JOURNAL OF BACTERIOLOGY, Apr. 1988, p. 1651–1657
0021-9193/88/041651-07$02.00/0

Vol. 170, No. 4

Tetracycline-Dependent Appearance of Plasmidlike Forms in *Bacteroides uniformis* 0061 Mediated by Conjugal *Bacteroides* Tetracycline Resistance Elements

NADJA B. SHOEMAKER* AND ABIGAIL A. SALYERS
Department of Microbiology, University of Illinois, Urbana, Illinois 61801

Received 17 August 1987/Accepted 20 January 1988

Some human colonic *Bacteroides* strains carry conjugal tetracycline resistance (Tc^r) elements, which are thought to be chromosomal. We have found that some of these Tc^r elements can mediate the appearance of plasmidlike forms in *Bacteroides uniformis* 0061. When *B. uniformis* 0061, containing a conjugal Tc^r element designated Tc^r ERL, was grown in medium containing tetracycline (1 μg/ml), two circular DNA forms were found in the alkaline plasmid preparations: NBU1 (10.3 ± 0.5 kilobases) and NBU2 (11.5 ± 0.5 kilobases). Restriction analysis of NBU1 and NBU2 showed that they were not identical, although Southern blot analysis indicated that they did contain some region(s) of homology. Results of Southern blot analysis also demonstrated that both NBU1 and NBU2 were normally integrated in the chromosome of *B. uniformis* or in some undetected large plasmid. Although we were unable to determine the exact structure and location of the integrated forms of NBU1 and NBU2 in *B. uniformis*, they appear to be in close proximity to each other. Neither NBU1 or NBU2 could be detected as a plasmidlike form in cells exposed to UV light, thymidine starvation, mitomycin C, or autoclaved chlortetracycline (50 μg/ml). Four conjugal Tc^r elements other than the Tc^r ERL element were able to mediate the appearance of NBU1 alone, and two Tc^r elements did not mediate the excision of either NBU1 or NBU2. Three strains from different *Bacteroides* species contained some DNA sequences which had homology to NBU1 and NBU2.

Two types of conjugal elements have been found in *Bacteroides* strains isolated from the human colon. One type consists of large self-mobilizing plasmids such as pBF4 and pBI136 (11, 15). Both of these plasmids carry a gene which encodes resistance to erythromycin (Em^r) and clindamycin. The Em^r genes of pBF4 and pBI136 have been shown to reside on related transposons (9, 12).

A second type of *Bacteroides* conjugal element is associated with the transfer of tetracycline resistance (Tc^r). Some of these elements transfer Tc^r only, whereas others transfer both Tc^r and Em^r (2, 4, 5, 13). These conjugal Tc^r elements are thought to be located in the host chromosome, because no plasmid DNA has been found to be associated consistently with Tc^r transfer. In many cases, transfer of Tc^r or Tc^r Em^r is enhanced by pregrowth of the donor on tetracycline (2, 5, 8, 15), but the mechanism of the tetracycline enhancement of transfer is unknown. To date, none of the Tc^r or Tc^r Em^r elements has been cloned and analyzed. As a result, nothing is known about the structure of the Tc^r elements or whether elements found in different strains are related to each other.

One of the conjugal Tc^r elements we have studied in some detail is a Tc^r element that was transferred to *Bacteroides uniformis* 0061 from a clinical isolate, *Bacteroides fragilis* ERL. We have designated this Tc^r element Tc^r ERL. We have shown previously that Tc^r ERL not only transfers itself from *Bacteroides* sp. to *Bacteroides* sp., but also transfers *Bacteroides-Escherichia coli* shuttle vectors from *Bacteroides* donors to *E. coli* or *Bacteroides* recipients (8) and R751::Tn*4351* derivatives from their chromosomal insertion sites in the *Bacteroides* donors to *E. coli* recipients (10). In all cases, the transfer frequency is enhanced up to 100-fold by pregrowth of the donor on tetracycline. In the course of some experiments designed to detect low levels of plasmids or circular intermediates in a *B. uniformis* strain which carried the Tc^r ERL element, we observed two low-copy-number plasmidlike bands on the agarose gels. These closed-circular DNA elements were observed only when the strain was grown in medium containing tetracycline. In this report, we present evidence (i) that these two plasmidlike elements are normally integrated in *B. uniformis* 0061, either in the chromosome or on some undetected coresident replicon; (ii) that they are excised and appear as closed-circular forms only if *B. uniformis* 0061 contains the Tc^r ERL element and is grown in tetracycline; and (iii) that none of the other conjugal *Bacteroides* Tc^r or Tc^r Em^r elements we tested could mediate the excision of both of these *B. uniformis* integrated elements. Finally, we show that strains from three other human colonic *Bacteroides* species contain chromosomal DNA sequences that cross-hybridize with the plasmidlike elements seen in *B. uniformis*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. A list of the strains and plasmids used in this study is given in Table 1. Unless otherwise indicated, *Bacteroides* strains were grown in prereduced Trypticase (BBL Microbiology Systems, Cockeysville, Md.)-yeast extract-glucose (TYG; 9) medium under an atmosphere of 80% nitrogen–20% carbon dioxide or on TYG agar plates in a BBL GasPak jar. Strains of *E. coli* were grown in Luria broth (LB) or on LB agar. Strains of *B. uniformis* 0061 which carry different *Bacteroides* Tc^r elements were constructed by mobilizing the Tc^r element from the strain of origin into *B. uniformis* BU1001, a spontaneous rifampin-resistant (Rif^r) derivative of *B. uniformis* 0061. Mating conditions for *Bacteroides*-to-*Bacteroides* matings have been described previously (8).

