ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1998, p. 1563–1567 Vol. 42, No. 7
0066-4804/98/$04.00+0

Chloramphenicol Resistance in *Clostridium difficile* Is Encoded on Tn*4453* Transposons That Are Closely Related to Tn*4451* from *Clostridium perfringens*

DENA LYRAS,* CHRISTINE STORIE, ANDREA S. HUGGINS, PAUL K. CRELLIN, TRUDI L. BANNAM, AND JULIAN I. ROOD

Department of Microbiology, Monash University, Clayton, Victoria 3168, Australia

Received 22 December 1997/Returned for modification 20 February 1998/Accepted 16 April 1998

The chloramphenicol resistance gene *catD* from *Clostridium difficile* was shown to be encoded on the transposons Tn*4453a* and Tn*4453b*, which were structurally and functionally related to Tn*4451* from *Clostridium perfringens*. Tn*4453a* and Tn*4453b* excised precisely from recombinant plasmids, generating a circular form, as is the case for Tn*4451*. Evidence that this process is mediated by Tn*4453*-encoded *tnpX* genes was obtained from experiments which showed that in *trans* these genes complemented a Tn*4451tnpXΔ1* mutation for excision. Nucleotide sequencing showed that the joint of the circular form generated by the excision of Tn*4453a* and Tn*4453b* was similar to that from Tn*4451*. These results suggest that the Tn*4453*-encoded TnpX proteins bind to similar DNA target sequences and function in a manner comparable to that of TnpX from Tn*4451*. Furthermore, it has been shown that Tn*4453a* and Tn*4453b* can be transferred to suitable recipient cells by RP4 and therefore are mobilizable transposons. It is concluded that, like Tn*4451*, they must encode a functional *tnpZ* gene and a target *oriT* or RS_A site. The finding that related transposable elements are present in *C. difficile* and *C. perfringens* has implications for the evolution and dissemination of antibiotic resistance genes and the mobile elements on which they are found within the clostridia.

Clostridium difficile is the major etiological agent of pseudomembranous colitis and also causes a more common, but less severe, form of this disease, known as antibiotic-associated diarrhea (13, 17). *C. difficile* causes disease when the normal intestinal flora is altered as a result of antimicrobial therapy. Although these organisms probably become a part of the normal intestinal flora, during antibiotic treatment they proliferate, which disrupts other endogenous flora (30). Since it is usually necessary to administer further antibiotics to treat the resultant infection, the presence of antibiotic-resistant *C. difficile* isolates may complicate the treatment of the diseases caused by this organism (13).

Chloramphenicol resistance in *C. difficile* and *Clostridium perfringens* may be mediated by the *catD* (31, 32) and *catP* (5, 27) genes, respectively, both of which encode chloramphenicol acetyltransferases. The *C. perfringens catP* gene is located on the transposons Tn*4451* and Tn*4452* (2). Tn*4451* is found on the conjugative tetracycline resistance plasmid pIP401 and excises precisely upon conjugative transfer in *C. perfringens* and when it is present on multicopy plasmids in both *C. perfringens* and *Escherichia coli* (2, 4, 5). The products of both excision events are identical, indicating that the same precise deletion event is occurring in both organisms (3). Transposition of Tn*4451* has been demonstrated in *E. coli* but occurs only at a very low frequency (2). Transposition has not been demonstrated in *C. perfringens* because of the lack of a detection method with sufficient sensitivity.

Tn*4451* has been completely sequenced (6,338 bp) and has been shown to contain six genes (5). One of these genes, *tnpX*, encodes a *trans*-acting site-specific recombinase which is responsible for the excision of Tn*4451* in both *C. perfringens* and *E. coli* (5). The TnpX protein catalyzes the excision of Tn*4451* as a circular molecule (5); this molecule may function as the transposition intermediate, as do the equivalent circular molecules from the well-characterized conjugative transposons Tn*916* and Tn*1545* (24). The TnpX recombinase is a large member of the resolvase-invertase family of site-specific recombinases (5) and site-directed mutagenesis studies have shown that the resolvase-invertase domains are functional in the excision of Tn*4451* (8). Tn*4451* is flanked by directly repeated GA dinucleotides, and GA residues are also found at the joint of the circular form, where the left and right termini of Tn*4451* are fused (3, 5, 8). Analysis of a number of Tn*4451* transposition target sites revealed that they resemble the joint of the circular form and that insertion occurs at a GA dinucleotide (8). On the basis of these data a model for the excision and insertion of Tn*4451* which involves the resolvase-invertase domain of TnpX that introduces 2-bp staggered cuts at the GA dinucleotides has been proposed (8).

