM105 to ampicillin and erythromycin resistance. Several resistant transformants were identified, and one isolate, JIR917, was selected for further studies. Plasmid DNA was prepared from this strain and digested with *Sac*I. The results showed that the resistance determinant (*ermP*) was located on a 5.2-kb *Sac*I fragment of the recombinant plasmid pJIR122. A detailed restriction map of pJIR122 was deduced after digestion with various combinations of restriction enzymes (Fig. 1). A feature of the cloned 5.2-kb *Sac*I fragment was the absence of restriction sites for 19 of the 33 enzymes used. Digestion of pJIR122 with *Sac*I and ligation of the resultant fragments led to the construction of pJIR190, which contained the 5.2-kb *Sac*I fragment in the opposite orientation to pJIR122. As pJIR190 was able to confer erythromycin resistance upon DH5α, it was concluded that the 5.2-kb *Sac*I fragment encoded all the regulatory and structural sequences necessary for the expression of *ermP* in *E. coli*.

The cloned 5.2-kb *Sac*I fragment in pJIR122 was found to undergo spontaneous deletion of approximately 2.7 kb, including the erythromycin resistance determinant, in both *recA*$^+$ (IY38) and *recA* (JFM65) *E. coli* strains. Restriction analysis of the resultant plasmid pJIR155 showed that the deleted region was located between two 0.35-kb *Cfo*I fragments and that one of these fragments was retained in pJIR155.

Localization of the erythromycin resistance determinant. To determine the location of the *ermP* gene, a series of subclones was constructed as described in Materials and Methods. Deletion derivatives pJIR208 and pJIR211 (Fig. 1) lacked the 2.75-kb *Ava*I fragment and the 2.9-kb *Acc*I fragment, respectively. Both plasmids conferred erythromycin resistance, which indicated that the *ermP* gene was located within the 2.3-kb *Acc*I-*Sac*I fragment of pJIR122. Deletion derivative pJIR212, which contained the 2.1-kb *Hin*cII-*Sac*I fragment, did not confer erythromycin resistance. It was therefore concluded that the *Hin*cII site located at 3.1 kb on the pJIR122 map was within the *ermP* structural gene or its regulatory region. From these results, one end of *ermP* was localized to the 0.2-kb region between the *Acc*I site (2.9 kb) and the *Hin*cII site (3.1 kb) of pJIR122.

To determine the location of the other end of the gene, a series of BAL 31-derived deletion plasmids was generated from the *Eco*RI site of pJIR208 as described in Materials and Methods. The smallest deletion derivative still able to confer

FIG. 1. Restriction map of pJIR122 and derivatives. No sites in the cloned 5.2-kb region were found for the enzymes *Apa*I, *Bgl*II, *Bcl*I, *Eco*RI, *Eco*RV, *Hae*III, *Hpa*II, *Kpn*I, *Mlu*I, *Nco*I, *Nru*I, *Pst*I, *Pvu*II, *Sph*I, *Sal*I, *Sma*I, *Stu*I, *Xba*I, and *Xho*I. Multiple sites for the enzymes *Alu*I, *Bst*EII, *Dra*I, *Hae*II, *Hin*fI, *Sau*IIIa, and *Taq*I were not mapped. Part of the pUC18 region of the plasmids is indicated by the bold lines. The scale is indicated in kilobase pairs. The approximate location of the *ermP* gene is indicated. Restriction sites enclosed by parentheses indicate sites that were not reconstructed during cloning.

erythromycin resistance was pJIR229, which contained a 1.0-kb fragment of pJIR208 (Fig. 1). Deletion derivative pJIR231, which contained a 0.85-kb fragment of pJIR208 (Fig. 1), did not confer erythromycin resistance. It was therefore concluded that the other end of the *ermP* gene must be located between the deletion endpoints in pJIR231 and pJIR229, at 3.6 and 3.75 kb, respectively, on the pJIR122 map. From these results, the *ermP* gene was calculated to be between 0.5 and 0.85 kb in size (Fig. 1).

Hybridization analysis of erythromycin-resistant *C. perfringens* isolates. To carry out comparative hybridization studies, it was necessary to construct a plasmid from which an *ermP*-specific DNA hybridization probe could be readily isolated. As both the BAL 31 deletion endpoint in pJIR231 (3.6 kb) and the *Hin*cII site (3.1 kb) were located within *ermP*, deletion of the 0.35-kb *Hin*cII fragment from pJIR231 resulted in a plasmid, pJIR233, containing a 0.5-kb fragment from within the *ermP* gene (Fig. 1). This fragment was isolated from *Hin*dIII-*Eco*RI digests of pJIR233, labeled with ^{32}P, and used as an *ermP*-specific probe in DNA hybridization experiments.

