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Two Independent Conjugal Transfer Systems Operating in *Bacteroides fragilis* V479-1

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Bacteroides fragilis V479-1 (also designated strain 92) has previously been shown to contain a conjugative plasmid, pBF4, that specifies resistance to clindamycin (Cc). A report of inducible tetracycline (Tc) resistance in this strain suggested that this phenotype was also plasmid associated (G. Privitera et al., Nature [London] **278**:657–659, 1979) and prompted further investigation. Mating experiments with V479-1 and a Cc-sensitive derivative of V479-1, V598, showed that Tc resistance transfer occurred by a conjugation-like event which was insensitive to DNase, was not mediated by donor culture cell-free filtrates, and required cell-to-cell contact. Results from transfer experiments with V479-1 indicated that Tc and Cc resistance determinants were not linked and segregated independently in matings. Progeny recovered from matings with the V479-1 or V598 donor strain were able to transfer the Tc resistance marker in secondary crosses. Tc resistance transfer from V479-1 or V598 was greatly stimulated by pregrowth in the presence of Tc but not Cc. pBF4-mediated Cc resistance transfer was not affected by pregrowth in the presence of Cc or Tc. Filter blot DNA hybridization studies revealed that pBF4 sequences were not involved in either the Tc resistance phenotype or its associated conjugal transfer properties. The Tc resistance transfer element was not associated with pBF4 or any other extrachromosomal DNA element.

Bacteroides fragilis, an anaerobic, gram-negative, nonsporeforming rod, is a common inhabitant of the gastrointestinal tract of humans (2, 6) and is also an important opportunistic pathogen (1). Recently, several reports have appeared which have described self-transmissible antibiotic resistance plasmids in this organism (12, 19, 23).

Because such plasmids encode resistance to clindamycin, the drug of choice against *Bacteroides* infections, the medical implications of their dissemination are serious. On the other hand, the relatively broad host range of these plasmids (24) promises to catalyze the development of genetic transfer systems in *B. fragilis* and related species. One such resistant strain of *B. fragilis*, V479-1 (designated strain 92 by M. Sebald [12, 23]) has been studied extensively. Its 27 × 10^6-dalton (27-Mdal) plasmid pBF4 (also called pIP410) was shown to mediate transferable, constitutively expressed resistance to the macrolide-lincosamide-streptogramin (MLS) antibiotics, including clindamycin (Cc) (12, 23). Physical analyses of pBF4 and its MLS-sensitive deletion derivatives have made possible the construction of a restriction endonuclease cleavage site map of the plasmid containing the putative location of the MLS resistance determinant(s) (24, 25).

Following the initial reports of plasmid-mediated MLS resistance in *B. fragilis* V479-1, Privitera et al. (13) discovered that this strain possessed an inducibly expressed tetracycline (Tc) resistance. Interestingly, this resistance was transferable only from donor cultures that had been grown in the presence of Tc. Their claim that this transfer system was also plasmid linked prompted us to reexamine the V479-1 strain. In this report, we confirm the presence of an inducible Tc resistance phenotype in V479-1. Further, we show that this Tc resistance determinant is not carried by pBF4 or by any other detectable extrachromosomal element. In addition, we demonstrate that Tc resistance is transferred by a conjugation-like process that operates independently of the pBF4-mediated conjugal transfer system.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study and their relevant characteristics are given in Table 1. The parental donor strain, V479-1, containing

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TABLE 1. Bacterial strains

Organism[a]	Designation	Relevant phenotype[b]	Size of plasmids (Mdal)
B. fragilis	V479-1	Cc^r Tc^r Rf^s Rha^- Cat^+ Ara^-	27[c]
B. fragilis	V598[d]	Cc^s Tc^r Rf^s Rha^- Cat^+ Ara^-	None
B. uniformis	V528	Cc^s Tc^s Rf^r Rha^- Cat^- Ara^+	None[e]
B. ovatus	V211	Cc^s Tc^s Rf^s Rha^+ Cat^- Ara^+	None[e]
B. uniformis	V852[f]	Cc^s Tc^r Rf^r Rha^- Cat^- Ara^+	None
B. uniformis	V853[g]	Cc^s Tc^r Rf^r Rha^- Cat^- Ara^+	None

[a] An earlier report referred to the *B. fragilis* group with these strains being termed subspecies. Based on physiological traits and DNA-DNA hybridization studies, these strains have now been elevated to the species level (6).

