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Evidence that the Clindamycin-Erythromycin Resistance Gene of *Bacteroides* Plasmid pBF4 Is on a Transposable Element

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We constructed a shuttle vector, pE5-2, which can replicate in both *Bacteroides* spp. and *Escherichia coli*. pE5-2 contains a cryptic *Bacteroides* plasmid (pB8-51), a 3.8-kilobase (kb) *Eco*RI-D fragment from the 41-kb *Bacteroides fragilis* plasmid pBF4, and RSF1010, an IncQ *E. coli* plasmid. pE5-2 was mobilized by R751, an IncP *E. coli* plasmid, between *E. coli* strains with a frequency of 5×10^{-2} to 3.8×10^{-1} transconjugants per recipient. R751 also mobilized pE5-2 from *E. coli* donors to *Bacteroides uniformis* 0061RT and *Bacteroides thetaiotaomicron* 5482 with a frequency of 0.9×10^{-6} to 2.5×10^{-6}. The *Bacteroides* transconjugants contained only pE5-2 and were resistant to clindamycin and erythromycin. Thus, the gene for clindamycin and erythromycin resistance must be located within the *Eco* RI-D fragment of BF4. A second recombinant plasmid, pSS-2, which contained 33 kb of pBF4 (including the *Eco*RI-D fragment and contiguous regions) could also be mobilized by R751 between *E. coli* strains. In some transconjugants, a 5.5-kb (±0.3 kb) segment of the pBF4 portion of pSS2 was inserted into one of several sites on R751. In some other transconjugants this same 5.5-kb segment was integrated into the *E. coli* chromosome. This segment could transfer a second time onto R751. Transfer was RecA independent. The transferred segment included the entire *Eco*RI-D fragment, and thus the clindamycin-erythromycin resistance determinant, from pBF4.

Resistance to clindamycin (Cc) and erythromycin (Em) can be transferred by conjugation among strains of human colonic *Bacteroides* (4, 7, 9, 10, 12, 14–19). Four conjugative plasmids associated with Cc resistance transfer have been reported (4, 7, 12, 14–19). These plasmids are self-transmissible and vary considerably in size; pBFTM10 (17) and pCP1 (4), isolated separately but probably identical to pBFTM10, are 14.6 kilobases (kb). pBF4 (also called pIP410) is 41 kb (12, 19), and pBI136 is 82 kb (15). The Cc^r determinants of pBF4 and pBFTM10 have each been tentatively localized to 3.8- and 4-kb *Eco*RI fragments, respectively. This localization was based on restriction mapping of some natural deletions of pBF4 and pBFTM10 which no longer conferred Cc resistance (14, 17, 19). Since there was no way of reintroducing cloned DNA into *Bacteroides* spp., it was not possible to determine whether the entire Cc^r determinant was located within these 3.8- and 4-kb *Eco*RI fragments. However, the 3.8-kb *Eco*RI-D fragment of pBF4 (see Fig. 1) hybridized with the corresponding 4-kb *Eco*RI fragments of pBFTM10 and pCP1 on Southern blots (4, 14). Since the plasmids were otherwise different in size and restriction pattern, the homology between the 3.8- and 4-kb *Eco*RI fragments and their similar restriction patterns indicated that the Cc^r determinant might be on a transposable element (4, 9, 14).

Further indirect evidence that the Cc^r determinant might be on a transposable element has been reported by Smith and Macrina (15). They described a Cc^r strain of *Bacteroides ovatus* which contained two plasmids. The *Eco*RI-D fragment of pBF4 hybridized to pBI136 which transferred the Cc^r, whereas the other *Eco*RI fragments of pBF4 hybridized to the second resident plasmid which did not carry Cc^r. Finally, the 3.8- and 4-kb *Eco*RI fragments from pBF4, pBFTM10, and pCP1 have been shown to hybridize with DNA from a number of Cc^r *Bacteroides* strains (4, 7, 9, 10).

In this report, we describe the construction of a shuttle vector which can be conjugated from *Escherichia coli* to *Bacteroides* spp. Using this plasmid, pE5-2, we were able to localize the Cc^r determinant from pBF4. Also, we present the first direct evidence that the Cc^r determinant may be located on a transposable element.

MATERIALS AND METHODS

Bacterial strains and media. A list of the strains used in this study is given in Table 1. *Bacteroides* strains were grown either in prereduced Trypticase-yeast extract-glucose broth (TYG [5]) under an 80% N_2CO_2 atmosphere or on TYG agar plates in a GasPak jar. *E. coli* stains were grown in Luria broth, on Luria broth plates (*E. coli*-to-*E. coli* matings), or on TYG agar plates (*E. coli*-to-*Bacteroides* matings).

Bacterial matings. Donors and recipients were grown to early log (1×10^8 to 2×10^8 CFU per ml); 1 ml of each was mixed, pelleted, suspended in 100 μl of TYG broth and cospotted on nitrocellulose filters on Luria broth on TYG agar plates as described previously (16). The filters were incubated at 37°C for 10 to 12 h aerobically for *E. coli*-to-*E. coli* matings and anaerobically in GasPak jars for matings with *Bacteroides* strains. After incubation, the cells were suspended in Luria broth or TYG by vortexing and plated on selective medium. Abbreviations and concentrations for antibiotics used were as follows: tetracycline (Tc), 5 to 10 μg/ml; erythromycin (Em) or clindamycin (Cc), 10 to 20 μg/ml; rifampin (Rm), 20 μg/ml; geneticin or G418 (Gn), 400 μg/ml; nalidixic acid (Nx), 100 μg/ml; sulphanilamide (Su), 400 to 800 μg/ml. Preparation of competent *E. coli* and transformation of *E. coli* with plasmid DNA were performed as described by Lederberg and Cohen (6).