Another gene carried by Tn*4451*, *tnpZ*, encodes the 50-kDa TnpZ protein, which has amino acid sequence similarity to those of a group of plasmid mobilization and recombination proteins that comprise the Mob-Pre family (5). These proteins interact with an upstream palindromic sequence known as the RS_A site to mediate plasmid mobilization and the formation of plasmid multimers and cointegrates. In the presence of the conjugative IncP plasmid RP4, TnpZ has been shown to promote RS_A-dependent plasmid mobilization in *cis* and the in *trans* mobilization of a coresident plasmid carrying an RS_A site (9). In addition, TnpZ was found to modulate the conjugative transfer of plasmids from *E. coli* to *C. perfringens* (9, 15).

The chloramphenicol resistance gene *catD* from *C. difficile* has also been cloned (31). Hybridization studies indicated that this chromosomal gene is very closely related to *catP* (23). The CATD and CATP monomers have 97% amino acid sequence

* Corresponding author. Mailing address: Department of Microbiology, Monash University, Clayton, Victoria 3168, Australia. Phone: 61 3 9905 4821. Fax: 61 3 9905 4811. E-mail: dena.lyras@med.monash.edu.au.

TABLE 1. Properties of recombinant plasmids

Plasmid(s)	Relevant characteristic(s)	Reference or source
pUC18 and pUC19	Ap^r	29
pJIR45	Ap^r Cm^r; Tn*4451* in pUC18	4
pJIR62	Ap^r Cm^r; *catP* from Tn*4451* in pUC18	4
pJIR639	Ap^r; *tnpX* from Tn*4451* in pBluescript-II	5
pJIR773	Cm^r Km^r; Tn*4451tnpXΔ1* in pSU39	5
pJIR1377	Ap^r Cm^r; Tn*4453a* in pUC18	This study
pJIR1378	Ap^r Cm^r; Tn*4453b* in pUC19	This study
pJIR1488	Ap^r; *tnpX* from Tn*4453a* in pUC18	This study
pJIR1489	Ap^r; *tnpX* from Tn*4453b* in pUC18	This study

TABLE 2. Synthetic oligonucleotide primers

Primer	Nucleotide sequence	Coordinates[a]
94	5′-GAAAATGTCAAGGACTT-3′	72–88
211	5′-CGTTCCTTGTCCTGCT-3′	5542–5527
212	5′-TCGGGGACTATTACTA-3′	2173–2188
220	5′-CATCAATCACAATCTC-3′	5025–5010
221	5′-TGGTGCGGTAGAGTGG-3′	4131–4116
274	5′-AACCTGTGGTTATGTAT-3′	3407–3391
300	5′-GGGCTATACTTTAATAG-3′	1–17
1727	5′-GGGGTCGAGTTTGTCAAG-3′	6338–6321
3277[b]	5′-AGTATTCCGCAAAGGTTTTTCTTCTGCGG-3′	2220–2200
4675	5′-AATCAAGCAGGACAAGGAACGA-3′	5522–5543
4676	5′-TGAAGTCCTTGACATTTTCTTA-3′	86–75

[a] All primers were derived from Tn*4451*, the nucleotide sequence of which was published previously (5).

[b] Note that the first 8 nucleotide bases at the 3′ end of this oligonucleotide primer are not derived from Tn*4451*.

identity (11). In contrast to *catP*, the *catD* gene appears to be present in at least two copies on the *C. difficile* chromosome (31). There is no evidence that *catD* can be transferred by conjugation, either within or between species (12, 33).

The aim of this study was to determine if the similarity between the *catP* and *catD* determinants extended beyond the resistance genes and therefore to determine if *catD* was located on an element similar to Tn*4451*. In this paper we report the cloning and detailed genetic analysis of two *catD* transposons that are closely related to Tn*4451*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth media. The *E. coli* strains used in this study were derivatives of DH5α (Bethesda Research Laboratories), S17-1 (26), or LT101 (20). The *C. perfringens* isolate used in this study was CP590, which carries Tn*4451* as part of the conjugative plasmid pIP401 (2, 7). The chloramphenicol-resistant *C. difficile* strains included isolates from Belgium (SGC0545), England (W1), Holland (3026), and Italy (C250) (31) and two isolates from Japan (KZ1606 and KZ1613 [19]). The properties of the plasmids used in this study are presented in Table 1.