Induction conditions. In most cases, *Bacteroides* strains

* Corresponding author.

TABLE 1. Strains used in this study

Strain	Relevant phenotype[a]	Source or reference
Bacteroides clinical isolates		
B. fragilis V479	Tc^r Em^r (pBF4)	13; also called strain 92 (5)
B. fragilis CEST	Tc^r Em^r	T. England, Mercy Hospital, Urbana, Ill.
B. fragilis ERL	Tc^r Em^r	T. England
B. thetaiotaomicron DOT1	Tc^r Em^r Ap^r	T. England
B. thetaiotaomicron DOT2	Tc^r Em^r Ap^r	T. England
B. fragilis 12256	Tc^r Em^r Pn^r	VPI Anaerobe Laboratory Blacksburg, Va.; also designated V503 (4)
B. uniformis strains		
0061		VPI Anaerobe Laboratory
BU1001	Rif^r	Spontaneous Rif^r derivative of *B. uniformis* 0061 (9)
BU1002 (Tc^r V479)	Tc^r Rif^r	BU1001 Tc^r transconjugant from a mating with *B. fragilis* V479; this study.
BU1003 (Tc^r Em^r CEST)	Tc^r Em^r Rif^r	BU1001 Tc^r Em^r transconjugant from a mating with *B. fragilis* CEST; this study.
BU1004 (Tc^r ERL)	Tc^r Rif^r	BU1001 Tc^r transconjugant from a mating with *B. fragilis* ERL (8)
BU1005 (Tc^r Em^r ERL)	Tc^r Em^r Rif^r	BU1001 Tc^r Em^r transconjugant from a mating with *B. fragilis* ERL; this study.
BU1006 (Tc^r Em^r DOT1)	Tc^r Em^r Rif^r	BU1001 Tc^r Em^r transconjugant from a mating with *B. thetaiotamicron* DOT1; this study.
BU1007 (Tc^r Em^r DOT2)	Tc^r Em^r Rif^r	BU1001 Tc^r Em^r transconjugant from a mating with *B. thetaiotamicron* DOT2; this study.
BU1008 (Tc^r Em^r 12256)	Tc^r Em^r Rif^r	BU1001 Tc^r Em^r transconjugant from a mating with *B. fragilis* 12256; this study.

[a] Abbreviations: Ap^r, ampicillin resistance; Em^r, erythromycin resistance; Pn^r, penicillin G resistance; Rif^r, rifampin resistance; Tc^r, tetracycline resistance.

were grown in TYG broth containing 1 μg of tetracycline per ml. In some experiments, the concentration of tetracycline was varied to determine the minimum concentration needed to cause the appearance of the excised elements. Concentrations of tetracycline greater than 0.1 μg/ml significantly slowed the rate of growth of the Tc^s strain *B. uniformis* BU1001.

Chlortetracycline was added to TYG to final concentrations of 0.1, 0.3, 0.5, and 1.0 μg/ml to determine whether it caused excision of circular forms. In some experiments chlortetracycline at 50 or 100 μg/ml was autoclaved in TYG to create a nontoxic tetracycline derivative (1). This was tested for the ability to cause the excision of circular forms from the chromosomes of *B. uniformis* BU1001 and BU1004 (Tc^r ERL). In other experiments, the effect of UV light, thymidine starvation, or exposure to mitomycin C was tested. For UV light experiments, a mid-exponential-phase culture of *B. uniformis* was centrifuged, washed once, suspended in 1× M9 salts (10 mM Na_2HPO_4, 10 mM KH_2PO_4, 10 mM NaCl, 20 mM NH_4Cl, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$), and then exposed to UV light for times that gave either 1 to 5% or 50% killing. The cells were centrifuged, suspended in TYG, and allowed to grow for 4 h at 37°C. For thymidine starvation experiments, Thy^- derivatives of *B. uniformis* BU1001 and BU1004 were grown to mid-exponential phase in TYG plus thymidine (100 μg/ml), centrifuged, washed in M9 salts, and then inoculated into TYG which contained no added thymidine. After 0, 1, 3, or 5 h, thymidine was added to the culture and the cells were allowed to grow for 4 h before plasmid preparations were made. For the mitomycin C experiments, cells were either grown overnight in 5, 10, or 30 μg of mitomycin C per ml (concentrations that slowed growth but did not prevent it) or grown to mid-exponential phase and exposed for 2 h to a high enough concentration of mitomycin C to stop growth (100 to 300 μg/ml).

Isolation and characterization of plasmid and chromosomal DNA. To detect the low-copy-number circular forms from the *Bacteroides* strains, a plasmid preparation made from about 10^{10} cells was loaded in each well on the agarose gel. The plasmid forms were isolated by using the Ish-Horowitz modification of the alkaline lysis procedure of Birnboim and Doly (3) and run on 0.8% or 1% agarose slab gels in 1× GGB (0.04 M Tris, 20 mM sodium acetate, 2mM EDTA [pH 8.3]) at 50 V for 16 to 18 h or 200 V for 2 h. Chromosomal DNA was isolated by the method of Saito and Miura (6). Restriction digests were done as specified by the manufacturer (Bethesda Research Laboratories). For Southern blots of chromosomal DNA, 3 to 5 μg of digested DNA was loaded in each well and electrophoresed in 0.8% agarose gels in 4× GGB at 50 V for 16 to 18 h.