Plasmid isolation and analysis. Plasmids were isolated from *E. coli* and *Bacteroides* strains by the Ish-Horowitz modification of the method of Birnboim and Doly (8). Plasmid DNA was purified for cloning by CsCl-ethidium bromide density gradient centrifugation (8). Restriction endonuclease digestions and ligations with T4 DNA ligase were done by standard procedures (8). Restriction digests were resolved

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TABLE 1. Bacterial strains which were used in this study

Strain	Relevant traits[a]	Source (reference)[b]
E. coli		
K802	Rm^r Nx^r	W. Wood (20)
HB101	RecA Sm^r	H. Boyer (8)
EM24N	RecA Nx^r Sm^r	RecA Nx^r Sm^r derivative of LE392 (8), J. Cronan
DB11	Rm^r Cc^s Em^s	J. Davies
DB11N	Nx^r Rm^r Cc^s Em^s	Spontaneous Nx^r mutant of DB11
X760	Trp^- Sm^r	R. Curtiss
SF8	RecB RecC Sm^r	W. Reznikoff
B. fragilis ERL	Cc^r Tc^r	T. England, clinical isolate
B. uniformis 0061	Wild type	V.P.I. Anaerobe Laboratory
0061RT	Tc^r Rm^r	Cc^s Tc^r transconjugant of a mating between *B. fragilis* ERL and a spontaneous Rm^r mutant of *B. uniformis* 0061
B. thetaiotaomicron 5482	Nx^r	V.P.I. Anaerobe Laboratory
B. fragilis		
V479	Cc^r (pBF4) Tc^r	D. Guiney (12, 18)
V479C	Cc^s Tc^r	*B. fragilis* V479 cured of pBF4 (this lab)

[a] *E. coli* are naturally resistant to clindamycin and erythromycin except for special mutants like DB11. *Bacteroides* strains are resistant to all known aminoglycosides including geneticin.

[b] V.P.I. Anaerobe Laboratory; Virginia Polytechnic Institute Anaerobe Laboratory, Blacksburg, Va.

on 0.7% agarose slab gels in 4× GGB (0.16 M Tris, 0.08 M sodium acetate, 0.008 M EDTA [pH 8.3]). After electrophoresis for 10 to 15 h, gels were stained with ethidium bromide and photographed. For Southern blot analysis, the DNA from agarose gels was transferred to nitrocellulose paper by capillary blotting (8). Probes were labeled with ^{32}P by nick translation (13) and hybridized to the DNA on the nitrocellulose paper for 48 h at 42°C in a hybridization solution which contained 50% formamide (8). After hybridization, the blots were washed 2× for 30 min each with 0.2% sodium dodecyl sulfate in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and twice with 0.2% sodium dodecyl sulfate in 0.5× SSC. The filters were then autoradiographed.

RESULTS

Construction of chimeric plasmids. Parts of the 41-kb conjugative Cc^r *Bacteroides* plasmid, pBF4 (Fig. 1), were used in the construction of two chimeric plasmids, pE5-2 and pSS-2. The construction of pE5-2 is shown in Fig. 2. pB8-51 is a cryptic 4.4-kb plasmid originally isolated from *Bacteroides eggerthii* B8-51. pB8-51 was used because it hybridized on Southern blots with small cryptic plasmids of similar size and restriction pattern from strains representing eight colonic *Bacteroides* species. Thus, there should be no *Bacteroides* host range problem when pB8-51 is used as an origin of replication. The *E. coli* plasmid RSF1010 is a broad host range IncQ 8.9-kb plasmid which has an origin of transfer that is recognized by *E. coli* IncP plasmids (1). The sulfanilamide and streptomycin resistance (Su^r Sm^r) genes are part of a single transcriptional unit (1). The *Eco*RI-D fragment from pBF4 was included to furnish a drug resistance marker, Cc^r Em^r, that is expressed in *Bacteroides* spp. The *Eco*RI-D fragment is also known to contain a tetracycline resistance (Tc^r) gene which is not expressed in *Bacteroides* spp. but is expressed in *E. coli* only when the bacteria are grown aerobically (2). To differentiate this Tc^r determinant from other Tc^r determinants, we designated it *Tc^r. Strains of *E. coli* which contained pE5-2 were *Tc^r Su^r Sm^s. pE5-2 did not confer Cc^r Em^r to *E. coli* DB11, a mutant which is Cc^s Em^s.

pSS-2 (Fig. 3) was constructed in an attempt to clone as much of pBF4 as possible, including the regions flanking the *Eco*RI-D fragment. The 33-kb *Pst*I fragment of pBF4 was cloned into pEG155.1, an RSF1010 derivative which contains a gene from *Bacteroides fragilis* 12256 that complements *trpE* mutations of *E. coli* (S. Linn, unpublished results). The structure of pSS-2 was confirmed by a comparison of the *Eco*RI digestion patterns of pSS-2 with those of pEG155.1 and pBF4 and by probing blots of these digests with ^{32}P-labeled pBF4 and pEG155.1 (Fig. 4).

Stability of pE5-2 and pSS-2 in *E. coli*. pE5-2 was as stable as RSF1010 in *E. coli*. When cultures containing pE5-2 were grown without tetracycline selection in liquid cultures or on a nitrocellulose filter on agar plates during conjugation experiments, greater than 99% of the CFUs were Su^r and *Tc^r. However, pSS-2 was much less stable. In similar experiments, only 10 to 30% of the CFUs were still *Tc^r after overnight culturing without tetracycline. This was true even in the $RecA^-$ strains HB101 and EM24N.