[b] Minimum inhibitory concentrations for the antibiotic resistance markers were: Cc^r, >200 μg/ml; Tc^r, >20 μg/ml (resistance to Tc was always measured with cells that were previously grown in 0.1 μg of Tc per ml); rifampin (Rf^r), >20 μg/ml; Cc^s, <5 μg/ml; Tc^s, <1 μg/ml; Rf^s, <1 μg/ml. Rha, Ability to utilize rhamnose as sole carbon source; Cat, production of catalase; Ara, ability to utilize arabinose as sole carbon source.

[c] The 27-Mdal plasmid pBF4 has been previously described (24, 25).

[d] Obtained as a Cc^s variant of V479-1 after curing with ethidium bromide (24).

[e] Strain previously determined to be plasmid free (21).

[f] Obtained from mating between V479-1 and V528 (Table 2, experiment 4).

[g] Obtained from mating between V598 and V528 (Table 2, experiment 8).

the plasmid pBF4, was originally obtained from Madeline Sebald (Pasteur Institute; strain 92) and has been previously described (23, 25). All stock cultures were maintained anaerobically in chopped-meat medium (6). The complex medium used for routine culturing was brain heart infusion broth (BHI; Difco Laboratories, Detroit, Mich.) supplemented with L-cysteine (1.0 g/liter), hemin (5 mg/liter), and menadione (1.0 mg/liter). Broth cultures of supplemented BHI were incubated in stoppered tubes containing an atmosphere of 90% N_2 and 10% CO_2. For solid media, 15 g of agar per liter was added to BHI broth, and culture plates were incubated anaerobically in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.) with an atmosphere of 80% N_2, 10% CO_2, and 10% H_2. The defined medium used for secondary mating experiments was modified from Varel and Bryant (22) and contained the following components in 1 liter: mineral solution no. 3 (22), 50 ml; Casamino Acids, 1.0 g; yeast extract, 0.5 g; tryptone (Difco), 0.5 g; L-methionine, 20 mg; hemin, 5.0 mg; menadione, 1.0 mg; L-cysteine (free base), 1.0 g; $NaHCO_3$, 2.0 g; $(NH_4)_2SO_4$, 0.6 g; rhamnose, 2.5 g; and agar, 15 g. The pH of the medium was 7.1.

Antibiotic susceptibility testing was performed by the agar dilution method (8). For examination of Tc resistance, cells were pregrown overnight in the presence of 0.1 μg of Tc per ml before plating. Under these conditions, the minimum inhibitory concentration (<1% viability) of resistant strains was >20 μg of Tc per ml. Cells of V479-1 and V598 not pregrown in 0.1 μg of Tc per ml showed poor plating efficiency (<50%) on 5 μg of Tc per ml.

Filter matings were performed as previously described (16, 23), with the following minor modifications. Mid-log cultures of donor (0.5 ml, ~10^8 cells per ml) and recipient (1.0 ml) were sedimented separately in 1.5 ml of Eppendorf polypropylene tubes and suspended in 75 μl of sterile BHI broth. These cultures were then mixed, transferred to nitrocellulose filters, and incubated nonselectively in GasPak jars before plating on the appropriate selective medium (16).

