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Stability in *Escherichia coli* of an Antibiotic Resistance Plasmid from *Bacteroides fragilis*

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A *Bacteroides fragilis* strain resistant to penicillin G, tetracycline, and clindamycin was screened for the presence of plasmid deoxyribonucleic acid (DNA). Agarose gel electrophoresis of ethanol-precipitated DNA from cleared lysates of this strain revealed two plasmid DNA bands. The molecular weights of the plasmids were estimated by their relative mobility in agarose gel and compared with standard plasmids with known molecular weights. The molecular weights were $3.40 \pm 0.20 \times 10^6$ and $1.95 \pm 0.05 \times 10^6$ for plasmids pBY1 and pBY2, respectively. Plasmid DNA purified by cesium chloride-ethidium bromide gradient centrifugation was used to transform a restriction- and modification-negative strain of *Escherichia coli*. Penicillin G- and tetracycline-resistant transformants were screened for the presence of plasmid DNA. A plasmid band corresponding to a molecular weight of 1.95×10^6 was present in all transformants tested. Curing experiments demonstrated that the plasmid, referred to as pBY22 when present in transformants, was responsible for penicillin G and tetracycline resistance. Plasmid pBY22 was mobilized and transferred to other *E. coli* strains by plasmid R1*drd-19*. Stability of pBY22 was examined in different *E. coli* strains and was shown to be stably maintained in both restriction-negative and restriction-positive strains. Unexpectedly, pBY2 and pBY22 were resistant to digestion by 12 different restriction endonucleases.

More than 25% of the bacteria residing in the normal human colon are organisms belonging to the *Bacteroides fragilis* group (18). This group of gram-negative anaerobic bacteria is comprised of seven named *Bacteroides* species (*B. fragilis, B. thetaiotaomicron, B. vulgatus, B. ovatus, B. distasonis, B. uniformis, B. eggerthii*) and several unnamed species (20). Of the pathogens in this group, *B. fragilis* is the most frequently encountered in clinical specimens (10, 26). *Bacteroides* spp. are characteristically resistant to several antimicrobial agents, particularly penicillins and aminoglycosides, and are moderately resistant to erythromycin and tetracycline. In recent years, an increase in the frquency of tetracycline resistance has been noted (22, 36). One of the most useful antibiotics for *Bacteroides* infections is clindamycin, but recently clindamycin-resistant strains (27, 37, 40) have been isolated from clinical specimens.

The role played by plasmids in the spread and acquisition of antibiotic resistance among pathogenic bacteria is well known (9). Plasmids have been described in *Bacteroides* spp. (13, 35, 38), and there is good evidence that antibiotic resistance of *Bacteroides* spp. is plasmid mediated (21, 27, 37, 40, 42). It has been suggested that the *Bacteroides* spp. in the colon of humans or animals may represent a significant repository of antibiotic-resistant plasmids (13, 32, 38) capable of being transferred to more conventional pathogens. In this respect, Mancini and Behme (21) and Young and Mayer (42) have demonstrated the transfer of antibiotic resistance markers from *B. fragilis* to *Escherichia coli*; however, they did not conclusively demonstrate either plasmid transfer or stability of the markers.

A prerequisite for the hypothesis that the *Bacteroides* spp. of the colon represents a repository of antibiotic-resistant plasmids capable of transfer and expression in other pathogens is that the plasmids be stable in the recipient. In this paper we report the isolation and partial characterization of a small plasmid from *B. fragilis* which is stably maintained in *E. coli*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. Bacterial strains and plasmids used are listed in Table 1. *B. fragilis* VPI12256 was identified by L. V. Holdeman (personal communication) at the Virginia Polytechnic Institute Anaerobe Laboratory. Its identity was reconfirmed (unpublished data) by using species-specific bacteriophages (3). Stock cultures of *B. fragilis* were propagated in prereduced anaerobically sterilized