E. coli strains were grown on 2YT agar medium (16) supplemented with ampicillin (100 μg/ml), chloramphenicol (20 μg/ml), kanamycin (20 μg/ml), or rifampin (150 μg/ml). *C. perfringens* strains were cultured in Trypticase-peptone-glucose broth (21), brain heart infusion broth (Oxoid), fluid thioglycolate medium (Difco), or nutrient agar (22) supplemented with chloramphenicol (10 μg/ml). The *C. difficile* strains were grown in BHIS medium (28) supplemented with chloramphenicol (10 μg/ml). Clostridial agar cultures were grown in an atmosphere of 10% H_2–10% CO_2–80% N_2. All bacterial strains were grown at 37°C.

DNA isolation and general molecular techniques. Plasmid DNA from *E. coli* was isolated by an alkaline lysis procedure (18). PCR amplifications were performed with *Taq* DNA polymerase (Boehringer Mannheim). PCR products for nucleotide sequencing and cloning were purified by isolation from a low-melting-temperature agarose gel (Seaplaque; FMC BioProducts) with the Magic PCR Preps DNA Purification System (Promega). Total genomic DNA from the clostridial isolates was prepared by a method developed for *C. perfringens* (1). Transformation of *E. coli* cells was done as described before (25). The primers used for PCR or nucleotide sequencing were synthesized on an Applied Biosystems 392 DNA/RNA Synthesizer and are shown in Table 2 and Fig. 1.

Transposon stability assays. To determine the stabilities of Tn*4451*, Tn*4453a*, and Tn*4453b* on recombinant plasmids, assays were performed as described previously (5, 8), with modifications as follows. Each strain was cultured on solid medium supplemented with ampicillin and chloramphenicol, and a single colony was transferred to 10 ml of broth with the same antibiotics, which select for the vector plasmid and the transposon, respectively. After overnight incubation at 37°C, plasmid DNA was extracted and was used to transform competent *E. coli* DH5α cells to ampicillin resistance. Single colonies ($n = 120$) were then patched onto media containing chloramphenicol or ampicillin and were incubated at 37°C overnight. The stability of the transposon carried by each plasmid was defined as the percentage of ampicillin-resistant colonies that were resistant to chloramphenicol. The values presented are the averages of three independent experiments.

To determine the ability of recombinant plasmids carrying *tnpX* genes from Tn*4453a* and Tn*4453b* to complement the Tn*4451tnpXΔ1* mutation carried on pJIR773, *trans*-complementation assays were performed as described previously (8).

***E. coli* spot mating experiments.** Matings were carried out with late-exponential-phase *E. coli* cultures as follows (10). A 1-in-2 dilution of the rifampin-resistant recipient LT101 (20) was used to flood the selective agar medium, and the surface was allowed to dry. Samples (20 μl) of serially diluted donor cultures were then spotted onto the surface and, when absorbed, were incubated overnight at 37°C.

Cloning of PCR-generated DNA fragments. Purified PCR products were treated with T4 polynucleotide kinase and T4 DNA polymerase (Boehringer Mannheim). Following phenol-chloroform extraction and ethanol precipitation (25) the products were ligated to the appropriate restriction endonuclease-digested and alkaline phosphatase-treated vector DNA.

Nucleotide sequencing. Nucleotide sequence analysis was performed with the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems) and an ABI 373 A automated fluorescent sequencing apparatus (Applied Biosystems). The sequences were compiled with Sequencher software (Gene Codes Corporation).

RESULTS

Identification of a Tn*4451*-like transposon in *C. difficile*. To see if *catD* was located on an element similar to Tn*4451*, PCR analysis was performed with *C. difficile* SGC0545, W1, 3026, and C250, which are known to carry *catD* (31), as well as chloramphenicol-resistant strains KZ1606 and KZ1613. The Tn*4451*-carrying strain *C. perfringens* CP590 was included as a positive control. The Tn*4451*-derived oligonucleotide primers used for this analysis were chosen so that when combined they