To determine the distribution of the *ermP* determinant in *C. perfringens*, target DNA for dot blot hybridization studies was prepared from 40 erythromycin-, clindamycin-, and lincomycin-resistant *C. perfringens* isolates. With the exception of CP592, all of these isolates were obtained from porcine feces, with 17 from Wisconsin (33) and 22 from Western Australia (31). When examined with the *ermP*-specific probe, only five strains, including the parent strain CP592, were found to contain hybridizing DNA sequences (data not shown). All the hybridizing strains except CP592, which is of human origin from France, were Wisconsin isolates.

These hybridizing strains were studied further by Southern hybridization analysis of purified chromosomal DNA from each strain. The target DNA from each isolate was cleaved with *Sac*I or *Cfo*I, and the fragments were separated by agarose gel electrophoresis. The results showed that a hybridizing *Sac*I fragment, of the same size as the 5.2-kb fragment cloned in pJIR122, was detected in DNA from CP592 (Fig. 2). In the other strains (CW469, CW471, CW472, and CW631), hybridization was restricted to fragments of greater than 20 kb. However, these four strains contained a hybridizing *Cfo*I fragment which was only slightly larger than the 1.15-kb *ermP*-containing *Cfo*I fragment from pJIR122 and CP592 (Fig. 2).

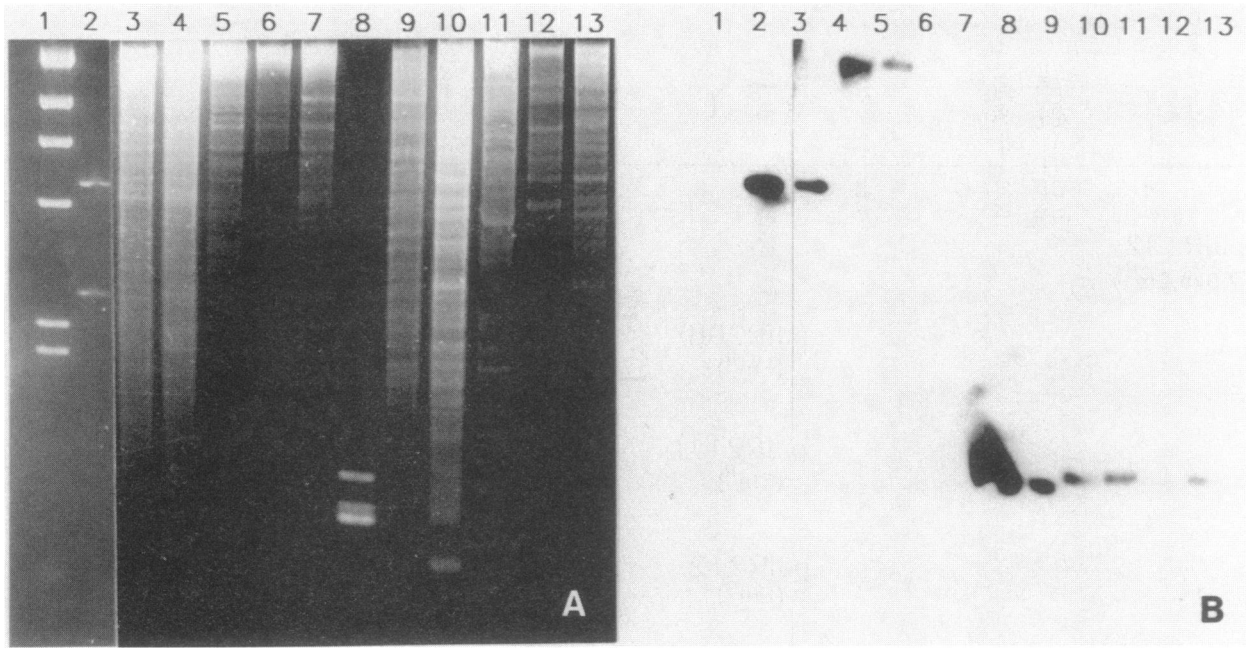

FIG. 2. Southern hybridization analysis of erythromycin resistance determinants from *C. perfringens*. Chromosomal DNA from the hybridizing *C. perfringens* isolates identified by dot blot hybridization was digested with *Sac*I or *Cfo*I, bound to nitrocellulose filters, and examined with the *ermP* probe. The profiles of the agarose gel (A) and autoradiograph (B) are shown. DNA in lanes 2 to 7 was digested with *Sac*I, and DNA in lanes 8 to 13 was digested with *Cfo*I. Lane 1, λ *cI857* (*Hin*dIII digested); lanes 2 and 8, pJIR122; lanes 3 and 9, CP592; lanes 4 and 10, CW469; lanes 5 and 11, CW471; lanes 6 and 12, CW472; lanes 7 and 13, CW631. Note that the apparent hybridization at ca. 1.2 kb in lane 7 is due to overexposure of the strongly hybridizing band in lane 8.