TABLE 1. *Bacterial strains and plasmids*

Strain	Plasmid content	Mol wt (10^6)	Resistance markers or phenotype[a]	Origin or source	Reference
B. fragilis VPI12256			Pen G, Tc, Cc	T. D. Wilkins	39
	pBY1	3.40 ± 0.20[b]		This study	
	pBY2	1.95 ± 0.05[b]	Pen G, Tc	This study	
E. coli					
1485	RSF1030	5.5	Ap	P.R.C.[c]	16
J53	S-a	25	Cm, Km, Sm, Su	P.R.C.	23
C600	RP4	34	Ap, Tc, Km	P.R.C.	15
C600	R1*drd-19*	62	Ap, Sm, Cm, Km, Su	P.R.C.	33
SC182			Rif, Nal, *hsdS*	P.R.C.	
EL401			Nal	P.R.C.	
J53-1			Nal	P.R.C.	
JP990			Nal	G.R. Miller	1
C600 185NX			Nal	S. Falkow	
HB101			Lac$^-$, Sm, *hsdS*	S. Falkow	4
HB101-1			Spontaneous nalidixic acid-resistant mutant of HB101	This study	

[a] Ap, ampicillin; Tc, tetracycline; Pen G, penicillin G; Cc, clindamycin; Cm, chloramphenicol; Su, sulfonamide; Rif, rifampin; Nal, nalidixic acid; Sm, streptomycin; or Km, kanamycin resistance; Lac, lactose fermentation; *hsdS*, restriction and modification negative.

[b] Determined by agarose gel electrophoresis.

[c] Plasmid Reference Center, Stanford University, Stanford, Calif.

chopped-meat broth (17). Routine growth of *Bacteroides* spp. was in brain heart infusion broth (or agar) supplemented as described by Holdeman et al. (17). For transformation experiments, *E. coli* was grown in L-broth supplemented with glucose and $CaCl_2$ as described by Elwell and Falkow (8). Routine growth of *E. coli* was in brain heart infusion broth. All incubations were at 37°C.

Preparation of crude lysates for plasmid screening. Cells were grown in 50 ml of brain heart infusion (*E. coli*) or brain heart infusion-supplemented (*B. fragilis*) broth overnight and were harvested by centrifugation. The cells were suspended in 1.5 ml of 25% sucrose buffered with 0.05 M Tris and 1 mM EDTA (pH 8). Cells were lysed by addition of 0.2 ml of lysozyme (1% in 0.25 M Tris-hydrochloride, pH 8), 0.4 ml of EDTA (0.25 M, pH 8) and sodium dodecyl sulfate to a final concentration of 1%. This preparation was adjusted to 1 M NaCl and was placed in ice for 4 h or overnight. Cell debris and high-molecular-weight chromosomal DNA was removed by centrifugation at 34,000 × *g* at 4°C for 30 min (9). The volume of supernatant was doubled by the addition of distilled water and treated with RNase (bovine pancreas, type IA, Sigma Chemical Co., St. Louis, Mo.) for 1 h at 37°C. DNA was precipitated with ethanol after phenol extraction. The precipitated DNA was suspended in 0.1 ml of TES buffer (0.05 M Tris–5 mM EDTA–0.05 M NaCl, pH 8) and either analyzed immediately by agarose gel electrophoresis or stored at −20°C (23). This procedure was used for initial screening for plasmids in *B. fragilis* and for screening for plasmids in *E. coli* after transformation experiments.

Plasmid purification. Cell lysis was performed by a modification of the procedures described by Clewell and Helinski (5). Cultures were grown overnight in brain heart infusion or brain heart infusion-supplemented broth. The cells were harvested and washed with TES buffer. Cells from a 300-ml culture were suspended in 3 ml of cold 25% sucrose in 0.05 M Tris and 1 mM EDTA (pH 8) and placed on ice for 30 min. A 0.6-ml portion of a 5-mg/ml solution of lysozyme in 0.25 M EDTA (pH 8) was added, and the cells were incubated on ice. After 10 min, 1.2 ml of 0.25 M EDTA was added, and the preparation was incubated on ice for another 10 min. The cells were lysed by addition of 4.8 ml of lytic mix (0.1% Triton X-100–0.06 M EDTA–0.05 M Tris, pH 8). A cleared lysate was obtained by centrifugation at 34,000 × *g* for 30 min at 4°C.