DNA preparation and analysis. Routine screening for plasmid content was performed as previously described (25), except 5 M potassium acetate replaced 5 M sodium chloride throughout the procedure. Purified plasmid DNA was prepared by cesium chloride-ethidium bromide ultracentrifugation (23), and bulk cellular DNA was isolated by the technique described by Marmur (9). Restriction endonuclease digestions were performed at 37°C for 4 h in the buffers recommended by the manufacturer (Bethesda Research Laboratories, Gaithersburg, Md.). DNA preparations were analyzed by agarose slab gel electrophoresis (11) with the appropriately sized reference DNA molecules as standards (7).

For filter blot hybridizations, restricted bulk cellular DNA was transferred to nitrocellulose filters as described by Southern (17), followed by hybridization with ^{32}P-labeled probe DNA and autoradiography by the method of Thayer (20). Radiolabeling of probe DNA was performed by in vitro nick translation (14) with the materials and protocol supplied by New England Nuclear Corp., Boston, Mass.

RESULTS

Antibiotic resistance expression and transfer. Transfer of Tc resistance was investigated in the plasmid-containing strain V479-1 (pBF4) and in its plasmidless derivative V598. Although V598 was cured of pBF4 and was Cc sensitive, it retained a Tc resistance phenotype which was identical to the parent V479-1 in both level of resistance and expression. Expression of Tc resistance was inducible (Fig. 1). Cells of either strain grown overnight in the presence of a subinhibitory concentration of Tc (0.1 μg/ml) did not show a lag in growth when challenged with 5 μg of Tc per ml. However, cultures not previously exposed to Tc demonstrated a significant lag when challenged with 5 μg of Tc per ml (Fig. 1). In addition to this inducible expression of resistance, both strains were capable of growth in broth cultures containing 40, but not 60, μg of Tc per ml if previously induced with Tc overnight.

Transfer of Tc resistance from either V479-1 or V598 appeared to be dependent upon preexposure to Tc. Little or no transfer of the Tc

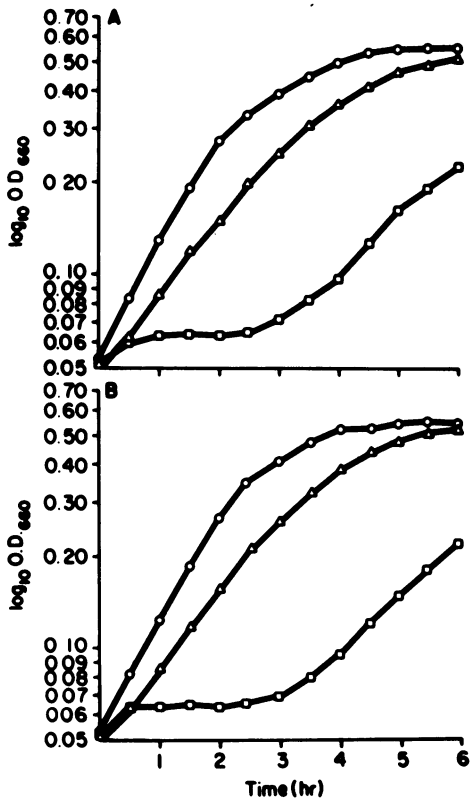

FIG. 1. Growth response of V479-1 and V598 in cultures containing Tc. (A) Response for V479-1; (B) growth response for V598. O, Case in which an overnight culture without Tc was inoculated at a 20-fold dilution into broth without tetracycline; □, case in which the same 20-fold-diluted inoculum was added to broth containing 5 μg of Tc per ml; △, case in which the overnight inoculum was grown in the presence of 0.1 μg of Tc per ml and then inoculated at a 20-fold dilution into broth containing 5 μg of Tc per ml.

resistance marker was observed unless cultures were first grown in the presence of Tc (Table 2). Cultures exposed to Tc for 18 to 36 h (5 to 10 generations) before mating displayed a significant increase in the frequency of Tc resistance transfer, and this increase was dependent on the concentration of the antibiotic used during the preexposure period. This was particularly apparent for strain V598, which showed an increase in transfer with each increase of Tc used during the preexposure (Table 2, experiments 6 through 9).