Conjugal transfer of pNIL5 and pE5-2. R751, a 52-kb conjugative IncP plasmid that carries trimethoprim resistance (Tp^r) (11) was used to mobilize pE5-2 from *E. coli* to *E.*

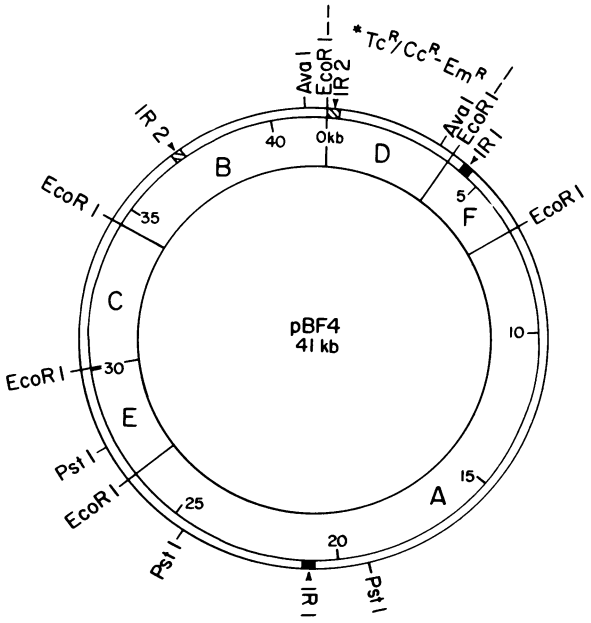

FIG. 1. Partial restriction map of pBF4 taken from Welch and Macrina (19). The *Eco*RI fragments are labeled A to F in order of descending size. Pairs of inverted repeats (IR1 and IR2) were located by electron microscopy of self-hybridized pBF4. The *Eco*RI-D fragment contains a tetracycline resistance determinant that is expressed in *E. coli* but not in *Bacteroides* spp. (2). To distinguish it from other tetracycline resistance determinants, we designated it *Tc^r.

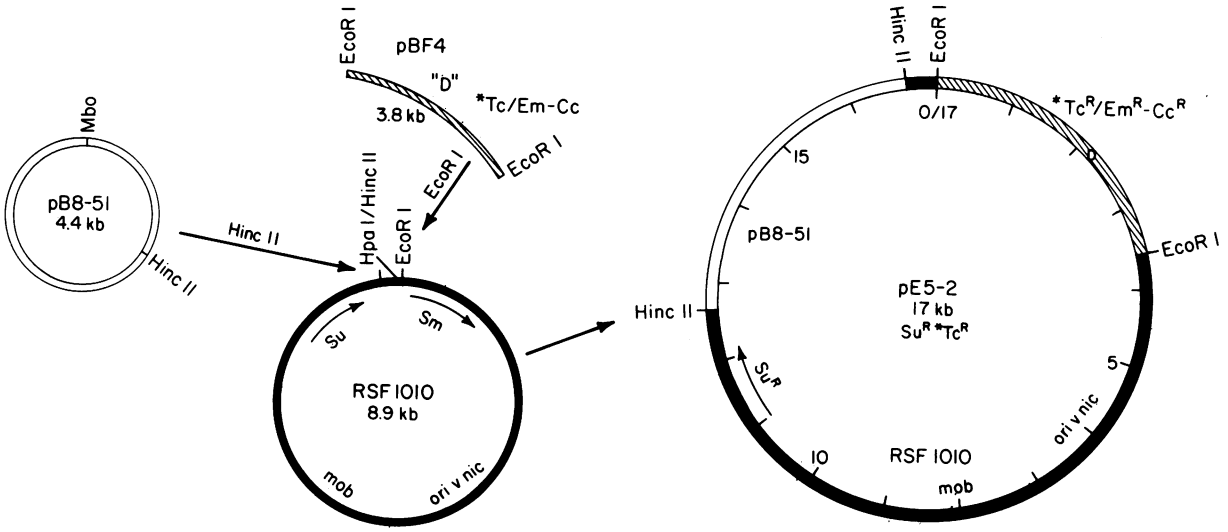

FIG. 2. Construction of pE5-2. The cryptic *Bacteroides* plasmid pB8-51 was cloned into the *Hpa*I-*Hin*cII site, and the 3.8-kb *Eco*RI-D fragment of pBF4 (Fig. 1) was cloned into the *Eco*RI site of RSF1010. The resultant 17-kb chimeric plasmid expressed the Su^r and *Tc^r determinants in *E. coli* and the Cc^r Em^r determinant in *Bacteroides* spp. A variant of pE5-2, pNIL5 (not shown), contained RSF1010 and the *Eco*RI-D fragment of pBF4 but not pB8-51.

coli and from *E. coli* to *Bacteroides* spp. R751 and other IncP plasmids mobilize RSF1010 at very high frequencies (1). R751 mobilized both pE5-2 and a variant of pE5-2 that did not contain pB8-51, pNIL5, at frequencies of 2×10^{-2} to 5×10^{-2} to 3.8×10^{-1} transconjugants per recipient in *E. coli*-to-*E. coli* transfer (Table 2). pE5-2, but not pNIL5, was detected in *Bacteroides* recipients, and these recipients were Cc^r Em^r. pE5-2 was transferred at frequencies of 10^{-6} per recipient to *B. uniformis* 0061RT and *B. thetaiotaomicron* 5482A. We did not detect transfer of pE5-2 to *B. fragilis* V479C from either of two different *E. coli* donors, HB101 and SF8.

pE5-2 was recovered from the Cc^r Em^r transconjugants of matings between *E. coli* and *B. uniformis* 0061RT or between *E. coli* and *B. thetaiotaomicron* 5482 (Fig. 5). Although the *Bacteroides* transconjugants were Em^r and Cc^r (10 μg/ml), they were Su^s (400 μg/ml), i.e., the Su^r determinant of RSF1010 was not expressed in *Bacteroides* spp. None of the 25 *Bacteroides* transconjugants tested contained R751 plasmid DNA.