Plasmid DNA was purified by CsCl-ethidium bromide gradient centrifugation, essentially as described by Clewell and Helinski (5). A 6-ml portion of cleared lysate obtained by the Triton X-100 lysis procedure was brought to 7.8 ml with TES buffer. After the addition of 8.0 g of CsCl, ethidium bromide was added to a final concentration of 500 μg/ml using a 10-mg/ml stock solution of ethidium bromide in distilled water. The gradients were centrifuged in a Beckman SW41 Ti rotor for 44 h at 40,000 rpm and 15°C. Gradients were examined with a shortwave UV lamp, and plasmid DNA bands were collected through the side of the tube with a syringe and 20-gauge needle. The ethidium bromide was removed by extracting the DNA fractions four times with CsCl-saturated isopropanol. The CsCl was removed by overnight dialysis against TES buffer at 4°C.

Preparation of molecular-weight standard plasmids. Cells from a 500-ml culture of *E. coli* were lysed with Triton X-100 as described above. The cleared lysates were adjusted to final concentrations of 0.5 M NaCl and 10% (wt/vol) polyethylene glycol (19). After overnight incubation at 4°C, precipitated

DNA was removed by centrifugation at 5,000 × *g* for 5 min and dissolved in 1 ml of 7 M CsCl in TES. After removal of polyethylene glycol and CsCl by dialysis, DNA was used immediately as molecular weight markers in agarose gel electrophoresis or stored at −20°C.

Agarose gel electrophoresis. Ethanol- or polyethylene glycol-precipitated DNA (50 to 100 μl) was subjected to vertical slab gel electrophoresis in 0.7% agarose gels (Bethesda Research Laboratories, Gaithersburg, Md.). The DNA samples were electrophoresed for 3.5 h at 100 V (constant voltage) and 50 mA. After electrophoresis, gels were stained with a solution of ethidium bromide (1 μg/ml) for 20 min. Gels were examined with a shortwave UV transilluminator (Ultra Violet Products) and photographed with a red filter (Wratten no. 25) and Kodak Tri X-Pan film.

Transformation experiments. Transformation experiments were performed as described by Cohen et al. (7). *E. coli* HB101 was grown in L-broth to an optical density at 590 nm of 0.85 to 0.9. The cells were harvested and washed once with sterile 0.01 M NaCl, suspended in 0.3 M $CaCl_2$, and incubated on ice for 20 min. The suspension was centrifuged, and the cells were suspended in one-tenth the original volume of cold, sterile 0.03 M $CaCl_2$. A 0.2-ml portion of purified plasmid DNA was added to a test tube containing 0.2 ml of competent cells and 60 μl of cold sterile 0.3 M $CaCl_2$. The tubes were incubated on ice for 1 h, then warmed to 42°C and gently shaken for 2 min. After rechilling, the cells were diluted in fresh L-broth and incubated for 6 h at 37°C. Appropriate dilutions were made and plated onto brain heart infusion-supplemented plates containing 16 μg of tetracycline per ml or 100 μg of penicillin G per ml.

Restriction endonuclease digestion. DNA for restriction endonuclease analysis was prepared as described by Finkelstein and Rownd (11). Plasmid DNA bands were sliced from 0.7% agarose gels. Each gel slice was placed into 0.1 ml of 0.1 M Tris (pH 5.95) and crushed by passing through a 1-ml plastic syringe. Agarase (Calbiochem, La Jolla, Calif.) was added at a final concentration of 50 μg per gel slice and incubated at 37°C for 2 h. The agarose was removed by centrifugation at 50,000 × *g* for 30 min. Ethidium bromide was extracted with isopropanol, and the DNA was concentrated by sodium acetate-ethanol precipitation.

Restriction endonucleases *Bam*I, *Hin*dIII, and *Eco*RI were purchased from Miles Laboratories, and *Eco*RII, *Cfo*I, *Alu*I, *Hin*dII, *Hin*fI, *Dde*I, *Hpa*II, *Sau*96I, and *Ava*II were purchased from Bethesda Research Laboratories. Approximately 1 μg of DNA (50 μl) was mixed with 1 to 2 U of restriction endonuclease and incubated at 37°C for 90 to 120 min. The reaction was halted by addition of 5 μl of stop mixture (5% sodium dodecyl sulfate, 25% glycerol, 0.025% bromophenol blue). Analysis of DNA fragments was by electrophoresis in 1.2% slab agarose gels for 5.5 h at 80 V.