It is interesting to note that V598 transferred Tc resistance after preexposure to 0.1 μg of Tc per ml, whereas V479-1 did not consistently transfer the resistance marker at this level of preexposure. In addition, V598 seemed to transfer Tc resistance at slightly higher frequencies for all levels of Tc preexposure, relative to the parental strain V479-1.

In contrast to Tc resistance transfer, Cc resistance (and resistance to other MLS antibiotics) transfer from V479-1 was not dependent on preexposure to Cc (Table 2, experiments 1 and 5). Furthermore, transfer frequencies for the Cc resistance marker were not affected by preexposure to Tc at any of the concentrations tested (Table 2, experiments 1 through 4). Additional evidence for the independence of these two antibiotic resistance transfer systems was obtained by analysis of transconjugant clones isolated after the matings described in Table 2, experiments 1 through 4. Progeny cells obtrained by selection for the Tc resistance marker never displayed the Cc resistance phenotype (400 colonies tested), nor did clones originally isolated on Cc Rf media possess the Tc resistance phenotype (500 colonies tested).

Progeny isolated from all matings were shown to reflect the genetic background of the recipient strain based on their ability to utilize arabinose, their lack of catalase, and their sensitivities to antibiotics (see Table 1). In addition, representative Tc-resistant transconjugants were shown to possess an inducible resistance which was identical to those of the parental strains (data not shown).

Characterization of Tc resistance transfer. Previous work has shown that the transfer of Cc resistance from V479-1 was the result of a conjugation-like event which requires cell-to-cell contact between the recipient and donor (23). A similar approach suggested that Tc resistance transfer from both V479-1 and V598 also required cell-to-cell contact (Table 3). These data show that the transfer was not mediated by cell-free filtrates of donor cells, nor did transfer occur in broth cultures when donor and recipient were mixed and incubated. Furthermore, when donor and recipient cells were separated by a membrane filter, transfer did not occur at detectable frequencies. Finally, when the mating mixture and nonselective mating agar plate were treated with DNase, a reduction in the transfer frequency was observed relative to the nontreated control. However, a similar reduction was noted when only the $MgSO_4$ buffer was added to the mating mixture and the nonselective agar plate (Table 3, treatments 1, 2, and 3).

Noninvolvement of pBF4 in Tc resistance transfer. Studies from this laboratory have shown that the 27-Mdal plasmid, pBF4, is responsible for the transferable Cc resistance phenotype in V479-1. To determine the role of pBF4 in Tc resistance transfer, progeny isolated from matings with V479-1 or V598 were examined for the presence of plasmid DNA after growth in the presence of Tc (5 μg/ml). With standard plasmid

TABLE 2. Transfer of antibiotic resistance from V479-1 and V598

Expt	Donor	Recipient	Treatment (μg/ml)[a]	Marker transfer frequency[b]	
				Tc	Cc
1	V479-1	V528	None	$<1 \times 10^{8}$	$6.6 (\pm 0.7) \times 10^{-6}$
2	V479-1	V528	0.1 Tc	$<1 \times 10^{-8}$[c]	$6.0 (\pm 1.0) \times 10^{-6}$
3	V479-1	V528	1.0 Tc	$4.1 (\pm 1.2) \times 10^{-6}$	$4.8 (\pm 1.5) \times 10^{-6}$
4	V479-1	V528	10.0 Tc	$8.6 (\pm 1.1) \times 10^{-6}$	$5.5 (\pm 1.2) \times 10^{-6}$
5	V479-1	V528	10.0 Cc	$<1 \times 10^{-8}$	$5.5 (\pm 1.7) \times 10^{-6}$
6	V598	V528	None	$<1 \times 10^{-8}$ [c]	$<1 \times 10^{-8}$
7	V598	V528	0.1 Tc	$2.0 (\pm 1.1) \times 10^{-6}$	$<1 \times 10^{-8}$
8	V598	V528	1.0 Tc	$1.7 (\pm 2.1) \times 10^{-5}$	$<1 \times 10^{-8}$
9	V598	V528	10.0 Tc	$3.9 (\pm 0.7) \times 10^{-5}$	$<1 \times 10^{-8}$

[a] The treatments consisted of growth in broth cultures for 36 h (two overnight transfers) in the presence of the indicated concentration of Tc before mating.