Conjugal transfer of pSS-2. Since pBF4 is self-transmissible and since pSS-2 contains 75% of pBF4, we tested the ability of pSS-2 to mobilize itself from *E. coli* to *E. coli*. No transfer was detected ($<10^{-9}$). R751 was able to mobilize pSS-2 between *E. coli* strains. The frequencies varied from 2.5×10^{-2} to 1.2×10^{-7}, depending upon whether the recipient was RecA$^-$ or RecA$^+$ (Table 3).

We screened over 100 of the *Tc^r transconjugants from various matings between HB101 RecA$^-$ (R751, pSS-2) and RecA$^-$ or RecA$^+$ recipients. The highest frequency of *Tc^r transfer was to the RecA$^-$ strain EM24N (Table 3). Of 47 *Tc^r transconjugants tested, 46 were both *Tc^r and Tp^r. The plasmids from 25 of the *Tc^r transconjugants were examined on agarose gels; 17 transconjugants had both R751 and pSS-2 intact, 7 contained R751 and a deleted form of pSS-2, and one contained R751 with an insert. The frequency of transfer of *Tc^r from HB101 (R751, pSS-2) was 10^2-fold lower to K802 (RecA$^+$) than to EM24N (RecA$^-$) (Table 3). Of 40 *Tc^r transconjugants, 39 were also Tp^r. Plasmids from 24 *Tc^r transconjugants were analyzed. No transconjugants (0 of 24) received pSS-2 intact. Of these, 20 had only R751, 3 had R751 and a deleted form of pSS-2, and 1 had no plasmid DNA. When the plasmids from four transconjugants of a mating in which DB11 was the recipient were examined, three transconjugants had R751 with an insert and one had only R751; the *Tc^r was apparently in the chromosome.

Analysis of *Tc^r transconjugants. One type of *Tc^r Tp^r transconjugant contained what appeared to be R751 with an insert. Transformation of SF8 or HB101 with plasmid DNA from strains of this type yielded transformants that were both *Tc^r and *Tp^r and contained only a single plasmid. When strains that contained these plasmids were used as donors in conjugations, *Tc^r was cotransferred with Tp^r to the recipient at frequencies of 0.5 to 1.0 (Table 3). These findings indicated that the *Tc^r was now on R751. A second type of *Tc^r Tp^r transconjugant contained only R751.

Figure 6 shows the *Eco*RI restriction patterns of both types of *Tc^r Tp^r transconjugants compared to that of pSS-2 (lane A) and R751 (lane I). The *Eco*RI fragments of plasmids from the second type of transconjugants (Fig. 6, lanes C and G) were indistinguishable from those of R751. Moreover, the plasmid in lane G did not hybridize to ^{32}P-labeled pBF4 in Southern blots (Fig. 7, lane E). Thus the *Tc^r determinant from pSS-2 was not on the R751. However, the *Eco*RI digest of the chromosomal DNA from one of these strains hybridized with ^{32}P-labeled pBF4 (Fig. 7, lane D). Apparently, the *Tc^r determinant had inserted in the chromosome (Ω*Tc^r).

Two transconjugants that carried chromosomal insertions of the *Tc^r element were streaked and single-colony isolates were picked to eliminate the possibility of very low levels of contamination with strains harboring pSS-2 or pSS-2 derivatives. These strains were used as donors in conjugation experiments. They transferred Tp^r to recipients at frequencies of 0.5 to 1.0 and *Tc^r Tp^r at frequencies of 10^{-6} to 10^{-7} (e.g., Table 3). The Tp^r transconjugants contained R751 (Fig. 6, lane F) and the *Tc^r Tp^r transconjugants contained R751 *Tc^r plasmids (Fig. 6, lanes B, D, and E).

The *Eco*RI restriction patterns of R751 *Tc^r plasmids (Fig. 6, lanes B, D, E, and H) all have one R751 *Eco*RI fragment missing and contain three new *Eco*RI fragments. In each