Comparative hybridization analysis of other clostridial species. The distribution of the *ermP* determinant in isolates of other clostridial species was also examined. All of the isolates examined were resistant to erythromycin, but some were susceptible to either clindamycin or lincomycin (Table 1). Purified chromosomal DNA was prepared from eight *C. difficile* isolates, one *Clostridium sporogenes* isolate, one *C. paraputrificum* isolate, and an erythromycin-susceptible *C. difficile* strain, cleaved with *Sac*I, and examined by Southern hybridization with the *ermP*-specific probe. Only the six isolates (five *C. difficile*, one *C. paraputrificum*) which were resistant to all three antibiotics contained sequences that hybridized to *ermP* (Fig. 3). In each of these DNA preparations, the hybridizing *Sac*I fragments were greater than 20 kb in size.

Comparative hybridization analysis of other genera. To determine the relationship between *ermP* and *erm* determinants from other genera, plasmids representing each of the known *erm* hybridization classes (*ermA* to *ermG*) were isolated and Southern blots of the digested plasmids were

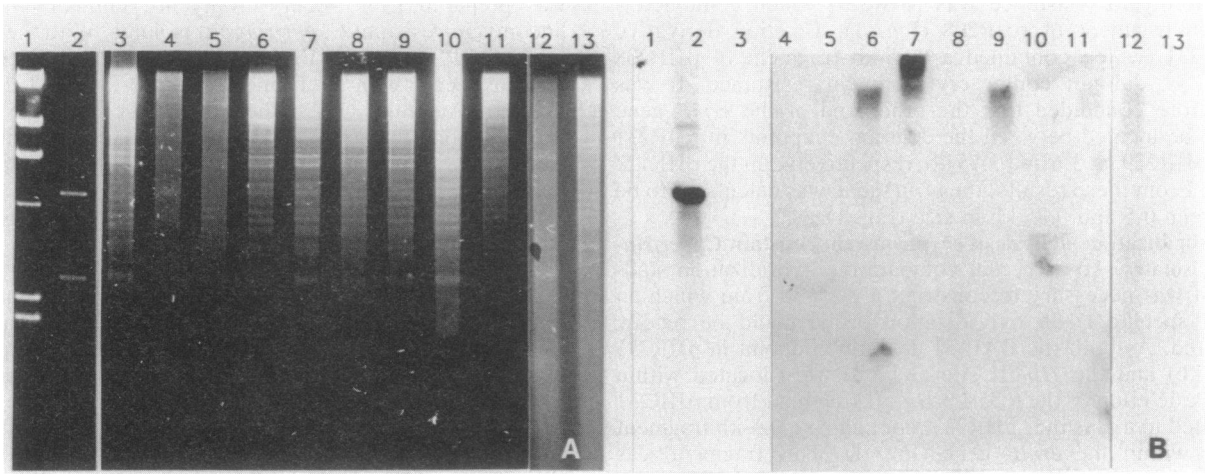

FIG. 3. Southern hybridization analysis of clostridial erythromycin resistance determinants. Purified chromosomal DNA from *C. difficile* (lanes 3 to 11), *C. paraputrificum* (lane 12), and *C. sporogenes* (lane 13) was cleaved with *Sac*I, transferred to nitrocellulose, and probed with the 0.5-kb *ermP*-specific fragment. Both the agarose gel (A) and autoradiograph (B) are shown. Lane 1, λ *cI857* (*Hin*dIII digested); lane 2, pJIR122 (*Sac*I digested); lane 3, CD18; lane 4, L253; lane 5, L289; lane 6, 630; lane 7, 662; lane 8, AM480; lane 9, AM1180; lane 10, AM1182; lane 11, AM1185; lane 12, CW498; lane 13, CW486. Note that the apparent hybridization in lane 10 is due to a nonspecific artifact.