Southern hybridizations. The Southern blots were done as previously described (9), essentially as described by Maniatis et al. (3), with 50% formamide in the prehybridization and hybridization solutions. About 10^7 cpm of ^{32}P-labeled probe (R751-INS1 or R751-INS2) was used in each hybridization. To visualize the *Hin*dIII λ standard fragments on the autoradiograms, 10^4 to 10^5 cpm of ^{32}P-labeled λ DNA was included with the test probe in the initial hybridization step. If the Southern blot contained DNA which had not been previously tested for homology to λ DNA, the blot was probed with the test probe first, autoradiograms were made, and the blot was then reprobed with ^{32}P-labeled λ DNA. Large probes, such as the R751 derivatives (>50 kilobases [kb]) were partially digested with *Ava*I before the nick translation reaction to ensure even labeling.

RESULTS

Visualization of NBU1 and NBU2. In our previous work with *B. uniformis* 0061, we had observed an approximately 50-kb, low-copy-number, cryptic plasmid (pBU50), but had never seen any other plasmids. Recently, we found that when *B. uniformis* BU1004, which was constructed by conjugating the Tc^r ERL element into *B. uniformis* BU1001 (Table 1), was grown in 0.3 to 1 μg of tetracycline per ml, two plasmidlike forms (NBU1 and NBU2) appeared (Fig. 1). No such plasmidlike forms were seen when the parent strain, BU1001, was exposed to the same concentrations of tetracycline (Fig. 1). It should be noted, however, that strain BU1001 grows very poorly in concentrations of tetracycline greater than 0.1 to 0.2 μg/ml and that the plasmid prepara-

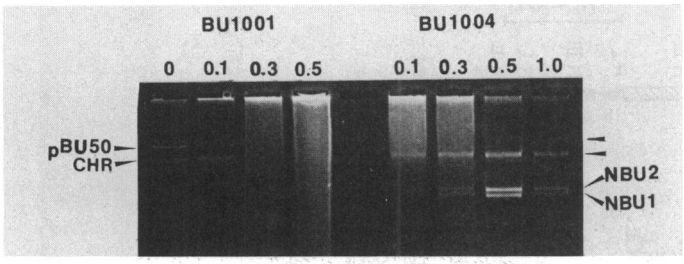

FIG. 1. Agarose gel (0.8%) of plasmid preparations made from *B. uniformis* BU1001 and BU1004 which were grown in increasing concentrations of tetracycline. The concentration of tetracycline in micrograms per milliliter is given at the top of each lane. Each lane contains an alkaline plasmid preparation made from about 10^{10} cells from overnight BU1001 or BU1004 cultures grown in the indicated amount of tetracycline. The locations of the cryptic 50-kb plasmid (pBU50) and the contaminating chromosomal DNA band (CHR) are indicated on the left. The locations of NBU1 and NBU2 are indicated at the right of the gel.

tions of the cells grown at 0.5 μg of tetracycline per ml contain contaminating material that interferes with visualization of pBU50 (Fig. 1).

NBU1 and NBU2 were readily cut by restriction enzymes. Preliminary restriction maps could be determined without separating the two elements because several restriction enzymes had sites that were unique to either NBU1 or NBU2 (e.g., *Eco*RV, *Eco*RI, etc.) (Fig. 2). From the restriction maps, it is clear that NBU1 is not a smaller form of NBU2 (Fig. 2). NBU1 and NBU2 appear to be about 10.3 and 11.5 kb, respectively.

NBU1 and NBU2 in R751 derivatives. The results shown in Fig. 1 did not rule out the possibility that NBU1 and NBU2 were already in the strain before introduction of the Tc^r ERL element. To test this, and for other experiments, we needed a hybridization probe that contained NBU1 and NBU2 sequences. Fortuitously, we found that we had isolated such plasmids in previous experiments involving mobilization of the IncP plasmid R751 (14) out of *Bacteroides* spp. (10). R751 does not replicate in *Bacteroides* recipients. However, if R751 carries the *Bacteroides* transposon Tn*4351*, R751::Tn*4351* can be inserted into the *B. uniformis* chromosome at different sites. These chromosomal insertions are flanked either by direct repeats of IS*4351* or by IS*4351* at one end and Tn*4351* at the other end (7, 10). When *B. uniformis* strains containing an integrated R751 and the conjugal Tc^r ERL were grown in tetracycline and mated with *E. coli*, plasmid derivatives of R751 were recovered from the *E. coli* transconjugants (10). From two separate *B. uniformis* donors, R751 derivatives were isolated that contained either 10 kb (R751-INS2) or 23 kb (R751-INS1) of extra DNA. Both inserts had homology to total cellular DNA from BU1001 (10). We have subsequently found from Southern blot analysis that these DNA inserts cross-hybridize with each other and with NBU1 and NBU2 (data not shown; see below).

We had shown previously that the 20- to 23-kb DNA insert in R751-INS1 was flanked by direct repeats of IS*4351* and that the 10-kb insert in R751-INS2 was flanked by IS*4351* at one end and Tn*4351* at the other (10). Further restriction enzyme mapping and Southern blot analysis gave the maps shown in Fig. 3. The restriction map of R751-INS2 (Fig. 3A) indicates that R751::Tn*4351* formed a cointegrate with NBU1 through one of the IS*4351* elements of Tn*4351*. The NBU1 has a small deletion at one end of the insertion, which includes the *PstI* site (Fig. 2). The larger R751 derivative, R751-INS1, appears to contain most or all of both NBU1 and NBU2. However, NBU1 and NBU2 do not map as serial cointegrates (i.e., NBU1 and NBU2 separated by IS*4351* copies) but, rather, NBU2 maps within NBU1 (Fig. 3B). Although R751-INS2 contains only NBU1, it also hybridizes to the 8-kb *Hin*dIII fragment of NBU2 (data not shown). Thus, NBU1 contains some region that is homologous to NBU2.