[b] Transfer frequencies were estimated by averaging at least three independent experiments by the following formula: frequency of transfer = (number of resistant progeny)/(viable input donor cell). Selective media contained 5 μg of Tc and 10 μg of Cc per ml. Rifampin was used at a concentration of 20 μg/ml in all selective media to kill the donor strain.

[c] An occasional authentic Tc-resistant progeny was observed, but results were sporadic and accurate transfer frequencies could not be determined.

TABLE 3. Evidence implicating cell-to-cell contact in the Tc transfer event

Treatment[a]	Transfer frequency[b]	
	V479-1	V598
None	3.0×10^{-6}	8.1×10^{-6}
100 μg of DNase per ml in 5 mM $MgSO_4$ buffer added to the mating mixture and the nonselective mating plate	1.5×10^{-6}	1.7×10^{-6}
5 mM $MgSO_4$ buffer added to the mating mixture and the nonselective mating agar plate	1.1×10^{-6}	2.3×10^{-6}
Cell-free filtrates of donor added to recipient[c]	$<1 \times 10^{-8}$	$<1 \times 10^{-8}$
Donor and recipient separated by membrane filter[d]	$<1 \times 10^{-8}$	$<1 \times 10^{-8}$
Liquid broth matings[e]	$<1 \times 10^{-8}$	$<1 \times 10^{-8}$

[a] Results represent one experiment, but similar observations were made during additional trials.

[b] Transfer frequencies are as described in Table 2. The standard matings were done with the donor strain indicated and V528 as recipient. Donor cell cultures were preexposed to 1 μg of Tc per ml for 36 h, as described in Table 2. Transconjugants were selected on media containing Tc (5 μg/ml) and rifampin (20 μg/ml).

[c] Cell-free filtrates of the donor strain were prepared by filtration of log-phase cells through 0.45-μm pore size nitrocellulose filters.

[d] The donor strain and recipient were collected separately on 0.45-μm pore size filters and then placed, one on top of the other, cell-side up on the nonselective mating plate. These filter sandwiches were then treated as normal matings.

[e] Liquid broth matings were performed by adding log-phase cells of both donor and recipient to BHI broth and incubating overnight. The resulting culture was sedimented and plated on selective media.

screening methods as well as cesium chloride-ethidium bromide centrifugation, *B. fragilis* V598 was not found to contain detectable plasmid DNA. Tc-resistant transconjugants from V598 × V528 or V479-1 × V528 matings (up to 10 per experiment) were also plasmidless. In contrast, Cc-resistant transconjugants from V479-1 × V528 crosses, similarly examined, always contained pBF4 (data not shown).

Although pBF4 was not detected in V598 or any Tc-resistant progeny, the possibility remained that all or part of the plasmid was present as an integrated segment in the host chromosome. Filter blot hybridizations of *Eco*RI-restricted bulk cellular DNA from V479-1, V598, V528, V852, and V853 probed with radiolabeled pBF4 were used to investigate this possibility (Fig. 2). As expected, hybridization to the six *Eco*RI fragments of the pBF4 present in V479-1 was seen (lane A), and there were no hybridizing sequences in the recipient strain V528 (lane B). Surprisingly, in V598 (lane C), two weakly hybridizing fragments were observed; however, the molecular weights of these fragments did not correlate specifically to those of any of the pBF4 *Eco*RI fragments (lane A). Finally, no hybridization with DNA from the two Tc-resistant progeny, V852 and V853, was seen (Fig. 2, lanes D and E).