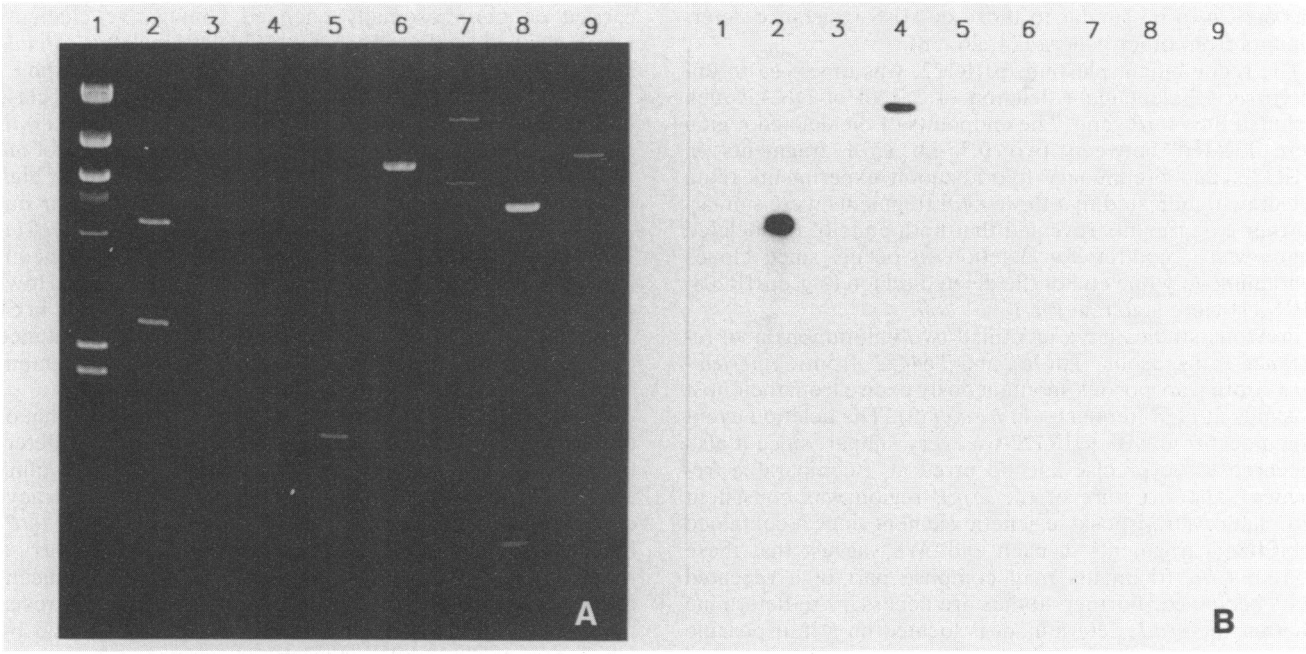

FIG. 4. Southern hybridization analysis of *erm* determinants from other genera. Plasmid DNA representative of each of the known *erm* classes was purified and digested with restriction enzymes to isolate the *erm* genes on readily identifiable fragments. The digested DNA was electrophoresed, transferred to nitrocellulose, and probed with the 0.5-kb *ermP*-specific probe. The agarose gel (A) and corresponding autoradiograph (B) are shown. Lane 1, λ *cI857* (*Hin*dIII digested); lane 2, pJIR122 (*Sac*I); lane 3, pEM9592 (*Eco*RI), *ermA*; lane 4, pAMβI (*Eco*RI), *ermB*; lane 5, pE194 (*Cfo*I), *ermC*; lane 6, pBD90 (*Hin*dIII), *ermD*; lane 7, pIJ43 (*Pst*I), *ermE*; lane 8, pFD214 (*Eco*RI), *ermF*; lane 9, pBD364 (*Bam*HI), *ermG*.

probed with the *ermP*-specific fragment. Hybridizing sequences were detected in the 21.5-kb *Eco*RI fragment from the streptococcal plasmid pAMβI (Fig. 4). This plasmid is representative of the *ermB* hybridization class, and the hybridizing *Eco*RI fragment is known to contain the *erm* determinant (22). The *ermP* probe did not hybridize to any of the other *erm* genes.

A series of dot blot hybridization experiments incorporating positive hybridization controls and individual radiolabeled probes representing each of the *erm* classes were used to determine whether DNA from any of the 40 erythromycin-resistant *C. perfringens* isolates hybridized with the other *erm* genes. DNA from the five strains which hybridized with the *ermP* probe also hybridized with the *ermB* class probe; however, the other *erm* probes did not hybridize to any of the *C. perfringens* DNA preparations (data not shown).