Location of NBU1 and NBU2 in *B. uniformis*. R751-INS1 hybridizes to total cellular DNA of *B. uniformis* BU1001 on Southern blots (10). To determine the location of NBU1 and NBU2 in *B. uniformis*. [^{32}P]-labeled R751-INS1 was used as the probe for the Southern blot in Fig. 4. The uncut and *Hin*dIII-digested plasmid forms of NBU1 and NBU2 from tetracycline-grown strain BU1004 are shown in lanes 2 and 3. The hybridization patterns of *Hin*dIII-digested total cellular DNA from strains BU1001 and BU1004 were the same (lanes 4 and 5). However, the patterns seen in cellular DNA from cells not exposed to tetracycline were significantly different from the pattern observed for the tetracycline-induced plasmid forms of NBU1 and NBU2 (lane 3). The

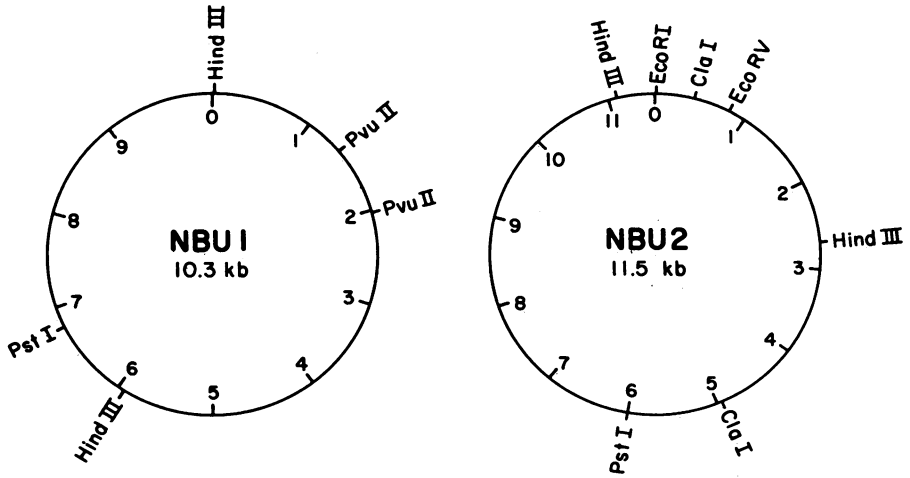

FIG. 2. Partial restriction maps of NBU1 and NBU2.

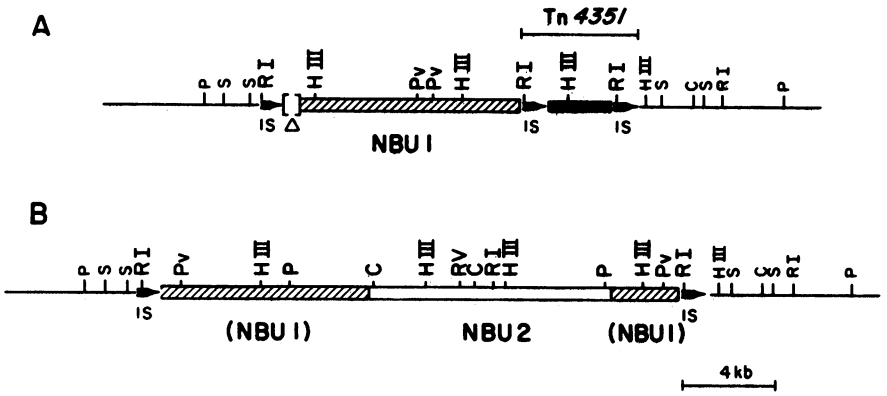

FIG. 3. Restriction maps of R751 derivatives which contain NBU1 or NBU1 and NBU2. (A) R751-INS2, which contains an insert of 10 kb, was isolated in *E. coli* HB101 from the *B. uniformis* BU1004 ΩR751::Tn*4351* donor DI-2 (10). Restriction mapping and Southern blot analyses (10; data not shown) indicated that the inserted material was NBU1 flanked by IS*4351* (arrow on the left and Tn*4351* (7, 10) on the right. The *Pst*I site of NBU1 is missing, and the small deletion is indicated by the brackets. (B) A larger R751 derivative (R751-INS1) was isolated in HB101 from the *B. uniformis* donor D4-6 (10). R751-INS1 contains 20 to 23 kb of inserted material. Restriction enzyme mapping and Southern blot analyses (10; data not shown) indicate that the inserted material includes both NBU1 and NBU2. NBU2 maps within NBU1, and R751::IS*4351* appears to have inserted into NBU1 between the *Pvu*II sites. The inserted material is flanked by copies of IS*4351* (10). The relevant restriction sites are indicated. The thin line and smaller letters indicate R751 sequences (14). Abbreviations: C, *Cla*I; H III, *Hin*dIII; P, *Pst*I; Pv, *Pvu*II; RI, *Eco*RI; RV, *Eco*RV; S. *Sal*I.

5.8-kb fragment of NBU1 and the 3.5-kb fragment of NBU2 are missing and a 3.1-kb fragment is present in the chromosomal patterns. Occasionally, very faint bands (one of about 23 kb, indicated by an arrow in lane 5, and a second fragment of about 1 kb, which is not visible in Fig. 4) can be seen in the total DNA digests. These results are consistent with the hypothesis that NBU1 and NBU2 are not normally in separate plasmidlike forms in *B. uniformis* and that they appear to be integrated into the chromosome or are part(s) of a coresident replicon. R751-INS1 did not hybridize to plasmid preparations from BU1001 or BU1004 which were not grown in tetracycline (data not shown). Therefore, NBU1 and NBU2 are not present in these plasmid preparations, and R751-INS1 does not hybridize to the cryptic plasmid pBU50.