Conjugation experiments. Conjugation experiments involving *Bacteroides* strains were performed on membrane filters as described by Welch et al. (40).

Conjugation experiments between *E. coli* strains were performed as described previously (28). Cultures of donor and recipient strains were grown in brain heart infusion broth overnight. One milliliter of overnight culture was added to 50 ml of fresh broth and incubated at 37°C with shaking until the absorbance (550 nm) was 0.5 for the donor strain (approximately 10^8 colony-forming units/ml) and 1.5 for the recipient (approximately 5×10^8 colony-forming units/ml). One-milliliter volumes of donor and recipient cells were mixed and incubated at 37°C without shaking. After 15 h of incubation, tubes containing mating mixtures were placed on ice, and appropriate dilutions were plated onto selective media.

Plasmid stability. Cells were grown in brain heart infusion broth (containing no antibiotics) for at least 50 generations, diluted, and plated onto brain heart infusion-supplemented plates for selection of single clones (34). Each individual colony was then tested on selective media to determine the proportion of clones which had retained or lost the antibiotic resistance marker.

RESULTS

Screening for plasmid DNA. *B. fragilis* strain VPI12256, which is resistant to clindamycin, tetracycline, and penicillin G (39), was examined for the presence of plasmid DNA. Ethanol-precipitated DNA from cleared lysates of cells was prepared and analyzed by agarose gel electrophoresis. This strain was found to harbor two species of plasmid DNA (Fig. 1). The plasmids were designated as pBY1 and pBY2, respectively, after the proposal for nomenclature of plasmids by Novick et al. (25). The molecular weight of each plasmid was estimated from its electrophoretic migration relative to the molecular weight standard plasmids. The molecular weight of each of the *B. fragilis* plasmids was estimated to be 3.4×10^6 for pBY1 and 1.95×10^6 for pBY2. The electrophoretic bands corresponding to open circular forms of plasmid DNA (Fig. 1) were identified and confirmed by nicking the covalently closed circular DNA with ethidium bromide and visible light.

Conjugation experiments. Attempts of conjugal transfer of antibiotic resistance from *B. fragilis* VPI12256 to other *B. fragilis* strains and to restriction-deficient strains of *E. coli* were unsuccessful. We therefore used transformation to transfer antibiotic resistance to other cells.

Transfer of antibiotic resistance by genetic transformation. Purified plasmid DNA (lower satellite band in the CsCl-ethidium bromide gradient) was used to transform the $CaCl_2$-treated *E. coli* HB101 cells. After transformation, cells were allowed to grow in fresh L-broth for 6 h before being transferred to selective media. Transformants were isolated on Mueller-Hinton agar plates containing 16 μg of tetracycline per ml or 100 μg of penicillin G per ml (frequency of transfer was approximately 10^{-6} to 10^{-7} per viable cell). A DNA control was also

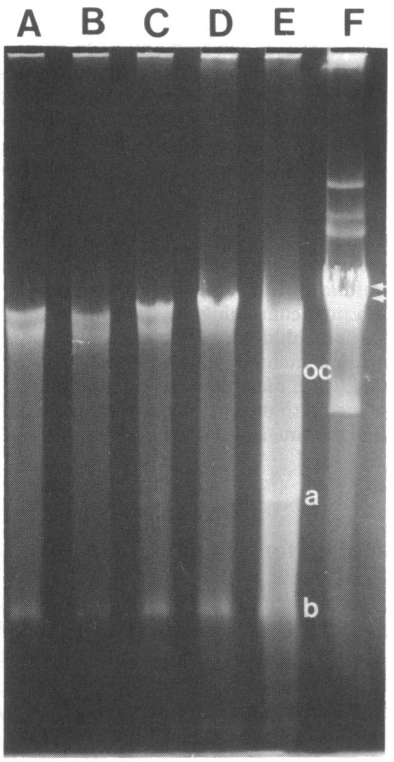

FIG. 1. *Agarose gel electrophoresis of ethanol-precipitated DNA from cleared lysates. (A, B) Penicillin-resistant E. coli HB101 transformants. (C, D) Tetracycline-resistant E. coli HB101 transformants. (E) B. fragilis VPI12256; upper band, open circular (OC) form of pBY1; (a) middle band, pBY1 (3.40 Md); (b) lower band, pBY2 (1.95 Md). (F) Molecular weight standard plasmids R1drd-19 (62 Md), RP4 (34 Md), S-a (25 Md), and RSF1030 (5.5 Md). The diffuse band (double arrow) present in all samples represents the chromosomal DNA.*