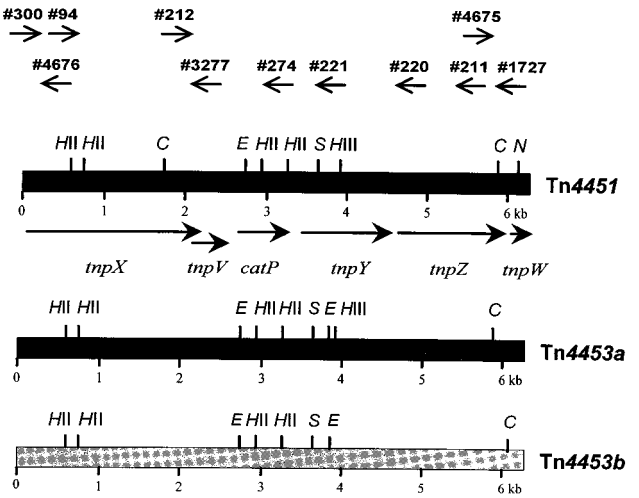

FIG. 1. Comparative linear maps of Tn*4451*, Tn*4453a*, and Tn*4453b*. The oligonucleotide primers used for PCR analysis are indicated by the short arrows. Restriction sites for *Cfo*I (C), *Eco*RV (E), *Hin*dII (HII), *Hin*dIII (HIII), *Nsi*I (N), and *Sau*3A (S) are indicated. The relative size, location, and direction of transcription of each of the Tn*4451*-encoded genes are indicated by the longer filled arrows.

TABLE 3. Stabilities of Tn*4453a* and Tn*4453b* in *E. coli*

Plasmid	Transposon	% Stability[a]
pJIR62	None[b]	100.0 ± 0
pJIR45	Tn*4451*	1.4 ± 0.9
pJIR1377	Tn*4453a*	80.8 ± 2.2
pJIR1378	Tn*4453b*	19.7 ± 3.3

[a] Each value is an average of three independent experiments and refers to the percentage of ampicillin-resistant colonies able to grow in the presence of chloramphenicol.
[b] Carries the *catP* gene only.

would span the entire transposon (Fig. 1). The primers were used in the combinations 300-274, 94-274, and 212-274 to amplify the left side of the transposon and 212-221, 212-220, 212-211, and 212-1727 to amplify the right side. PCR products of the appropriate size were obtained for all primer combinations from each of the six *C. difficile* strains (data not shown). These data therefore confirmed that the *C. difficile* isolates carried *catD* and provided evidence that in each isolate this gene was located on a transposon similar to Tn*4451*. We have designated this putative element Tn*4453*.

Cloning and restriction mapping of Tn*4453a* and Tn*4453b* from *C. difficile* W1. Since the *catD* gene from strain W1 had previously been cloned and sequenced (31, 32), further studies were restricted to this strain. Southern hybridization analysis of *Eco*RI-digested chromosomal DNA from strain W1 was carried out with a *catD*-specific probe. The results confirmed that there are two copies of this gene (31) and therefore two potential copies of Tn*4453* on the W1 chromosome (data not shown). These Tn*4453* variants were cloned into pUC18 and pUC19 as separate 15.5-kb and 11.0-kb *Eco*RI fragments, generating the recombinant plasmids pJIR1377 and pJIR1378, respectively. The putative transposons carried on these plasmids were designated Tn*4453a* and Tn*4453b*, respectively.

The restriction maps of both transposons were deduced and compared to the known map of Tn*4451* (Fig. 1). Both similarities and differences in the restriction profiles of the three elements were evident. These data also indicate that Tn*4453a* and Tn*4453b* are not identical, even though they are both found in the same strain. The differences between Tn*4451* and the two Tn*4453* variants suggest that although these elements probably have a common origin, they have subsequently evolved independently. Further studies were aimed at comparing the functional properties of these transposons.

Tn*4453a* and Tn*4453b* are excised as circular molecules in *E. coli* and *C. difficile*. Tn*4451* undergoes precise TnpX-mediated excision from multicopy plasmids in both *C. perfringens* and *E. coli* (2, 4, 5). To assess whether the *C. difficile* elements are also excised precisely in *E. coli*, transposon stability assays were performed with pJIR1377 and pJIR1378. The results (Table 3) showed that the recombinant plasmids carrying the Tn*4453* elements were unstable in *E. coli*, although they were more stable than plasmids carrying Tn*4451*. The *C. difficile* elements also differed in their stability, with Tn*4453a* being more stable than Tn*4453b*. The variation in transposon stability may reflect differences in flanking sequences rather than differences between the elements.