To determine whether NBU1 and NBU2 are closely associated in their integrated noninduced forms, total cellular DNA from BU1001 was digested with *Pst*I (one site in each element), *Eco*RI (one site in NBU2), and both *Sal*I and *Bam*HI (which do not cut in either element). The resulting Southern blot was probed with R751-INS1 (Fig. 5). The sizes and number of fragments seen for the *Pst*I and *Eco*RI digests were not consistent with intact NBU1 and NBU2 integrated side by side into a larger replicon. The single *Eco*RI fragment was only 18 kb. Since the NBU2 integration site appears to be within the 3.5-kb *Hin*dIII fragment (Fig. 4), which also contains the only *Eco*RI site on either plasmid (Fig. 2), we might expect to see a single *Eco*RI fragment in DNA from the uninduced cells if NBU1 and NBU2 were integrated side by side, but the size should be at least 22 kb. Similarly, since there is one *Pst*I site in each element, we would expect at least three *Pst*I fragments, totaling more than 22 kb, but only two *Pst*I fragments, totaling 15 kb, were seen. Finally, the three *Hin*dIII fragments (Fig. 3) added up to 16 to 18 kb rather than to 22 kb or more.

Effect of various treatments on the appearance of NBU1 and NBU2. The tetracycline derivative chlortetracycline could induce the appearance of NBU1 and NBU2 from BU1004 but not from BU1001. Autoclaved chlortetracycline is a nontoxic form of tetracycline that acts as an inducer of the regulated *E. coli* Tc^r gene on Tn*10* (1). However, growing

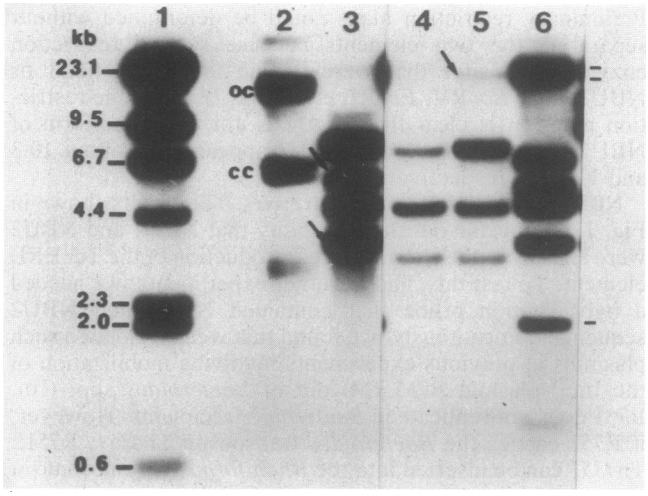

FIG. 4. Autoradiogram of *Hin*dIII-digested NBU1, NBU2, and total cellular DNA from *B. uniformis* BU1001 and BU1004 (Tc^r ERL) which was probed with ^{32}P-labeled R751-INS1 (Fig. 3). λ DNA, digested with *Hin*dIII, is in lane 1, and the fragment sizes in kilobases are indicated at the left of the autoradiogram. The λ DNA standards were visualized by including a small amount of ^{32}P-labeled λ DNA with the labeled R751-INS1 probe. λ DNA has no homology to the *B. uniformis* DNA used in this gel (data not shown). The uncut plasmid forms of NBU1 and NBU2, from a tetracycline-induced culture of BU1004, are in lane 2. The closed-circular (cc) and open-circular (oc) forms are indicated. NBU1 and NBU2, digested with *Hin*dIII, are in lane 3. The largest (8 kb) and smallest (3.5 kb) *Hin*dIII fragments are from NBU2, and the middle two fragments (5.8 and 4.6 kb) are from NBU1 (Fig. 2). The arrows in lane 3 indicate the bands that are not present in DNA from uninduced cells (lanes 4 and 5). *Hin*dIII-digested chromosomal DNA (3 to 5 μg) from BU1001 and BU1004 (not grown in tetracycline) are in lanes 4 and 5, respectively. A faint band, which could be a junction fragment due to the chromosomal insertion of NBU1 or NBU2, is indicated by an arrow in lane 5. The *Hin*dIII-digested probe (R751-INS1) is in lane 6. The R751 fragments of R751-INS1 are indicated by dashes to the right of the autoradiogram. R751::IS*4351* alone does not hybridize to NBU1, NBU2, or the *B. uniformis* chromosomal DNA (data not shown).

BU1001 and BU1004 in medium containing 50 or 100 μg of autoclaved chlortetracycline per ml did not lead to the appearance of NBU1 and NBU2. None of the other treatments we tested were able to cause the appearance of the plasmid forms of NBU1 and NBU2. These included (i) starvation for thymidine of thymidine-minus derivatives, (ii) low and high UV levels, and (iii) growth in various concentrations of mitomycin C (see Materials and Methods). Whenever the cells were grown under near-lethal conditions, there was a haze in the plasmid preparations that could have obscured visualization of the circular forms of NBU1 and NBU2 in stained agarose gels. However, Southern blots done on these preparations also indicated that there were no NBU1 and NBU2 sequences present in the plasmid preparations (data not shown).