Comparison of the restriction profiles of pAMβI (22) and pJIR122 (Fig. 1) revealed that in both plasmids the erythromycin resistance determinant was located on an approximately 1.15-kb *Cfo*I (*Hha*I) fragment. However, the plasmids appeared to differ significantly outside the resistance determinants, since pAMβI does not have the two 0.35-kb *Cfo*I fragments present in pJIR122. To confirm these observations, pAMβI and pJIR122 were cleaved with several combinations of restriction enzymes and Southern blots were probed with the *ermP*-specific fragment. The results showed that the *ermP* probe hybridized to similarly sized *Cfo*I fragments from both pAMβI and pJIR122 (Fig. 5). The results from the other digests indicated that the regions outside these *Cfo*I fragments were not identical.

DISCUSSION

Erythromycin resistance is frequently observed in *C. perfringens* isolates from both human and animal sources (7, 12, 31, 33). To facilitate comparative studies, we cloned the erythromycin resistance determinant, *ermP*, from one of these strains and showed that it was expressed in *E. coli*. On the basis of subcloning and deletion analysis, the gene was mapped, and the estimated size (between 0.5 and 0.85 kb)

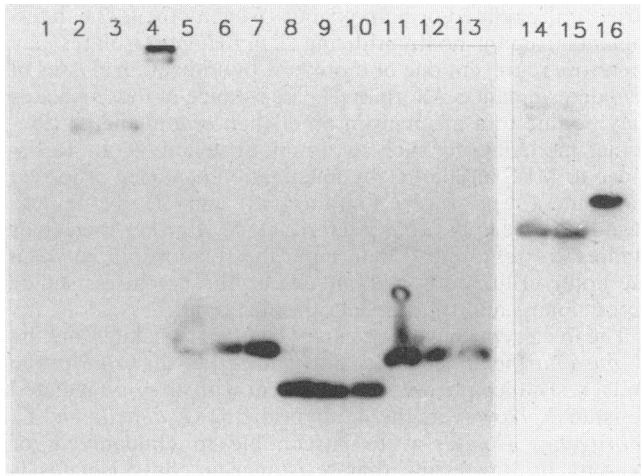

FIG. 5. Comparative hybridization analysis of pAMβI, pJIR122, and pJIR208. Plasmid DNA was isolated and cleaved with various restriction enzymes, and the resultant fragments were separated by electrophoresis. The fragments were then transferred to nitrocellulose and probed with the 0.5-kb *ermP* fragment. The resultant autoradiograph is shown. The restriction enzymes used were *Sac*I (lanes 2 to 4), *Cfo*I (lanes 5 to 7), *Acc*I-*Cfo*I (lanes 8 to 10), *Hinc*II-*Cfo*I (lanes 11 to 13), and *Acc*I-*Sac*I (lanes 14 to 16). In each group of three digests, the plasmid order was as follows: pJIR122, pJIR208, pAMβI. Lane 1, *Hin*dIII-digested λ *cI857* DNA.

was shown to be similar to those of MLS resistance determinants from other genera (24, 26, 38).

The recombinant plasmid, pJIR122, was observed to undergo *recA*-independent deletion of 2.7 kb of DNA which included the *ermP* gene. The endpoints of the deleted region were located between two 0.35-kb *Cfo*I fragments in pJIR122, and preliminary hybridization experiments (data not shown) indicated that these *Cfo*I fragments were similar. Further experiments revealed that both ends of the deleted region were required for deletion to occur, since clones containing only one end of the deleted region (e.g., pJIR208, pJIR211) were stable in *recA*$^+$ *E. coli*.

Previous studies have identified two chloramphenicol resistance transposons, Tn*4451* and Tn*4452*, from *C. perfringens*. Both transposons spontaneously excise from their host plasmids at high frequency in *E. coli* (3). The deletion event which occurred with pJIR122 was very similar, since it also appeared to be precise and occurred at a comparable frequency. The structure of the *ermP* region was consistent with that of a transposable genetic element since it contained hybridizing fragments at each end. We suggest that these 0.35-kb *Cfo*I fragments may compose part of a repeated DNA sequence. Further studies are necessary to determine whether the *ermP* determinant is located on a transposable element and whether such an element contains terminal repeated sequences.