included in which the DNA was substituted with sterile TES buffer. The transfer of the control cells to the selective media did not yield any tetracycline-resistant or penicillin G-resistant colonies. Several of the transformant colonies were restreaked on selective media and were tested for the phenotype of the recipient *E. coli* HB101 (Lac^-, streptomycin resistance). All the colonies tested were found to be Lac^- and streptomycin resistant. Three of the tetracycline-resistant transformants and three of the penicillin G-resistant transformants were screened for the presence of plasmid DNA. Agarose gel electrophoresis revealed one plasmid DNA band which had the same mobility as plasmid pBY2 (Fig. 1). This plasmid (presumably pBY2, but referred to as pBY22 when present in transformants) was found to be present in all of the tetracycline-resistant and penicillin G-resistant transformants tested. None of the tested transformants contained a plasmid having a molecular weight corresponding to that of pBY1. Several of the tetracycline-resistant transformants were tested for penicillin G resistance, and all were found to be resistant. The penicillin G-resistant transformants were also found to be resistant to tetracycline.

A spontaneous tetracycline- and penicillin-sensitive derivative of *E. coli* HB101(pBY22) was isolated after prolonged passage in nonselective media. The analysis of ethanol-precipitated DNA from cleared lysates of this strain revealed the loss of the 1.95-megadalton (Md) plasmid (pBY22; Fig. 2), which indicated that the genes responsible for resistance to tetracycline and penicillin G were carried on pBY22.

Restriction endonuclease analysis. Restriction endonuclease digestion was attempted to provide further evidence that the 1.95-Md plasmid (pBY22) present in *E. coli* HB101 transformants was identical to pBY2 in the parental *B. fragilis* strain VPI12256. Plasmid DNA was purified by extracting the covalently closed circular DNA from 0.7% agarose gels by the procedure described by Finkelstein and Rownd (11).

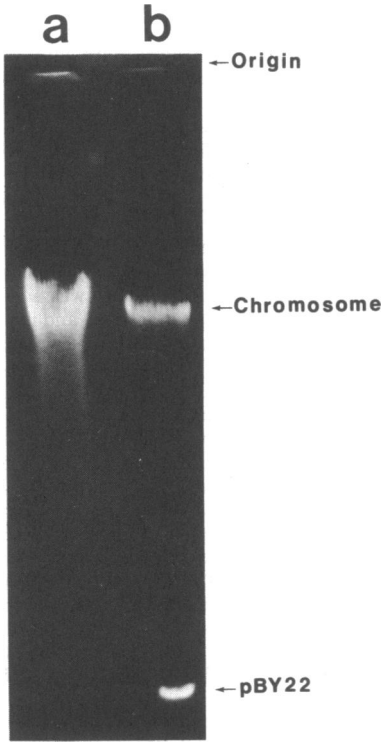

FIG. 2. *Agarose gel electrophoresis of ethanol-precipitated DNA from cleared lysates of (a) a spontaneous tetracycline- and penicillin G-sensitive revertant of E. coli HB101 (pBY22), and (b) tetracycline- and penicillin G-resistant E. coli HB101 (pBY22).*

The purified plasmid DNA was mixed with each of 12 restriction endonucleases (see above) and after appropriate incubation was analyzed by slab gel electrophoresis. Neither pBY2 nor pBY22 was digested by any of the restriction enzymes tested. Bacteriophage λ DNA was used as a positive control and produced the appropriate number of fragments (31) with each of the restriction enzymes.