Ability of other conjugal Tc^r elements to induce the appearance of NBU1 and NBU2. We transferred six Tc^r or Tc^r Em^r elements into *B. uniformis* BU1001 (Table 1) and grew the resulting transconjugants with and without 0.5 to 1 μg of tetracycline per ml. Four of the elements, Tc^r V479, Tc^r CEST, Tc^r DOT1 and Tc^r DOT2, mediated the appearance of NBU1 but not NBU2 when the cells were grown in tetracycline (Fig. 6). The identity of NBU1 was established by enzyme digestion patterns and hybridization to R751-INS2. The presence of NBU1 alone in the plasmid preparations allowed more accurate placement of the *Hin*dIII sites on the restriction maps of NBU1 and NBU2 in Fig. 2. Tc^r Em^r ERL caused the appearance of a plasmid form smaller than NBU1 (Fig. 6, lane 4). This 9.3-kb plasmid form contained one *Hin*dIII site and did not hybridize to R751-INS1 on Southern blots. Tc^r Em^r 12256 did not cause the appearance of any detectable NBU1 or NBU2 sequences by agarose gel (Fig. 6) or Southern blot (not shown) analysis.

The Tc^r elements that were not capable of excising NBU1 or NBU2 had not inserted within NBU1 or NBU2, because the *Hin*dIII, *Eco*RI, and *Pst*I digests of chromosomal DNA from BU1001 containing the various Tc^r or Tc^r Em^r conjugal elements gave the same hybridization patterns as BU1001

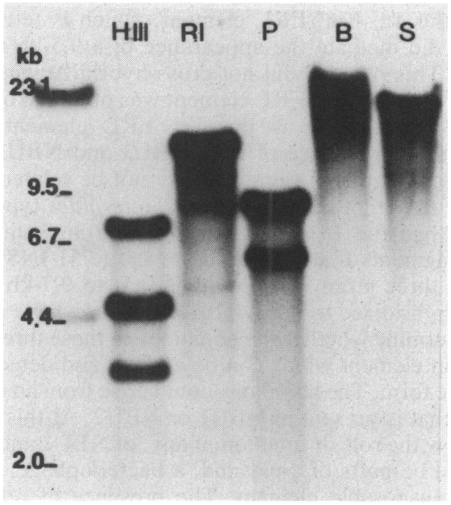

FIG. 5. Autoradiogram of chromosomal DNA from BU1001 which was digested with various restriction enzymes and probed with R751-INS1, a plasmid which contains both NBU1 and NBU2. Chromosomal DNA (3 to 5 μg) of BU1001 was digested with *Hin*dIII (lane HIII), *Eco*RI (lane RI), *Pst*I (lane P), *Bam*HI (lane B), or *Sal*I (lane S), and the fragments were resolved on a 0.8% agarose gel. The sizes in kilobases of the *Hin*dIII fragments of λ are indicated on the left.

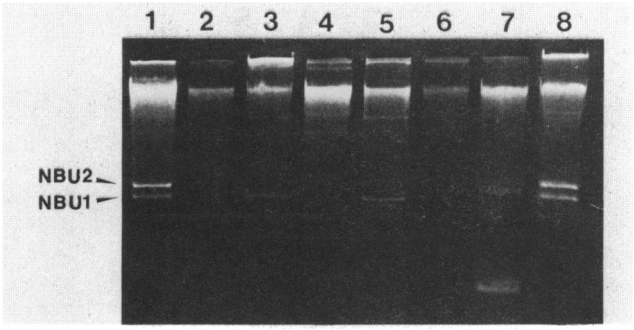

FIG. 6. Appearance of plasmidlike forms mediated by conjugal Tc^r or Tc^r Em^r elements other than Tc^r ERL. Six conjugal Tc^r or Tc^r Em^r elements were transferred into *B. uniformis* BU1001 (Table 1). The resultant BU1001 transconjugants were grown in TYG containing 1 μg of tetracycline per ml, and alkaline plasmid preparations were made (see Materials and Methods) and run on a 0.8% agarose gel. The plasmid preparation from BU1004 (Tc^r ERL) is shown for comparison in lanes 1 and 8. The locations of NBU2 and NBU1 are indicated at the left. The plasmid preparations of the other transconjugants are as follows: lane 2, BU1002 (Tc^r V479); lane 3, BU1001 (Tc^r Em^r CEST); lane 4, BU1005 (Tc^r Em^r ERL); lane 5, BU1006 (Tc^r Em^r DOT1); lane 6, BU1007 (Tc^r Em^r DOT2); lane 7, BU1008 (Tc^r Em^r 12256). The NBU1 band is very faint in lanes 2 and 6. This was seen in repeated experiments. The lower band in lane 7 is the closed-circular form of p12256, a cryptic plasmid from *B. fragilis* 12256 which cotransferred with Tc^r Em^r 12256. The open-circular form of this plasmid runs between NBU1 and NBU2 (lane 8). Note that the plasmid form in lane 4 (BU1005) is smaller than NBU1.

alone (Fig. 5) when R751-INS1 was used as the probe (data not shown). However, this result did not rule out the possibility that the Tc^r or Tc^r Em^r elements were interfering with the excision of NBU1 or NBU2 in some other way. To test this possibility, Tc^r Em^r 12256, Tc^r Em^r DOT1, and Tc^r Em^r DOT2 were conjugated into BU1004 from the original donors (Table 1), with selection for Em^r *B. uniformis* transconjugants. Since Tc^r Em^r is always cotransferred by these elements, the Em^r transconjugants should contain both the Tc^r ERL element and one of the Tc^r Em^{r}elements. In all cases, the plasmid preparations made from these double transconjugants, grown in tetracycline, contained the circular forms of both NBU1 and NBU2. Therefore, Tc^r Em^r 12256, Tc^r Em^r DOT1, and Tc^r Em^r DOT2 do not prevent the Tc^r ERL-mediated appearance of NBU1 and NBU2.

