

## Hybridization Studies Reveal Homologies Between pBF4 and pBFTM10, Two Clindamycin-Erythromycin Resistance Transfer Plasmids of *Bacteroides fragilis*

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Two clindamycin-erythromycin resistance transfer factors of *Bacteroides fragilis*, pBF4 and pBFTM10, were analyzed for regions of DNA homology. Although the plasmids were derived from different clinical isolates of *B. fragilis* and have different sizes, they showed homology in the clindamycin-erythromycin resistance region; no homology could be detected outside of this region.

Clindamycin (Cln)-erythromycin (Ery) resistance transfer in *Bacteroides fragilis* has been documented by several laboratories (8, 10, 13, 15). Two plasmids carrying Cln-Ery resistance, pBF4 (also called pIP410) (4) and pBFTM10, promote the transfer of drug resistance by conjugation. These plasmids were isolated from two different strains of *B. fragilis* and characterized independently (4, 14, 16). The plasmids differ in molecular size; pBF4 is 41 kilobases (kb), whereas pBFTM10 is 14.6 kb. Both plasmids give rise to Cln-Ery-sensitive derivatives by deletion of a specific segment of plasmid DNA that is presumed to include all or part of the determinant for Cln-Ery resistance. We report here the results of a collaborative study to determine whether there are any regions of homology in the two plasmids.

The bacterial plasmids used in this study and their strains of origin are listed in Table 1. *B. fragilis* was propagated by standard anaerobic methods (3). *Escherichia coli* strains were grown in Penassay broth (Difco Laboratories). Plasmid DNA was harvested by previously described techniques (1, 2). Digestions of pBF4 with *EcoRI* and *HpaI* (Bethesda Research Laboratories) and of pBFTM10 with *EcoRI* and *HindIII* (New England Biolabs) were conducted under the conditions recommended by the suppliers. Electrophoresis of pBF4 DNA was performed in 0.7% agarose gels with Tris-borate buffer (7). DNA from pBFTM10 was electrophoresed in

1% agarose gels with SB3 buffer (40 mM Tris, 5 mM sodium acetate, 1 mM EDTA adjusted to pH 7.9 with glacial acetic acid). Gels were stained with ethidium bromide (0.5 µg/ml), and DNA bands were visualized with long-wave UV light. DNA in agarose gels was transferred to nitrocellulose membrane filters by the method of Southern (11). [<sup>32</sup>P]DNA hybridization probes were obtained by *in vitro* nick translation (9). Autoradiograms were prepared by using Kodak X-Omat-AR film.

The restriction enzyme maps of pBF4 and its deletion derivative pBF4Δ1 are presented in Fig. 1A. Note that pBF4Δ1 lacks a 7.1-kb segment of DNA. An agarose gel of *EcoRI* digests of these plasmids is shown in Fig. 2A; *EcoRI* fragments D (3.6 kb) and F (2.5 kb) are not found in the digest of pBF4Δ1, and the *EcoRI* fragment A is reduced by about 1.0 kb as compared with pBF4. The restriction enzyme maps of pBFTM10 and the deletion derivative pBFTM10Δ1 are presented in Fig. 1B. *EcoRI* digests of these plasmids analyzed on agarose gels are shown in Fig. 2B. Plasmid pBFTM10Δ1 lacks the *EcoRI* fragment B' (4.3 kb) found in pBFTM10.

Results of the hybridization experiments are shown in Fig. 3. In Fig. 3A, labeled pBFTM10 DNA was used to probe pBF4 and pBF4Δ1 DNA. Strong homology can be detected in the *EcoRI*-D band of pBF4, and weak homology can be detected in the *EcoRI*-B band of this plasmid. Similarly, pBF4Δ1 shows weak homology in the *EcoRI*-B band; the *EcoRI*-D band is absent in this plasmid. These results are confirmed by the *HpaI* hybridization patterns. With this digest of