The data from this and several similar experiments suggested that pBF4 was not involved with Tc resistance per se, but a role for pBF4 in the mobilization of Tc resistance could not be ruled out, owing to the hybridizing sequences observed in V598. Therefore, the two Tc-resistant progeny, V852 and V853, lacking any pBF4 hybridizing sequences (Fig. 2, lanes D and E), were mated with *Bacteroides ovatus* V211. Both strains transferred Tc resistance at frequencies

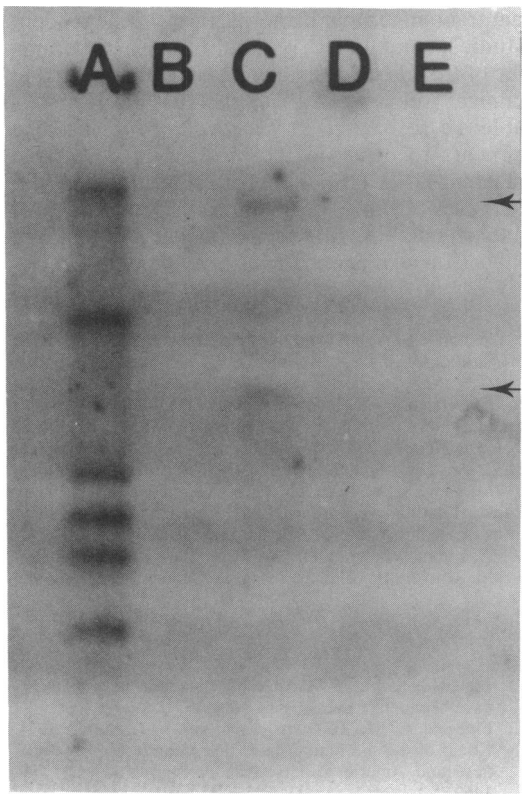

FIG. 2. Autoradiograph of filter-blotted bulk cellular DNA from V479-1, V598, V528, V852, and V853 probed with ^{32}P-labeled pBF4. *Eco*RI-restricted bulk cellular DNA was electrophoresed on a horizontal 0.8% agarose slab gel (28 by 18 by 1 cm) for 16 h at 40 V. The filter blot was prepared as described in the text and allowed to hybridize with ~2 × 10^7 cpm of ^{32}P-labeled pBF4 probe. Lane A, V479-1 (0.3 μg of DNA); the six *Eco*RI fragments of pBF4 ranging in size from 1.7 to 13.1 Mdal are shown. Lane B, V528 (1.5 μg of DNA). Lane C, V598 (1.5 μg of DNA); two weakly hybridizing fragments are indicated by arrows to the right of the photograph. Lane D, V852 (1.5 μg of DNA); lane E, V853 (1.5 μg of DNA).

comparable to those of the parental strains (Table 4). Interestingly, pregrowth in the presence of Tc was not required for transfer of resistance. However, Tc treatment did result in an increase in frequency of transfers.

DISCUSSION

The present study of antibiotic resistance transfer in *B. fragilis* V479-1 clearly documents the presence of two separate conjugal transfer systems in this strain. Previous work has shown pBF4-mediated conjugal Cc resistance transfer in this strain (23). We have shown here that Tc resistance transfer also fits the criteria for a conjugative-like genetic exchange (Table 3). Most notably, the process required cell-to-cell contact and was not inhibited by DNase. Depending on selection, transconjugant clones obtained from matings with the V479-1 donor were of two classes, Cc or Tc resistant (Table 2), thus demonstrating an independent segregation of these two antibiotic resistance determinants. In fact, the simultaneous transfer of both resistance markers was not observed under any of the conditions tested. Although Privitera et al. (13) were able to obtain transconjugant clones simultaneously resistant to Cc and Tc, this generally occurred at very low frequencies (~3 × 10^{-8}) or with donor cultures cycled through Tc enrichments (40 μg/ml) for 5 days. We found that Cc- and Tc-resistant progeny could be easily obtained by sequential matings of V479-1 and the same V528 host, suggesting that a similar phenomenon may occasionally occur in a single mating experiment.