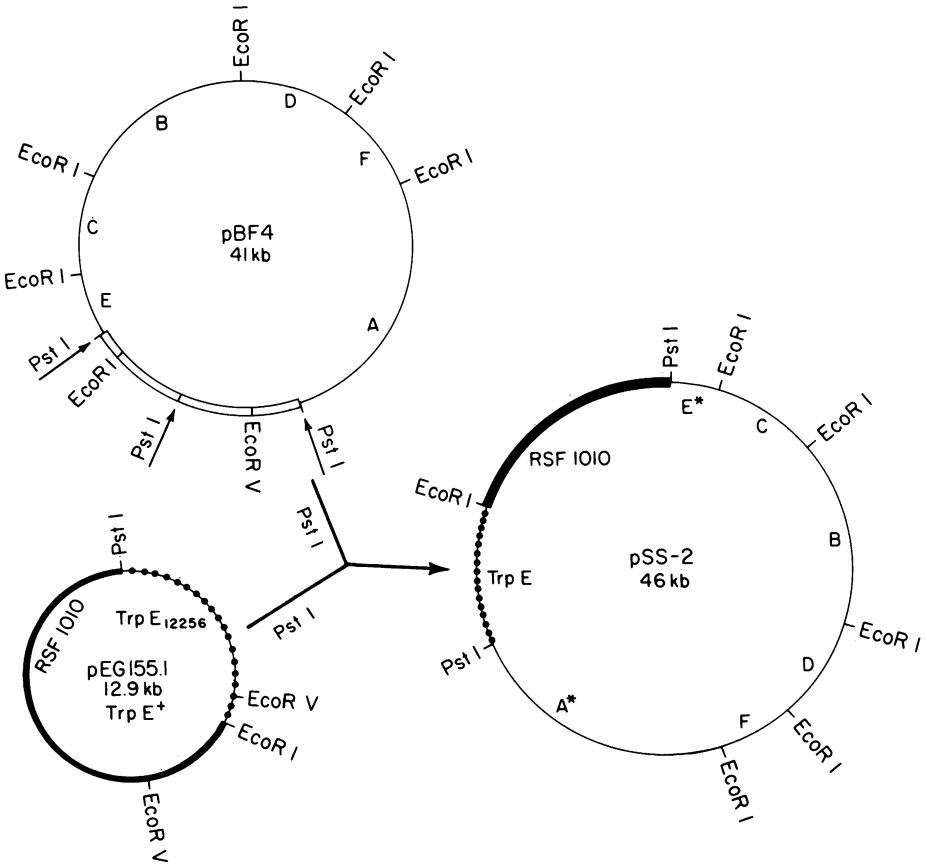

FIG. 3. Construction of pSS-2. pBF4 was digested with *Pst*I and the largest *Pst*I fragment was cloned into a unique *Pst*I site on pEG155.1. pEG155.1 contained part of RSF1010 (1) and a segment of *B. fragilis* 12256 DNA which complements *trpE* mutations in *E. coli* (S. Linn, unpublished results). The *Eco*RI fragments labeled B, C, D, and F on pSS-2 are identical to those from pBF4. *A and *E designated the segments of pBF4 which contain portions of the A and E fragments, respectively (Fig. 1).

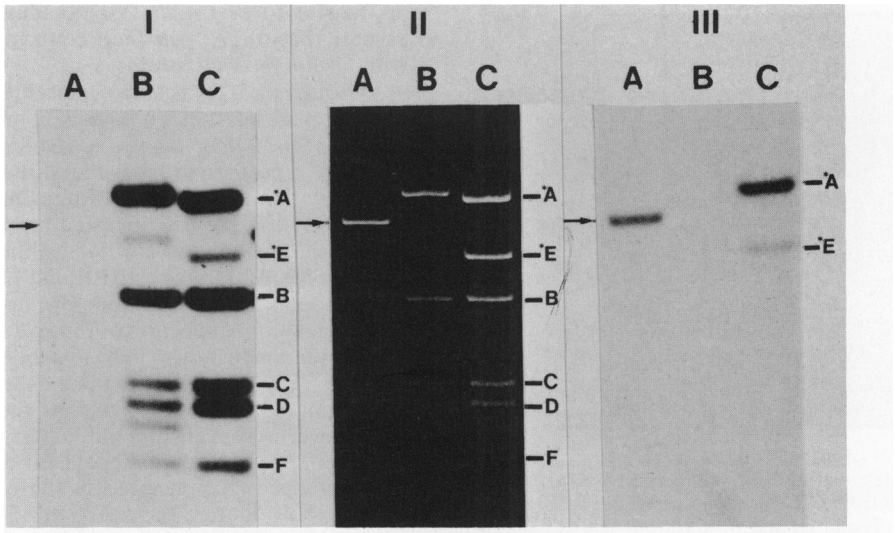

FIG. 4. Hybridization of *Eco*RI digest of pSS-2 with pBF4 and pEG155.1. The center panel (II) shows an *Eco*RI digest of pEG155.1 (A), pBF4 (B), and pSS-2 (C). Letters on the right of the gel indicate the migration distance of the various *Eco*RI fragments of pSS-2 which are shown schematically in Fig. 3. The left panel (I) is an autoradiogram of a Southern blot in which DNA from the gel shown in panel II was hybridized with ^{32}P-labeled pBF4. The right panel (III) is an autoradiogram of a Southern blot in which DNA from the gel shown in panel II was hybridized with ^{32}P-labeled pEG155.1.

TABLE 2. Frequency of mobilization by R751 of pE5-2 and pNIL5[a]

Plasmid mobilized	Donor strain	Recipient strain	Selection[b]	Frequency of transfer of *Tc^r or Em^r[c]
pE5-2	*E. coli* SF8	*E. coli* K802	Nx Tc	$2\text{-}5 \times 10^{-2}$
	E. coli K802	*E. coli* HB101	Sm Tc	4.5×10^{-1}
pNIL5	*E. coli* HB101	*E. coli* K802	Nx Tc	3.8×10^{-1}
pE5-2	*E. coli* SF8	*B. uniformis* 0061RT	Gn Em and Tc Em[d]	2.5×10^{-6}
		B. thetaiotaomicron 5482	Gn Em and Nx Em[d]	0.9×10^{-6}
		B. fragilis V479C	Gn Em	$<1.0 \times 10^{-9}$
pNIL5	*E. coli* HB101	*B. uniformis* 0061RT	Gr Em	$<1.4 \times 10^{-9}$