Excision of Tn*4451* results in the formation of a circular molecule (5). PCR analysis was used to determine whether similar molecules are produced by the excision of Tn*4453a* and Tn*4453b*. These studies used the outward-firing Tn*4451*-derived oligonucleotide primers 4675 and 4676 (Fig. 1). Binding of these primers to circular forms of Tn*4451*-like transposons would lead to the formation of 909-bp PCR products. Products of the appropriate size were observed when DNA from *E. coli* strains carrying pJIR45, pJIR1377, or pJIR1378 or DNA from the parent strain *C. difficile* W1 was used as the template (data not shown). These results provide clear evidence that the excision of Tn*4453a* and Tn*4453b* results in the formation of a circular molecule, as is the case with Tn*4451*. This process occurs both in *E. coli* and in the original *C. difficile* host, implying that excision occurs by a similar mechanism in both organisms.

Nucleotide sequence of the joint of the circular form of Tn*4453a* and Tn*4453b*. The 909-bp 4675-4676 PCR products generated from pJIR1377 and pJIR1378 were purified, partially sequenced, and compared to the sequence of the circular form of Tn*4451*. The results showed that both PCR products represented the joint of the circular form of the transposon, where the left and right termini of the elements are fused. At the fusion point these joints contained a GA dinucleotide, as does the circular form of Tn*4451* (Fig. 2). These residues are also found in consensus Tn*4451* target sequences (Fig. 2) and in the regions flanking Tn*4451* insertion sites, which indicates that they are important for Tn*4451* excision and insertion (5, 8). Site-directed mutagenesis studies have confirmed that the GA residues are important components of the TnpX target site (8).

Complementation in *trans* of the Tn*4451tnpXΔ1* mutation by the cloned Tn*4453a* and Tn*4453b tnpX* genes in *E. coli*. On the basis of the previous results it was postulated that Tn*4453a* and Tn*4453b* encoded TnpX proteins that functioned in a manner similar to that of TnpX from Tn*4451*. A *trans*-complementation assay was used to confirm the presence of functional *tnpX* genes. This assay is based on the finding that Tn*4451* derivatives carrying a *tnpX* gene that contains the internal *tnpXΔ1* deletion are stable on multicopy plasmids in *C. perfringens* and *E. coli*. Provision of a wild-type *tnpX* gene in *trans* restores the unstable phenotype of the Tn*4451tnpXΔ1* element (5).

PCR products encompassing the *tnpX* gene regions from Tn*4453a* and Tn*4453b* were generated with primers 300 and 3277 (Fig. 1). These fragments were cloned into *Sma*I-digested pUC18 DNA to construct pJIR1488 and pJIR1489, respectively. Partial nucleotide sequencing was used to confirm that the desired fragments had been cloned (data not shown). These plasmids were then introduced into DH5α derivatives carrying Tn*4451tnpXΔ1* on a compatible pSU39-derived replicon (5). The stability of the transposon derivative in the resultant transformants was then determined. The cloned *C. difficile*-derived *tnpX* genes were shown to be functional in that they could facilitate excision of the Tn*4451tnpXΔ1* element in the *trans*-complementation assay, although to somewhat different extents (Table 4). Excision was confirmed by detection of the circular form after PCR with the oligonucleotides 4675 and 4676 (data not shown).

Tn*4453a* and Tn*4453b* mediate mobilization of pJIR1377 and pJIR1378. To see if Tn*4453a* and Tn*4453b* also have a

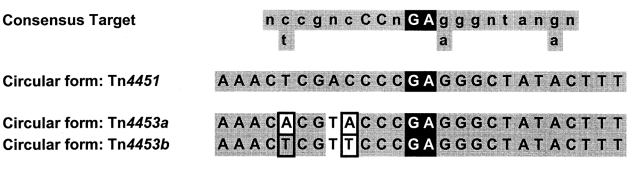

FIG. 2. Alignment of the joint of the circular forms of Tn*4451*, Tn*4453a*, and Tn*4453b*. Tn*4451* sequences are as reported previously (5, 8). The consensus target sequence which is cleaved by TnpX (8) is also shown. All of the sequences consist of central GA residues (black boxes). Residues which are the same as those found in Tn*4451* are shaded (grey boxes). Residues which differ between Tn*4453a* and Tn*4453b* are outlined in black.