The results of dot blot hybridization experiments showed that only 5 out of 40 erythromycin-, clindamycin-, and lincomycin-resistant *C. perfringens* isolates hybridized with the *ermP* and *ermB* probes. We therefore concluded that the *ermP* gene was not a common erythromycin resistance determinant in *C. perfringens*. Dubnau and Monod (11) suggest that MLS resistance determinants of the *ermC* class may be present in the clostridia. However, our hybridization experiments showed that none of the *C. perfringens* isolates hybridized to representatives of the other identified classes of MLS resistance determinants, including *ermC*. It is not known whether the erythromycin resistance determinants carried by the nonhybridizing *C. perfringens* isolates are structurally related or homologous to each other. The determinants from the nonhybridizing *C. perfringens* isolates may therefore represent one or more new hybridization classes of *erm* determinants. Alternatively, resistance in these isolates may be due to a mechanism other than *erm*-mediated ribosomal methylation, such as antibiotic inactivation. Resistance to MLS antibiotics by inactivation has been proposed for some *C. perfringens* strains (12) and clearly demonstrated to occur in other bacteria (4, 5). Further molecular studies are required to determine the relationship between the nonhybridizing *C. perfringens* erythromycin resistance determinants and those from other bacteria.

The five erythromycin-resistant *C. difficile* isolates and the *C. paraputrificum* isolate that hybridized to the *ermP* probe had a resistance phenotype consistent with an *erm*-mediated resistance. However, the nonhybridizing *C. difficile* and *C. sporogenes* isolates were susceptible to clindamycin or lincomycin, suggesting that resistance in these isolates is probably mediated by a non-*erm* mechanism.

The distribution of the *ermP*-like determinant appears to differ in *C. perfringens* and *C. difficile*. In *C. difficile*, all five of the erythromycin-, clindamycin-, and lincomycin-resistant isolates (from very diverse sources) had *ermP*-like determinants, which indicated that this determinant was widespread in *C. difficile*. Recent studies have shown that the MLS resistance determinants from two of the *C. difficile* isolates included in this study (630 and 662) appear to be carried on chromosomally encoded conjugative elements which can be transferred to susceptible strains of *C. difficile* and *Staphylococcus aureus*. Furthermore, like *ermP*, the *C. difficile* determinant (*ermZ*) is homologous to the *ermB* class of determinants (note that Hachler et al. [15] refer to *ermB* class genes as class A determinants). In contrast, all 40 of the *C. perfringens* isolates we examined were resistant to high levels of erythromycin, clindamycin, and lincomycin but only five isolates carried an *ermP*-like gene. In *C. perfringens*, the *ermP* determinant is either located on the nonconjugative plasmid pIP402 or is chromosomally encoded. However, unlike that of the chromosomal *ermZ* determinant in *C. difficile*, the conjugative transfer of erythromycin resistance from wild-type *C. perfringens* isolates has not been demonstrated (30, 31).

The distribution of *ermP* differed considerably from that of the analogous *C. perfringens* tetracycline resistance determinant, *tetP*, as only one hybridization group of tetracycline resistance genes is known in *C. perfringens* (1). Tetracycline-resistant strains of *C. difficile* do not hybridize to *tetP*, but the *C. paraputrificum* isolate studied here does carry a hybridizing *tet* determinant (1). Thus, there are significant differences in the mechanisms by which erythromycin resistance has spread in *C. perfringens* and *C. difficile* and by which tetracycline and erythromycin resistance have been acquired and have evolved in *C. perfringens*.

Representatives of the *ermB* hybridization class of MLS resistance determinants have been demonstrated in species as diverse as *Streptococcus faecalis*, *S. aureus*, *E. coli*, and *Klebsiella pneumoniae* (5). In particular, the plasmid pAMβI has been transferred by conjugation to a variety of gram-positive bacteria, including *Clostridium acetobutylicum* (28, 45), *Bacillus* spp. (21), *S. aureus* (13), and *Lactobacillus* spp. (14, 39). Our results showed that there was considerable restriction and DNA sequence similarity between the MLS resistance determinants of pAMβI and *ermP*. The *C. perfringens ermP* gene therefore belongs to the *ermB* class of MLS resistance determinants. This conclusion is supported by the results of Dubnau and Monod (11). The promiscuous nature of pAMβI suggests that the *ermP* gene may have been introduced into *C. perfringens* by conjugation with a bacterium carrying pAMβI or a similar plasmid. The resistance gene was presumably transferred by either homologous recombination or transposition to a nonconjugative plasmid (to form pIP402) or to the chromosome. Further studies are currently under way to determine the precise relationship between *ermP* and the MLS resistance gene of pAMβI.

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