Homology to NBU1 and NBU2 in DNA from other *Bacteroides* species. *Hin*dIII digests of DNA from three *B. uniformis* strains (0061, C7-17, and T1-1) and from the type strains of eight other human colonic *Bacteroides* species were tested for homology to NBU1 and NBU2 sequences by using R751-INS1 as a probe (Fig. 7). *B. uniformis* T1-1 DNA contained a single, large (>15-kb), weakly hybridizing fragment (lane 2). Three of the type strains from other *Bacteroides* species had a single *Hin*dIII band (about 9.6 kb) that hybridized to the probe (lanes 3 to 5). *B. uniformis* C7-17 and the other five type strains tested had no detectable homology to the R751-INS1.

DISCUSSION

We have shown that the conjugal *Bacteroides* Tc^r element Tc^r ERL mediates the tetracycline-dependent appearance of two plasmidlike forms, NBU1 and NBU2. Although these plasmidlike forms were seen only in *B. uniformis* 0061 after

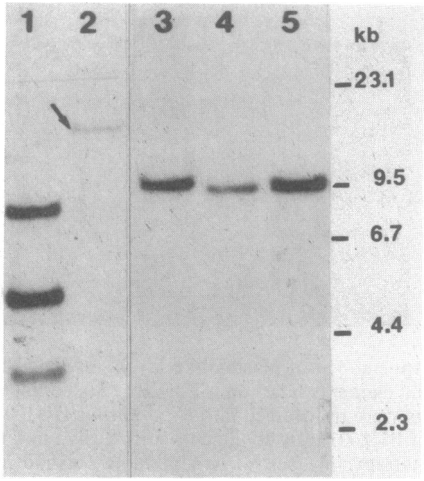

FIG. 7. Autoradiogram of *Hin*dIII-digested chromosomal DNA from human colonic *Bacteroides* strains that had homology to the NBU1-NBU2 probe. Chromosomal DNA from each of nine human colonic *Bacteroides* type strains and from two additional *B. uniformis* strains was digested with *Hin*dIII, and 3 to 5 μg of each digest was run on a 0.8% agarose gel. The Southern blot of the gel was hybridized to ^{32}P-labeled R751-INS1, which contains both NBU1 and NBU2 (Fig. 3 and 4). Only strains which contained a band(s) that hybridized to the probe are shown. The sizes in kilobases of the *Hin*dIII fragments of λ are indicated on the left. BU1001 (*B. uniformis* 0061) (lane 1) contains the three expected *Hin*dIII bands. *B. uniformis* T1-1 (lane 2) contains one weakly hybridizing band (16 to 18 kb), which is larger than any of the BU1001 bands. Three other type strains, *B. vulgatus* 4245 (lane 3), *B. stercoris* B5-21 (lane 4), and *B. eggerthii* B8-51 (lane 5), each contained one hybridizing *Hin*dIII fragment of about 9.5 to 9.7 kb. The remaining *Bacteroides* strains tested had no detectable homology to the probe: *B. uniformis* C7-17, *B. thetaiotaomicron* 5482, *B. ovatus* 0038, *B. caccae* 3452A, *B. fragilis* I 2553, and *B. fragilis* II 2393. All of the *Bacteroides* strains were obtained from the VPI Anaerobic Laboratory Culture Collection (Virginia Polytechnic Institute and State University, Blacksburg).

introduction of the Tc^r ERL element, results of Southern hybridization experiments with NBU1 and NBU2 sequences as a probe indicate that NBU1 and NBU2 are not brought into the strain with the Tc^r ERL element but are normally integrated either in the chromosome or in some unidentified large plasmid in *B. uniformis* 0061. Our results strongly support the hypothesis that the appearance of NBU1 and NUB2 is due to an excision event and that this excision event is associated with exposure to tetracycline. A number of treatments other than exposure to tetracycline, including exposure to UV light and mitomycin C, did not cause the appearance of the plasmidlike forms. Autoclaved chlortetracycline was also ineffective. It is interesting that the only conditions that cause the appearance of the plasmid forms are those that also enhance the transfer frequency of the Tc^r ERL element (8). Thus, the steps involved in the appearance of NBU1 and NBU2 could be connected with regulation by tetracycline of the inducible Tc^r elements.

The presence of the Tc^r ERL element, rather than just exposure to tetracycline alone, was necessary for the appearance of the plasmidlike forms of NBU1 and NBU2. First, NBU1 and NBU2 were not seen when strain BU1001 was grown in medium containing low concentrations of tetracycline. Second, the plasmidlike forms of NBU1 and NBU2 were not seen when BU1008, a derivative of BU1001 which carries the Tc^r Em^r 12256 element, was grown on tetracycline. The Tc^r Em^r 12256 element did not interfere with induction of the plasmidlike forms of NBU1 and NBU2 by the Tc^r ERL element when the two elements were in the same strain.

Although results of Southern blot analysis of total cellular DNA from BU1001 indicate that NBU1 and NBU2 are integrated close to each other, we were unable to deduce the structures of the two insertions from the Southern blot data. The orientation of NBU1 and NBU2 in R751-INS1 (Fig. 3B) suggests that IS*4351* had mediated R751 cointegration into a circular NBU1ΩNBU2 element somewhere between the two *Pvu*II sites of NBU1. Thus, NBU1 and NBU2 could be two parts of a larger element that resolves to produce NBU1 and NB2 . However, results of Southern blot analysis of integrated NBU1 and NBU2 in BU1001 are not consistent with the structure derived from Fig. 3B. The number and sizes of the restriction fragments seen on Southern blots (Fig. 5) are not consistent with either a simple cointegrate containing NBU1 and NBU2 or simple adjacent insertions of NBU1 and NBU2 into a larger replicon. The *Pst*I, *Hin*dIII, and *Eco*RI fragments all indicate a size of 16 to 18 kb versus the 22 to 23 kb of the two circular forms of NBU1 and NBU2. It is possible that there are missing junction fragments which are not well visualized by the probe used in the experiment. Alternatively, some restriction fragments in Fig. 5 could be doublets. However, it is more likely that the homology between NBU1 and NBU2 allows NBU1 and NBU2 to overlap in their integrated form. If this is true, NBU1 and NBU2 should not both be excised from the same replicon. In this case, the appearance of both NBU1 and NBU2 in preparations from strain BU1004 could be due to a mixture of NBU1 and NBU2 excisions from different cells in the population. We are investigating the nature and extent of the homology between NBU1 and NBU2.