TABLE 4. Complementation of Tn*4451tnpXΔ1* in *E. coli*

Strain[a]	pBS or pUC Replicon:		% Stability[b]
	Plasmid	Genotype[c]	
JIR5509	pUC18		100.0 ± 0
JIR2771	pJIR639	*tnpX*$^+$ (Tn*4451*)	48.0 ± 4.5
JIR5510	pJIR1488	*tnpX*$^+$ (Tn*4453a*)	4.4 ± 1.3
JIR5511	pJIR1489	*tnpX*$^+$ (Tn*4453b*)	14.0 ± 5.1

[a] In addition to the pBluescript-II KS (pBS), pUC18, or pUC19 (pUC) vector indicated, each strain carried the pSU39-based recombinant plasmid pJIR773, which carried Tn*4451tnpXΔ1* (5).

[b] Each value is an average of three independent experiments and refers to the percentage of kanamycin-resistant colonies able to grow in the presence of chloramphenicol.

[c] Refers to the *tnpX* gene carried by the recombinant pBS- or pUC-derived plasmid only.

functional TnpZ-RS_A mobilization system, plasmids carrying the various transposons were introduced into *E. coli* S17-1, which carries a chromosomal RP4 derivative. These strains were used as donors in matings with *E. coli* LT101 (Table 5). The results showed that the Tn*4453* plasmids pJIR1377 and pJIR1378 were able to be mobilized to the recipient bacterium, as was the Tn*4451* plasmid pJIR45 but not the negative control plasmid pJIR62, which carried only *catP* (Table 5). These data provide evidence that Tn*4453a* and Tn*4453b* carry a TnpZ-RS_A mobilization system that is functionally equivalent to that of Tn*4451*.

DISCUSSION

In this study, two chloramphenicol resistance transposons which carried the *catD* gene were identified in a single *C. difficile* strain and were shown to be functionally and structurally related to Tn*4451* from *C. perfringens*. PCR analysis indicated that five other *C. difficile* isolates carried similar transposons. These strains were from diverse sources, indicating that Tn*4451*-like transposons not only are found in chloramphenicol-resistant strains of *C. perfringens* (2, 23) but are also common in *C. difficile*.

Two closely related but distinct *C. difficile*-derived elements, Tn*4453a* and Tn*4453b*, were cloned and, like Tn*4451*, were found to be unstable on multicopy plasmids in *E. coli*. There was variation in the stability levels observed with these three elements (Table 3). These differences could be due to differences in the expression levels of the *tnpX* gene, sequence differences between the three TnpX proteins, or differences in the TnpX-binding regions flanking the ends of each element.

It was found that excision of Tn*4453a* and Tn*4453b* resulted in the production of a circular form of the transposons in both *E. coli* and *C. difficile*. This form may represent the transposition intermediate, as has been suggested for the equivalent molecule produced from Tn*4451* (5). The joints of these circular molecules were sequenced and were found to be very similar to the corresponding region in the Tn*4451* circular form and to the consensus TnpX target site, with a GA dinucleotide located at the fusion point (Fig. 2). A model has been proposed for Tn*4451* whereby the resolvase-invertase domain of TnpX introduces 2-bp staggered cuts at GA dinucleotides, leading to the excision or insertion of Tn*4451* via a circular intermediate (8). The findings that Tn*4453a* and Tn*4453b* have similar GA residues at the joints of their circular forms and similar joint sequences imply that the three TnpX proteins have similar mechanisms of action and similar DNA binding and target sites. The latter suggestions were supported by comparison of the ends of each *C. difficile* element to those of Tn*4451* and also to the consensus target sequence (Fig. 2). A high level of similarity was evident, with only two or three sequence changes, all of which were at one end. The ends of Tn*4453a* and Tn*4453b* also closely match the consensus TnpX target sequence. Evidence that the TnpX proteins encoded by these transposons were functionally interchangeable was obtained by cloning the *tnpX* genes from Tn*4453a* and Tn*4453b* and showing that they could substitute for the Tn*4451*-derived *tnpX* gene in a *trans*-complementation excision assay (Table 4). Overall, these data suggest that the TnpX proteins encoded by each transposon bind to similar DNA target sequences and subsequently function in a comparable manner to promote excision or insertion.

TABLE 5. TnpZ-mediated plasmid mobilization by RP4 in *E. coli*

Plasmid[a]	Transposon	Mobilization frequency[b]
pJIR62	None[c]	$<5.0 \times 10^{-8}$
pJIR45	Tn*4451*	1.0×10^{-2}
pJIR1377	Tn*4453a*	1.8×10^{-5}
pJIR1378	Tn*4453b*	1.2×10^{-2}

[a] All donor strains were derivatives of *E. coli* S17-1 (26) and contained the indicated plasmid.