Transfer of antibiotic resistance to other *E. coli* strains. One of the tetracycline-resistant transformants was selected and used as the recipient in mating with *E. coli* C600 (R1*drd-19*). Mating was performed by mixing 0.5-ml portions of *E. coli* C600 (R1*drd-19*; donor) and *E. coli* HB101 (pBY22, tet^r, $penG^r$; recipient) on a brain heart infusion agar plate for 2 h. The cells were then streaked onto selective media containing chloramphenicol (50 μg/ml) and tetracycline (16 μg/ml). The chloramphenicol- and tetracycline-resistant transconjugants were examined by agarose gel electrophoresis and were shown to harbor plasmids R1*drd-19* and pBY22. One of these transconjugants [*E. coli* HB101(R1*drd-19*, pBY22)] was used as the donor in mating with other *E. coli* strains. The mating was performed for 15 h, and selection was made on (i) plates containing nalidixic acid (20 μg/ml) and chloramphenicol (50 μg/ml), and (ii) plates containing nalidixic acid (20 μg/ml) and tetracycline (16 μg/ml). The frequencies of transfer of R1*drd-19* and tetracycline resistance to restriction-negative and also restriction-positive strains of *E. coli* K-12 are shown in Table 2. It appeared that plasmid pBY22 could be mobilized and transferred to other recipient *E. coli* strains when it coexisted with plasmid R1*drd-19* in the same host. However, the frequencies of transfer of pBY22 (tetracycline resistance) were always lower than that of plasmid R1*drd-19*. Although the conditions of mating were the same for all strains, the frequencies of transfer were different. The comparison of frequencies of transfer of pBY22 to restriction-positive and restriction-negative strains of *E. coli* K-12 showed that the presence of a restriction system in *E. coli* did not reduce the frequency of transfer.

The Nal^r, Tc^r, Cm^r transconjugants of each different strain were analyzed by agarose gel electrophoresis for the presence of plasmid DNA. It was found that all of the tested transconjugants had acquired plasmids R1*drd-19* and pBY22.

Stability of pBY22 in *E. coli*. Stability of plasmid pBY22 was tested in different *E. coli* strains. Cells were grown in the absence of antibiotics, and individual clones were tested on plates containing 16 μg of tetracycline per ml. As shown in Table 3, tetracycline resistance was retained in most of the colonies tested. Several of the tetracycline-resistant clones grown in the absence of tetracycline were examined for their plasmid content. All of these were found to harbor plasmids R1*drd-19* and pBY22. The spontaneous tetracycline-sensitive derivatives (derived from C600 185 NX and J53-1, Table 3) were found to have lost pBY22. The loss of penicillin resistance was not determined in these revertants, since plasmid R1*drd-19* mediates resistance to penicillin.

DISCUSSION

B. fragilis strain 12256 is resistant to penicillin G, tetracycline, and clindamycin. This unusual resistance pattern prompted us to study the genetic basis of the antibiotic resistance in this strain. The agarose gel electrophoretic analysis

TABLE 2. *Frequencies of transfer of R1drd-19 and tetracycline resistance to different E. coli strains*[a]

Recipient strain	Selection for[b]	
	Nal^r Cm^r	Nal^r Tc^r
SC182	0.85	9.0×10^{-5}
185NX	0.92	1.7×10^{-3}
EL401	0.18	1.3×10^{-3}
J53-1	0.076	1.6×10^{-4}
HB101-1	0.13	6.5×10^{-3}

[a] Donor strain HB101 harbored plasmids R1*drd-19* and pBY22. Frequencies were calculated as the number of transconjugants per donor cell.

[b] Nal^r, Resistance to nalidixic acid; Cm^r, resistance to chloramphenicol; Tc^r, resistance to tetracycline.

TABLE 3. *Stability of plasmid pBY2 in different E. coli strains*

Strain	No. of clones tested	No. of tetracycline-resistant clones
SC182		
1[a]	41	41
2	43	43
EL401		
1	48	48
2	51	51
C600 185 NX		
1	51	50
2	53	53
J53-1		
1	45	45
2	50	49
HB101-1		
1	43	43
2	52	52
JP990		
1	58	58
2	61	61

[a] Two transconjugants from each strain were tested and were designated 1 and 2.