[a] Conjugations were done as described in the text. The donors all contained R751, a conjugative plasmid, used to mobilized pE5-2 and pNIL5. R751 transferred itself in *E. coli*-to-*E. coli* matings at frequency of 0.5 to 1.0 but no R751 transfer was detected into *Bacteroides* recipients ($<10^{-9}$).
[b] Nx, Nalidixic acid; Sm, steptomycin; Tc, tetracycline; Gc, geneticin; Em, erythromycin. Concentrations used are given in the text.
[c] Number of transconjugants (*E. coli* selected as *Tc^r and *Bacteroides* spp. selected as Em^r) per recipient.
[d] Both sets of selection were used.

case one of the new bands was 3.8 kb, the same size as the *Eco*RI-D fragment of pSS-2 and pBF4 which contains the *Tc^r and the Em^r Cc^r determinants. The Southern blot of two R751 *Tc^r plasmids (Fig. 7, lanes A and B) showed that the ^{32}P-labeled pBF4 probe hybridizes strongly to the 3.8-kb *Eco*RI band and slightly less to a second band for each. All the R751 *Tc^r plasmids in Fig. 6, lanes B, D, E, and H, showed strong hybridization to a 3.8-kb fragment and weaker hybridization to a second band which varied in size (data not shown). The fact that the *Eco*RI patterns of the R751 *Tc^r plasmids were different indicates that the *Tc^r element inserted into different sites. Among the R751 *Tc^r plasmids we isolated, we found the *Tc^r determinant inserted in six different sites of R751.

Although the R751 *Tc^r plasmids contained what appeared to be the entire *Eco*RI-D fragment of pBF4 and transferred at frequencies of 0.5 to 1.0 between *E. coli*, no transfer ($<10^{-9}$) of Cc^r or Em^r to *Bacteroides* recipients was detected (Table 3).

Size of *Tc^r element. Six R751 *Tc^r plasmids with the *Tc^r determinant inserted into different sites were analyzed by restriction enzyme digestions, and the size of the insert was calculated (data not shown). In each case the size of the *Tc^r determinant was 5.5 ± 0.3 kb. The inserted element always included the 3.8-kb *Eco*RI-D fragment of pBF4 (i.e., Fig. 6, lanes B, D, E. and H), and subcloning showed that it contained both the *Tc^r and Em^r Cc^r determinants. To determine where the additional 1.5 to 2 kb were located on pBF4, we hybridized one of the ^{32}P-labeled R751 *Tc^r plasmids with a Southern blot of *Eco*RI-digested pBF4. The results are shown in Fig. 8. Both the *Eco*RI-D and *Eco*RI-B fragments on pBF4 (Fig. 1) but not the *Eco*RI-F fragment, hybridized with ^{32}P-labeled R751 *Tc^r. Thus, if there is any DNA from the *Eco*RI-F fragment on the *Tc^r element, it is too small to be detected under the conditions used (see above).

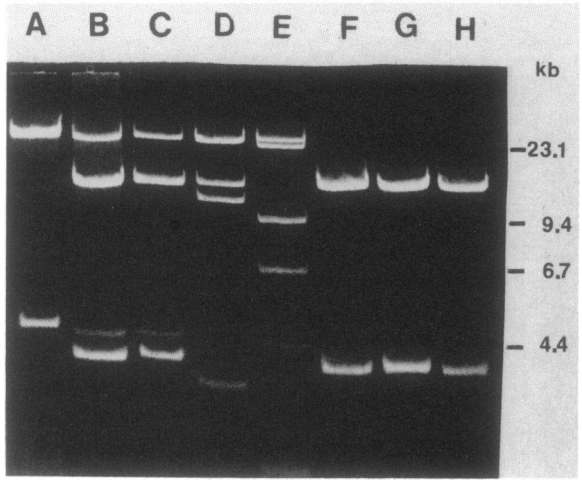

FIG. 5. *Eco*RI digests of plasmids in *Bacteroides* transconjugants containing pE5-2. After mating with *E. coli* SF8(R751, pE5-2), plasmids were isolated from Cc^r Em^r transconjugants in *B. thetaiotaomicron* 5482 (lanes B and C) and *B. uniformis* 0061RT (lanes F and G) and were digested with *Eco*RI. The *Eco*RI digest of p5482, the resident cryptic plasmid in 5482, is in lane A. Lanes D and H contain *Eco*RI digests of R751 and pE5-2, respectively. Lane E contains a *Hin*dIII digest of λ DNA. The sizes (in kb) of the λ *Hin*dIII fragments are indicated on the right.

DISCUSSION

When pE5-2 was mobilized by R751 from *E. coli* to *Bacteroides* spp., the transconjugants were Cc^r and Em^r. To our knowledge, this is the first direct evidence that the Cc^r Em^r determinant of pBF4 lies entirely within the *Eco*RI-D fragment of pBF4. pE5-2 is transferred at frequencies of 10^{-6} from *E. coli* donors to several *Bacteroides* species. The lack of transfer to *B. fragilis* V479C illustrates that we cannot assume that the *E. coli* IncP conjugal system will function with all *Bacteroides* strains.