Two conjugal Tc^r elements did not mediate the appearance of either NBU1 and NBU2: Tc^r Em^r 12256 and Tc^r Em^r ERL. Tc^r Em^r. 12256 is the only one of the elements tested that is not tetracycline inducible for tetracycline resistance or for conjugal transfer. It is also the only element that did not cause the appearance of some plasmidlike form. By contrast, the Tc^r Em^r ERL element, which is tetracycline inducible, did mediate the appearance of a 9.5-kb plasmidlike form. This plasmid did not cross-hybridize with NBU1 or NBU2. The Tc^r Em^r ERL element was obtained originally from the same strain as the Tc^r ERL element, which mediated the appearance of both NBU1 and NBU2. Thus, these two conjugal Tc^r elements may not be related.

None of the other human colonic *Bacteroides* type strains we tested had the BU1001 pattern of chromosomal DNA *Hin*dIII fragments that hybridized to the R751-INS1 probe. However, three strains had a single 9.5- to 9.7-kb *Hin*dIII band that hybridized to R751-INS1. So far, we have not been able to determine whether the homology in these three strains is due to an element which can be excised and detected as a plasmidlike form. The homology could arise from an insertion sequence that is present in NBU1 or NBU2. At this time we do not know the role or function, if any, of NBU1 and NBU2. They could be parts of a plasmid, a bacteriophage, or some form of transposable element. The presence of related sequences in other *Bacteroides* species gives some indication that they belong to a group of related elements or carry a common element, perhaps an insertion element.

ACKNOWLEDGMENT

This work was supported by Public Health Service grant AI 22383 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Bochner, B. R., H.-C.Huang, G. L. Schieven, and B. N. Ames.** 1980. Positive selection for the loss of tetracycline resistance. J. Bacteriol. **143**:926–933.
2. **Malamy, M. H., and F. P. Tally.** 1981. Mechanisms of drug-resistance transfer in *Bacteroides fragilis*. J. Antimicrob. Chemother. **8**(Suppl. D):59–75.
3. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. **Mays, J. D., C. J. Smith, R. A. Welch, C. Delfine, and F. L. Macrina.** 1982. Novel antibiotic resistance transfer in *Bacteroides*. Antimicrob. Agents Chemother. **21**:110–118.
5. **Privitera, G., M. Sebald, and F. Fayolle.** 1979. Common regulatory mechanism of expression and conjugative ability of a tetracycline resistance plasmid in *Bacteroides fragilis*. Nature (London). **278**:657–659.
6. **Saito, H., and K.-I. Miura.** 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. Biochim. Biophys. Acta **72**:619–629.
7. **Shoemaker, N. B., C. Getty, J. F. Gardner, and A. A. Salyers.** 1986. Tn*4351* transposes in *Bacteroides* spp. and mediates the integration of plasmid R751 into the *Bacteroides* chromosome. J. Bacteriol. **165**:929–936.
8. **Shoemaker, N. B., C. E. Getty, E. P. Guthrie, and A. A. Salyers.** 1986. Regions in *Bacteroides* plasmids pBFTM10 and pB8-51 that allow *Escherichia coli-Bacteroides* shuttle vectors to be mobilized by IncP plasmids and by a conjugative *Bacteroides* tetracycline resistance element. J. Bacteriol. **166**:959–965.
9. **Shoemaker, N. B., E. P. Guthrie, A. A. Salyers, and J. F. Gardner.** 1985. Evidence that the clindamycin-erythromycin resistance gene of *Bacteroides* plasmid pBF4 is on a transposable element. J. Bacteriol. **162**:626–632.
10. **Shoemaker, N. B., and A. A. Salyers.** 1987. Facilitated transfer of IncPβ R751 derivatives from the chromosome of *Bacteroides uniformis* to *Escherichia coli* recipients by a conjugative *Bacteroides* tetracycline resistance element. J. Bacteriol. **169**:3160–3167.
11. **Smith, C. J., and F. L. Macrina.** 1984. Large transmissible clindamycin resistance plasmid in *Bacteroides ovatus*. J. Bacteriol. **158**:739–741.
12. **Smith, C. J., and H. Spiegel.** 1987. Transposition of Tn*4551* in *Bacteroides fragilis*: identification and properties of a new transposon from *Bacteroides* spp. J. Bacteriol. **169**:3450–3457.
13. **Smith, C. J., R. A. Welch, and F. L. Macrina.** 1982. Two independent conjugal transfer systems operating in *Bacteroides fragilis* V479-1 J. Bacteriol. **151**:281–287.
14. **Thomas, C. M., and C. A. Smith.** 1987. Incompatability group P plasmids: genetics, evolution, and use in genetic manipulation. Annu. Rev. Microbiol. **41**:77–101.
15. **Welch, R. A., and F. L. Macrina.** 1981. Physical characterization of *Bacteroides fragilis* R plasmid pBF4. J. Bacteriol. **145**: 867–872.