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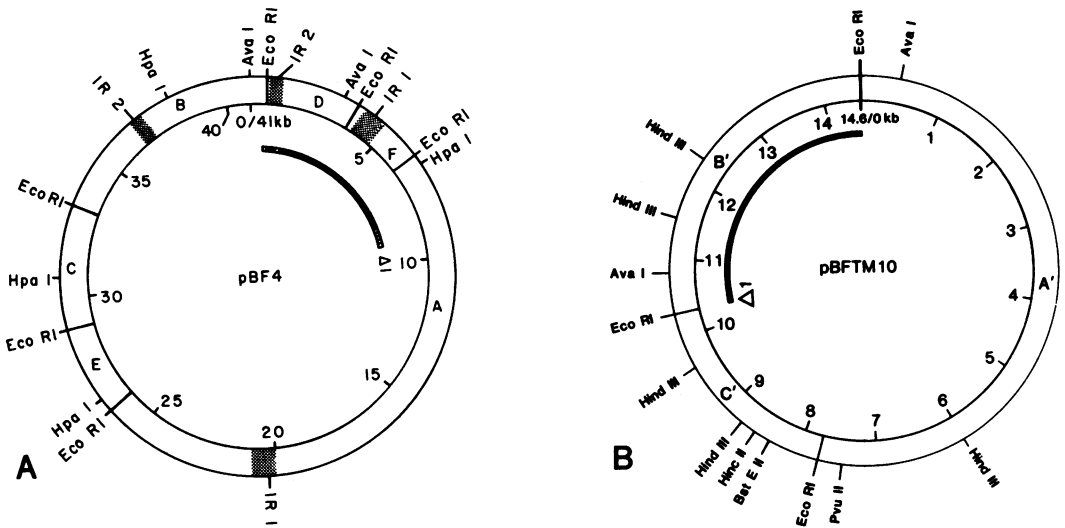


FIG. 1. Restriction enzyme cleavage maps. Size is denoted in kb. (A) Cleavage map of pBF4. The two pairs of inverted repeat (IR) sequences are shaded. The deletion carried by pBF4Δ1 is denoted by the heavy line (0.5 to 9 kb) on the inside of the map. The location of the deletion was determined by restriction endonuclease mapping, and the broken lines reflect the inability to define the precise endpoints of the deletion by this method. (B) Cleavage map of pBFTM10. The heavy line (10.3 to 14.6 kb) denotes the area of deletion in pBFTM10Δ1. Although the deletion is drawn to represent loss of the *EcoRI*-*AvaI* fragment at 10.4 to 10.9 kb, it could also represent loss of the *EcoI*-*AvaI* fragment at 0 to 0.5 kb.

pBF4, only *HpaI* fragment B, which includes all of the *EcoRI*-D fragment, shows homology to pBFTM10. This *HpaI* fragment is missing from pBF4Δ1, and no corresponding region of homology was detected.

In a similar manner, digests of pBFTM10 were probed with labeled DNA from plasmid pBR325::pBF4 *EcoRI*-D, which contains the *EcoRI*-D fragment of pBF4 cloned into the

unique *EcoRI* site of pBR325. Previous hybridization studies (6) with two deletion-bearing pBF4 derivatives and a *B. fragilis* strain carrying a chromosomal *Cln* determinant have provided strong evidence that the pBF4 *EcoRI*-D fragment contains all or part of the *Cln*' gene. Fig. 3B, lane 6, demonstrates homology only in the *EcoRI*-B' fragment of pBFTM10. This observation is confirmed by hybridization to the two

TABLE 1. Bacterial plasmids and strains

Plasmid	Strain or host	Pertinent phenotype <sup>a</sup>	Strain source or reference
<i>B. fragilis</i> pBF4	V479-1	Cln <sup>r</sup>	M. Sebald, Paris, France
pBF4Δ1	V479-1	Cln <sup>s</sup>	Derivative of V479-1 (6,15)
pBFTM10	TM4003	Cln <sup>r</sup>	12
pBFTM10Δ1	TM4292	Cln <sup>s</sup>	Derivative of TM4003
<i>E. coli</i> pBR325::pBF4 <i>EcoRI</i> -D	C600	Ap <sup>r</sup> Tc <sup>r</sup> Cm <sup>s</sup> 6	

<sup>a</sup> Cln<sup>r</sup>, Clindamycin, erythromycin resistant; Cln<sup>s</sup>, clindamycin, erythromycin sensitive; Ap<sup>r</sup>, ampicillin resistant; Tc<sup>r</sup>, tetracycline resistant; Cm<sup>s</sup>, sensitive to chloramphenicol by virtue of insertional inactivation of the Cm<sup>r</sup> determinant.

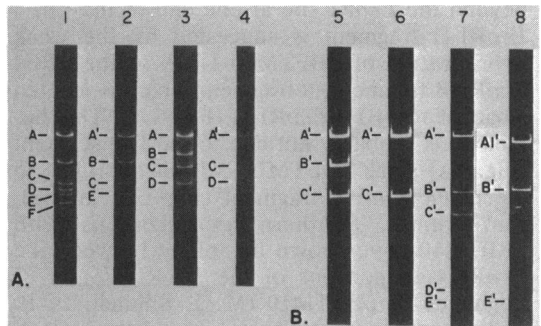


FIG. 2. Agarose gel electrophoresis of plasmid DNA cleaved with restriction enzymes. Letters indicate fragments. (A) *EcoRI* digests of pBF4 (lane 1) and pBF4Δ1 (lane 2); *HpaI* digests of pBF4 (lane 3) and pBF4Δ1 (lane 4). (B) *EcoRI* digests of pBFTM10 (lane 5) and pBFTM10Δ1 (lane 6); *HindIII* digests of pBFTM10 (lane 7) and pBFTM10Δ1 (lane 8).