Although pE5-2 was stable in both *E. coli* and *Bacteroides* spp., a plasmid that contained a much larger portion of pBF4, pSS-2, was highly unstable in *E. coli*. Pairs of inverted repeats and possible direct repeats have been located within the 33-kb *Pst*I fragment of pBF4 contained in pSS-2 ([19] Fig. 1). The inverted repeats could be responsible for the instability of pSS-2. Although pSS-2 was more stable in RecA$^-$ strains (HB101 and EM24N) than in RecA$^+$ strains, spontaneous deletions did occur in RecA$^-$ strains. These deletions appear to remove one of the inverted repeats (data not shown). The instability of pSS-2, especially in RecA$^+$ *E. coli* strains, enabled us to detect the transfer of *Tc^r from pSS-2 to R751 and to the chromosome. Since these insertion events could occur in RecA$^-$ cells, the insertion appears to be independent of RecA. When strains that contained *Tc^r inserted in the chromosome and R751 were used as donors, the resultant *Tc^r Tp^r transconjugants contained an R751 *Tc^r plasmid. The *Tc^r determinant could be retransferred from the *E. coli* chromosome onto R751. The R751 *Tc^r plasmids had the *Tc^r element inserted in one of the several different sites. The insert size was calculated to be 5.5 ± 0.3 kb in each case, and the inserts always

TABLE 3. Frequency of transfer of pSS-2, mobilized by R751, and frequency of transfer of the *Tc^r Em^r elements which appeared to have inserted in R751 or in the chromosome of some of the *E. coli* transconjugants

Donor strain (plasmids)	Recipient strain	Selection[a]	Frequency of transfer of *Tc^r or Em^r[b]
E. coli HB101 (R751, pSS-2)	*E. coli* K802	Nx Tc	1.5×10^{-4}
	E. coli EM24N	Nx Tc	2.5×10^{-2}
	E. coli DB11N	Nx Tc	1.2×10^{-7}
E. coli HB101 (R751, pSS-2)	*B. uniformis* 0061RT	Gn Em	$<3.0 \times 10^{-9}$
	B. thetaiotaomicron 5482	Gn Em	$<2.5 \times 10^{-9}$
E. coli K802 (R751 [Ω*Tc^r])[c]	*E. coli* X760	Sm Tc	2.8×10^{-6}
	E. coli HB101	Sm Tc	4.0×10^{-7}
E. coli SF8 (R751[*Tc^r])[d]	*E. coli* K802	Nx Tc	1.0
	B. uniformis 0061RT	Gn Em	$<4.0 \times 10^{-9}$
	B. thetaiotaomicron 5482	Gn Em	$<1.0 \times 10^{-8}$

[a] Nx, Nalidixic acid; Tc, tetracycline; Gn, geneticin; Em, erythromycin. Concentrations used are given in the text.
[b] Transconjugants (*Tc^r in the case of *E. coli* and Em^r in the case of *Bacteroides* spp.) per recipient.
[c] Strain which contains a single plasmid (R751) and which appears to have *Tc^r inserted in the chromosome. Ω*Tc^r is used to indicate chromosomal insertion of the *Tc^r element.
[d] Strain carrying an R751 which has a *Tc^r insertion.

contained the 3.8-kb *Eco*RI-D fragment of pBF4 that codes for Em^r Cc^r. Thus, the *Tc^r determinant and the Cc^r Em^r element appear to be on a transposon. This transposon has been assigned the designation Tn*4351*. Although there has been some indirect evidence that the Cc^r Em^r determinant on pBF4 is on a transposon (14), this is the first direct evidence for such a transposable element.

No transposition or recombinational events were detected when the *Eco*RI-D fragment was cloned into RSF1010(pNIL5) or into a nonmobilizable *E. coli* plasmid, pBR328 (data not shown). This indicates that there is some DNA outside this 3.8-kb fragment which is necessary for transposition. Shimell et al. (14) have described what may be direct repeats which include the *Eco*RI-*Ava*I sites on pBF4, postulating that these repeats may be involved in transposition. One of these direct repeat sites lies within the *Eco*RI-D fragment, whereas the other extends into the *Eco*RI-B fragment. Guiney et al. (2) have cloned the 550-base-pair *Eco*RI-*Ava*I fragment from pCP1 and found that it hybridized to the *Eco*RI-D and the *Eco*RI-B (Fig. 1) of pBF4. Our estimate of the size of the putative transposable element, together with the results of the experiment in which *Eco*RI-digested pBF4 is hybridized with ^{32}P-labeled R751 *Tc^r, indicate that the element contains the *Eco*RI-D fragment and may extend as much as 2 kb into the *Eco*RI-B fragment but not detectably into the *Eco*RI-F fragment.

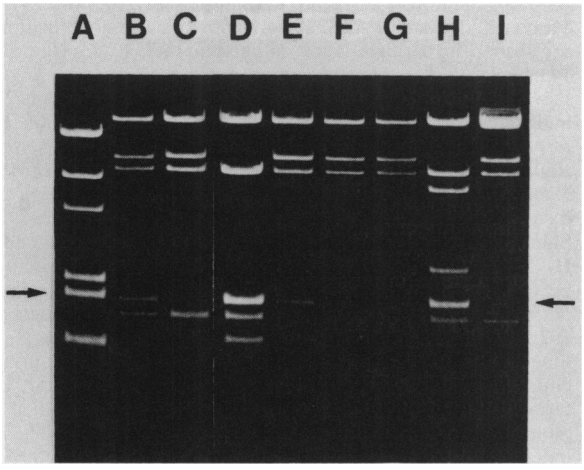

FIG. 6. *Eco*RI digests of plasmids from *Tc^r Tp^r *E. coli* transconjugants. Lanes A and I contain the *E. coli* digests of pSS-2 and R751, respectively. The migration distance of the 3.8-kb *Eco*RI-D fragment of pBF4 is indicated by arrows on both sides of the figure. Lane H is R751 *Tc^r isolated from a *Tc^r Tp^r transconjugant that was obtained from a mating of HB101(R751, pSS-2) with DB11 (Em^s Cc^s). Lanes C and G are the plasmids found in *Tc^r Tp^r transconjugants that appear to contain only R751. An example of the plasmids found in Tp^r transconjugants, when the strain that appeared to have a chromosomal *Tc^r (lane G) is used as donor, is in lane F. Two examples of plasmids from *Tc^r Tp^r (R751 *Tc^r) transconjugants that resulted from matings with the same donor are shown in lanes D and E. The plasmid from a *Tc^r Tp^r (R751 *Tc^r) transconjugant from the donor shown in lane C is in lane B.