[b] Each value is an average of two independent experiments and refers to the number of chloramphenicol-resistant transconjugants per donor cell.

[c] Carries the *catP* gene only.

The Tn*4451*-encoded TnpZ protein is the only known MobPre protein encoded on a transposable element from a gram-positive bacterium (9). On the basis of the results of this study, it is concluded that Tn*4453a* and Tn*4453b* encode equivalent TnpZ proteins and RS_A sites since these transposons also facilitated RP4-mediated mobilization of their host plasmids (Table 5). The observed differences in mobilization frequencies are probably the result of differences in the TnpZ proteins or RS_A sites encoded by these transposons. Further studies are required to determine the role that this mobilization system plays in the dissemination of Tn*4451*- and Tn*4453*-like transposons to different bacterial genera and species.

The comparative analysis of these chloramphenicol resistance elements provides clear evidence that genetic exchange between *C. difficile* and *C. perfringens* may have occurred either directly or through an intermediate bacterial host. Not only is there near identity between the *catD* and *catP* genes, but there is also a high degree of similarity between the transposons which carry these genes. The probability that gene transfer may occur directly or indirectly between these species is also supported by the comparative analysis of the erythromycin resistance determinants *ermBP* and *ermBZ* from *C. perfringens* and *C. difficile*, respectively (6, 14). However, direct and reproducible exchange of genetic information between *C. difficile* and *C. perfringens* has not been demonstrated. Further studies are required to elucidate the mechanism of transfer of Tn*4451*-like elements, especially with regard to the transposition process. Such studies will lead to a greater understanding of how these transposons are disseminated among these important pathogenic bacteria and of the evolutionary relationships between clostridial transposons and those from other bacteria.

ACKNOWLEDGMENTS

We thank Pauline Howarth for excellent technical assistance.

This research was supported by a grant from the Australian National Health and Medical Research Council.