of ethanol-precipitated DNA from cleared lysates of this strain established the presence of two species of plasmids with molecular masses of 3.4 and 1.95 Md. Attempts at conjugal transfer of antibiotic resistance(s) from this strain to other *B. fragilis* strains and to *E. coli* were unsuccessful. These results are not unusual since plasmids with a molecular weight of less than 10 Md are normally not self-transmissible (6). Transformation experiments demonstrated that the 1.95-Md plasmid (referred to as pBY22 when present in *E. coli* transformants) was responsible for resistance to penicillin G and tetracycline. Since the *E. coli* HB101 transformants (containing only pBY22) could not transfer their antibiotic resistances to other *E. coli* strains, it was concluded that plasmid pBY22 was nonconjugative. However, when pBY22 coexisted with plasmid R1*drd-19* in the same host, it could be mobilized and transferred by conjugation to suitable recipient strains. The frequencies of transfer of pBY22 from *E. coli* HB101 to other *E. coli* strains were compared, and it was shown that the presence of a restriction system in *E. coli* had no effect on the transfer of pBY22. The study of the stability of pBY22 in different strains of *E. coli* K-12 established that this plasmid was stably maintained in *E. coli* K-12. Guiney and Davis (14) demonstrated that two plasmids from *Bacteroides ochraceus* were stable in *E. coli* after conjugation; however, this species of *Bacteroides* has recently been shown to be synonymous with the genus *Capnocytophaga* (24, 37, 41).

Although pBY22 in *E. coli* transformants is a nonconjugative plasmid, it could be mobilized by conjugative plasmid R1*drd-19*. Mobilization seems to be a very efficient mechanism for the dissemination of nonconjugative plasmids in other bacterial systems. For example, it appears that mobilization is the mechanism by which the non-self-transmissible plasmids of *Neisseria gonorrhoeae* are transferred (27, 29, 30). In addition, Barth and Grinter (2) have shown that the common plasmids conferring linked resistance to sulfonamide and streptomycin in *Enterobacteriaceae* are nonconjugative. These plasmids are presumably disseminated by mobilization (8).

Evidence that pBY2 from *B. fragilis* VPI-12256 was transferred to various *E. coli* strains was based primarily on the acquisition of a 1.95-Md plasmid and the simultaneous acquisition of penicillin G and tetracycline resistance. In addition, we observed that strains which were spontaneously cured of the plasmid reverted to penicillin G and tetracycline sensitive (Fig. 2). To further substantiate that pBY2 in *B. fragilis* was identical to pBY22 in *E. coli* transformants, we performed restriction endonuclease digestions on the purified plasmids. Unexpectedly, we found that neither pBY2 nor pBY22 was susceptible to any of the 12 restriction enzymes tested. Although we could not obtain DNA fragments for comparative analysis, the failure of either plasmid to be digested is highly suggestive that they are identical. We are currently investigating the mechanism of endonuclease resistance of the plasmids.

B. fragilis and *E. coli* both inhabit the intestinal tract of humans and animals. Since species of *Bacteroides* are a major constituent of the gastrointestinal tract of humans and animals, the possibility that the genus *Bacteroides* represents a significant repository of clinically and ecologically important plasmids has been raised (13, 32, 35, 38). Recently, Mancini and Behme (21) demonstrated transfer of multiple antibiotic resistance from *B. fragilis* to *E. coli*; however, the resistance markers were unstable in *E. coli* transconjugants. Similarly, Young and Mayer (42) described the transfer of antibiotic resistance from *B. fragilis* to *E. coli* by transformation. Although they were successful in transferring β-lactamase production to *E. coli* by using a purified plasmid preparation, they were not able to detect plasmids in the transconjugants. The two reports on the transfer of antibiotic resistance markers from *B. fragilis* to *E. coli* and the demonstration in this work that a plasmid of *B. fragilis* can be stably maintained in *E. coli* provide further evidence that *Bacteroides* in the colon may be reservoir for antibiotic-resistant plasmids. However, it still remains to be proven that the colonic *Bacteroides* actually possess antibiotic-resistant determinants and that they can be transferred under natural conditions to other bacteria. A recently described experiment (T. Butler, K. Joiner, M. Malamy, J. Bartlett, and F. P. Tally, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 216, 1980) demonstrated the transfer of antibiotic resistance among *B. fragilis* in an abscess in mice, lending support to the suggestion that *Bacteroides* can indeed transfer antibiotic resistance in vivo.

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