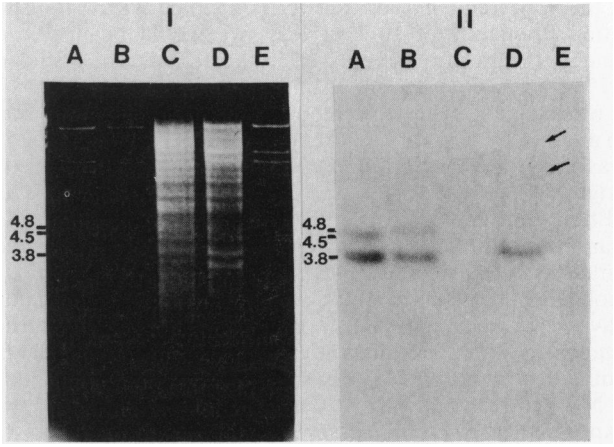

FIG. 7. Hybridization with ^{32}P-labeled pBF4 to locate and identify *Tc^r insertions into R751 and the *E. coli* chromosome. Panel I shows the agarose gel of the *Eco*RI digests of R751 *Tc^r plasmids from two *Tc^r Tp^r transconjugants (lanes A and B), K802 chromosomal DNA (lane C), chromosomal DNA of a K802 transconjugant that appears to have *Tc^r in the chromosome, and plasmid DNA from the same strain (lane E), which is also shown in Fig. 6 lane G. The Southern blot of the agarose gel in panel I hybridized with ^{32}P-labeled pBF4 is shown in panel II. The location of the 3.8-kb *Eco*RI-D band of pBF4 and the calculated sizes of two other bands of the R751-*Tc^r plasmids that hybridize to pBF4 are indicated at the left of panel I and panel II. Arrows indicate the positions of two large faint bands in lane D, panel II.

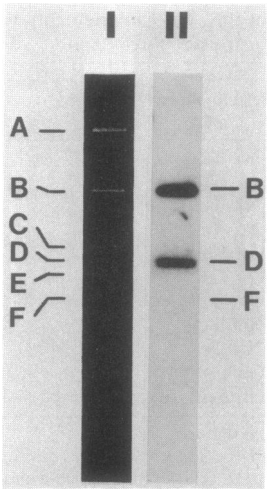

FIG. 8. An *Eco*RI digest of pBF4 (shown in I) was hybridized with ^{32}P-labeled R751 *Tc^r (II). The migration distances of the *Eco*RI fragments (A to F) are indicated by letters on the left side of the gel. The position of the *Eco*RI-B, D and F fragments are indicated by letters on the right side of the autoradiogram.

All of the evidence for transposition which has been presented here comes from insertions which occur in *E. coli*. The similarity of the Cc^r Em^r regions of pBFTM10, pCP1, and pBF4 provides indirect evidence that this element transposes in *Bacteroides* spp. Such transpositional events might be seen in *Bacteroides* spp. if we could transfer pSS-2 or one of our R751 *Tc^r plasmids (as suicide plasmids) into *Bacteroides* spp. Attempts to do this have so far been unsuccessful. This may be due to the low frequency with which R751 mobilizes other plasmids and itself from *E. coli* to *Bacteroides* spp. (e.g., 10^{-7} to 10^{-6} in the case of pE5-2). If this low frequency of transfer is coupled with a transposition frequency of 10^{-3} or less, we would be unable to detect transposition events after the transfer of pSS-2 or R751 *Tc^r to *Bacteroides* recipients.

pE5-2 and pNIL5 were mobilized from one *E. coli* strain to another by R751 at high frequencies (10^{-2}) but only pE5-2 was mobilized into *Bacteroides* spp. This indicates that the origin of replication for pE5-2 in *Bacteroides* spp. is on pB8-51 and not on RSF1010. Likewise, no *Bacteroides* transconjugants with R751 or R751 *Tc^r were detected. Since R751 transfers at least as frequently as the plasmids it mobilizes, transfer of R751 into *Bacteroides* spp. should have occurred at a frequency of 10^{-6} or higher. If this is true, *Bacteroides* spp. are the first gram-negative bacteria that do not maintain IncP (R751, RK2) or IncQ (RSF1010) plasmids.

Neither the Su^r of RSF1010 or the Tp^r (Tn*402*) of R751 was expressed in *Bacteroides* spp. Guiney and co-workers (3) have shown that the ampicillin resistance on pBR322, the Tc^r on RK2, and the *Tc^r (from pBF4) also are not express in *Bacteroides* spp. Similarly the Cc^r Em^r from pBF4 does not confer Cc^r Em^r resistance to susceptible strains of *E. coli* (DB11). To date, there are no known antibiotic resistances that are expressed in both *E. coli* and in *Bacteroides*. spp.

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