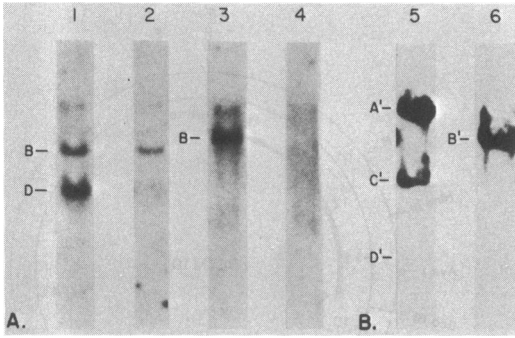


FIG. 3. Detection of DNA fragments containing sequences homologous to pBFTM10 (A) and pBR325::pBF4 *EcoRI*-D (B) probes. Letters indicate fragments. (A) Hybridization was performed in 0.9 M sodium chloride–0.09 M sodium citrate–0.2% Ficoll–0.2% polyvinylpyrrolidone–0.5% sodium dodecyl sulfate–0.02% bovine serum albumin V–100  $\mu$ g of calf thymus DNA per ml at 65°C for 20 h. *EcoRI* digests were of pBF4 (lane 1) and pBF4 $\Delta$ 1 (lane 2); *HpaI* digests were of pBF4 (lane 3) and pBF4 $\Delta$ 1 (lane 4). (B) Hybridization was performed as previously described (6). The nitrocellulose filters contained pBFTM10 digested with *HindIII* (lane 5) and *EcoRI* (lane 6). Fragments exhibiting homology were determined by comparison to the agarose gel from which the blot was derived.

junction fragments of *EcoRI*-B' (*HindIII*-A' and *HindIII*-C'). The internal fragment of *EcoRI*-B (*HindIII*-D'), although not apparent in Fig. 3B, lane 5, can be seen in an autoradiograph exposed for a longer period of time. Hybridization to pBFTM10 $\Delta$ 1 was not done.

The results of this study clearly illustrate the reciprocal homology of pBF4 and pBFTM10 in the region proposed to contain the *Cln*-Ery resistance determinant. That homology extends beyond the *EcoRI* site at one end of the pBF4 *EcoRI*-D fragment is suggested by the weak hybridization of pBFTM10 DNA to the pBF4 *EcoRI*-B fragment, a fragment which is located adjacent to pBF4 *EcoRI*-D (Fig. 1A). This homology is probably not due to the IR2 segment (Fig. 1A) since pBFTM10 did not hybridize to the pBF4 *HpaI*-C fragment (Fig. 3A, lane 3). Furthermore, Southern hybridizations with pBFTM10 have shown homology between the *EcoRI*-*AvaI* portions of the *EcoRI*-A' and B' fragments of pBFTM10 (M. J. Shimell, F. P. Tally, and M. H. Malamy, unpublished data). This suggests that the *Cln*-Ery gene(s) of pBFTM10 is flanked by direct repeats of DNA. The existence of similar *EcoRI*-*AvaI* segments around pBF4 *EcoRI* fragment D is striking and may explain the weak hybridization of pBFTM10 to pBF4 *EcoRI*-B. Likewise, pBR325::pBF4 *EcoRI*-D should hybridize weak-

ly to pBFTM10 *EcoRI*-A'. However hybridization to fragment B' is so intense that it would not be possible to see weak hybridization to fragment A'.

Similarities between pBF4 and pBFTM10 are confined to a small region of each plasmid. Thus, these plasmids may only share a common *Cln*-Ery resistance determinant and differ in transfer and replication functions. In addition, whereas pBF4 possesses two pairs of inverted repeat sequences (Fig. 1A), similar structures have not been detected in electron micrographs of "snap-back" pBFTM10 (J. Reichler, F. P. Tally, and M. H. Malamy, unpublished data). In two different studies with derivatives of pBF4 and pBFTM10 as specific probes, homologous sequences have been found in the chromosome or on plasmids of *Cln*<sup>r</sup> strains isolated from diverse geographical locations (5, 6, 12). The fact that the *Cln*-Ery resistance determinant can exist either in the chromosome or on different resistance plasmids strongly suggests that the *Cln*-Ery resistance determinant resides on a transposable genetic element. This observation together with the increasing incidence of *Cln*-Ery resistance in *B. fragilis* isolates makes the widespread dissemination of *Cln*-Ery resistance by conjugation and subsequent transposition a distinct possibility.

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