REFERENCES

1. **Abraham, L. J., and J. I. Rood.** 1985. Cloning and analysis of the *Clostridium perfringens* tetracycline resistance plasmid, pCW3. Plasmid **13:**155–162.
2. **Abraham, L. J., and J. I. Rood.** 1987. Identification of Tn*4451* and Tn*4452*, chloramphenicol resistance transposons from *Clostridium perfringens*. J. Bacteriol. **169:**1579–1584.
3. **Abraham, L. J., and J. I. Rood.** 1988. The *Clostridium perfringens* chloramphenicol resistance transposon Tn*4451* excises precisely in *Escherichia coli*. Plasmid **19:**164–168.
4. **Abraham, L. J., A. J. Wales, and J. I. Rood.** 1985. Worldwide distribution of the conjugative *Clostridium perfringens* tetracycline resistance plasmid, pCW3. Plasmid **14:**37–46.
5. **Bannam, T. L., P. K. Crellin, and J. I. Rood.** 1995. Molecular genetics of the chloramphenicol-resistance transposon Tn*4451* from *Clostridium perfringens*: the TnpX site-specific recombinase excises a circular transposon molecule. Mol. Microbiol. **16:**535–551.
6. **Berryman, D. I., and J. I. Rood.** 1995. The closely related *ermB*/*AM* genes from *Clostridium perfringens*, *Enterococcus faecalis* (pAMβ1), and *Streptococcus agalactiae* (pIP501) are flanked by variants of a directly repeated sequence. Antimicrob. Agents Chemother. **39:**1830–1834.
7. **Brefort, G., M. Magot, H. Ionesco, and M. Sebald.** 1977. Characterization and transferability of *Clostridium perfringens* plasmids. Plasmid **1:**52–66.
8. **Crellin, P. K., and J. I. Rood.** 1997. The resolvase/invertase domain of the site-specific recombinase TnpX is functional and recognizes a target sequence that resembles the junction of the circular form of the *Clostridium perfringens* transposon Tn*4451*. J. Bacteriol. **179:**5148–5156.
9. **Crellin, P. K., and J. I. Rood.** 1998. Tn*4451* from *Clostridium perfringens* is a mobilizable transposon that encodes the functional Mob protein, TnpZ. Mol. Microbiol. **27:**631–642.
10. **Fong, S. T., and V. A. Stanisich.** 1989. Location and characterization of two functions on RP1 that inhibit the fertility of the IncW plasmid R388. J. Gen. Microbiol. **135:**499–502.
11. **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.
12. **Ionesco, H.** 1980. Transfert de la résistance à la tétracycline chez *Clostridium difficile*. Ann. Inst. Pasteur Microbiol. **131A:**171–179.
13. **Johnson, S., and D. N. Gerding.** 1997. Enterotoxemic infections, p. 117–140. *In* J. I. Rood, B. A. McClane, J. G. Songer, and R. W. Titball (ed.), The clostridia: molecular biology and pathogenesis. Academic Press, London, United Kingdom.
14. **Lyras, D., and J. I. Rood.** 1997. Transposable genetic elements and antibiotic resistance determinants from *Clostridium perfringens* and *Clostridium difficile*, p. 73–92. *In* J. I. Rood, B. A. McClane, J. G. Songer, and R. W. Titball (ed.), The clostridia: molecular biology and pathogenesis. Academic Press, London, United Kingdom.
15. **Lyras, D., and J. I. Rood.** 1998. Conjugative transfer of RP4-*oriT* shuttle vectors from *Escherichia coli* to *Clostridium perfringens*. Plasmid **39:**160–164.
16. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Moncrief, J. S., D. M. Lyerly, and T. D. Wilkins.** 1997. Molecular biology of the *Clostridium difficile* toxins, p. 369–392. *In* J. I. Rood, B. A. McClane, J. G. Songer, and R. W. Titball (ed.), The clostridia: molecular biology and pathogenesis. Academic Press, London, United Kingdom.
18. **Morelle, G.** 1989. A plasmid extraction procedure on a miniprep scale. Focus **11:**7–8.
19. **Nakamura, S., K. Yamakawa, S. Nakhio, 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.
20. **Palombo, E. A., K. Yusoff, V. A. Stanisich, V. Krishnapillai, and N. S. Willetts.** 1989. Cloning and genetic analysis of *tra* cistrons of the Tra 2/Tra 3 region of plasmid RP1. Plasmid **22:**59–69.
21. **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.
22. **Rood, J. I.** 1983. Transferable tetracycline resistance in *Clostridium perfringens* strains of porcine origin. Can. J. Microbiol. **29:**1241–1246.
23. **Rood, J. I., S. Jefferson, T. L. Bannam, J. M. Wilkie, P. Mullany, and B. W. Wren.** 1989. Hybridisation analysis of three chloramphenicol resistance determinants from *Clostridium perfringens* and *Clostridium difficile*. Antimicrob. Agents Chemother. **33:**1569–1574.
24. **Salyers, A. A., N. B. Shoemaker, A. M. Stevens, and L.-Y. Li.** 1995. Conjugative transposons: an unusual and diverse set of integrated gene transfer elements. Microbiol. Rev. **59:**579–590.
25. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
26. **Simon, R., U. Priefer, and A. Pühler.** 1983. A broad host range mobilization system for *in vivo* genetic engineering: transposon mutagenesis in gram negative bacteria. Biotechnology **1:**37–45.
27. **Sloan, J., T. A. Warner, P. T. Scott, T. L. Bannam, D. I. Berryman, and J. I. Rood.** 1992. Construction of a sequenced *Clostridium perfringens-Escherichia coli* shuttle plasmid. Plasmid **27:**207–219.
28. **Smith, C. J., S. M. Markowitz, and F. L. Macrina.** 1981. Transferable tetracycline resistance in *Clostridium difficile*. Antimicrob. Agents Chemother. **19:**997–1003.
29. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:**259–268.
30. **Wilson, K. H.** 1993. The microecology of *Clostridium difficile*. Clin. Infect. Dis. **16**(Suppl.4)**:**S214–S218.
31. **Wren, B. W., P. Mullany, C. Clayton, and S. Tabaqchali.** 1988. Molecular cloning and genetic analysis of a chloramphenicol acetyltransferase determinant from *Clostridium difficile*. Antimicrob. Agents Chemother. **32:**1213–1217.
32. **Wren, B. W., P. Mullany, C. Clayton, and S. Tabaqchali.** 1989. Nucleotide sequence of a chloramphenicol acetyl transferase gene from *Clostridium difficile*. Nucleic Acids Res. **17:**4877.
33. **Wüst, J., and U. Hardegger.** 1983. Transferable resistance to clindamycin, erythromycin, and tetracycline in *Clostridium difficile*. Antimicrob. Agents Chemother. **23:**784–786.