The differential effect of preexposure to antibiotics on Tc and Cc resistance transfer frequencies also suggested that these were two independent systems. Whereas preexposure to Cc did not alter the frequencies of transfer for either marker, preexposure to Tc had a dramatic effect on transfer of Tc but not Cc resistance (Table 2). Tc resistance transfer increased significantly when either V479-1 or its cured derivative, V598, was pregrown in the presence of this antibiotic. This effect was not seen, however, in crosses involving Tc-resistant *Bacteroides uniformis* as the donor and *B. ovatus* as the recipient (Table 4), suggesting that the effects of Tc on Tc transmissibility are donor specific. Current-

TABLE 4. Transfer of Tc resistance in secondary crosses

Expt	Donor[a]	Recipient	Treatment (μg/ml)[b]	Selective[c] medium	Frequency of transfer[d]
1	V852	V211	None	Tc, Rha	7.3 (±0.5) × 10^{-6}
2	V852	V211	1.0 Tc	Tc, Rha	1.0 (±0.1) × 10^{-5}
3	V853	V211	None	Tc, Rha	5.4 (±0.1) × 10^{-6}
4	V853	V211	1.0 Tc	Tc, Rha	9.8 (±2.1) × 10^{-6}

[a] Strains V852 and V853 are Tc-resistant transconjugants isolated from matings with V528 and V479-1 or V598 (see Table 1).

[b] Treatments were as described in Table 2 with the indicated concentration of Tc.

[c] Selective medium was the semidefined medium described in the text containing rhamnose (Rha) as the major carbon source. Antibiotics were Tc (3 μg/ml) and Cc (10 μg/ml).

[d] Transfer frequencies were as described in Table 2 and are the average of at least two independent experiments.

ly, it is not possible to invoke a mechanism responsible for the effect of Tc preexposure on Tc resistance transferability. However, it does appear that this phenomenon is not linked to the inducibility of Tc resistance expression itself. Two observations support this theory. First, 10 times more drug (1.0 versus 0.1 μg/ml) was needed to induce transfer from V479-1 than was needed to induce Tc resistance expression (Table 2 and Fig. 1). Second, the transfer induction phenomenon was clearly dependent on the donor background (V479-1 versus V852 or V853 [Tables 2 and 4]), whereas the induction of resistance expression was similar in all donor backgrounds (V479-1, V852, V853; data not shown).

Our genetic evidence suggests that Tc resistance transfer is independent of the transferable Cc resistance mediated by pBF4. Furthermore, the lack of pBF4 DNA in all Tc-resistant transconjugants lends further support to this idea. Finally, Tc resistance expression and transfer in the plasmidless derivative V598 was nearly identical to that observed in V479-1. The possibility that part or all of pBF4 participated in mobilization of Tc resistance as an integrated segment of the V598 host chromosome was ruled out by the lack of pBF4 hybridizing sequences in transconjugant clones with the transferable Tc resistance phenotype (Fig. 2). The two weakly hybridizing fragments observed only in V598 DNA may represent some sequence homology of the pBF4 inverted repeats (25) with similar structures in the host chromosome. The apparent lack of these two hybridizing components in V479-1 chromosomal DNA is most likely due to the reduced amount of DNA in this lane (Fig. 2, lane A versus lanes B through E), but their absence cannot be ruled out. The significance of these observations is not known and will require further experimentation. The lack of detectable plasmid DNA in V598 and all Tc-resistant transconjugants leaves the question of cellular location for the Tc resistance determinant(s) open to interpretation. Although it is possible that a very large or difficult-to-detect plasmid is responsible, this seems unlikely in light of the various plasmid isolation techniques used (4, 5, 25) and the ease of pBF4 isolation. It is our hypothesis that the Tc resistance determinant is part of a self-transmissible, transposon-like genetic element which resides as an integrated element in the host genome. Similar models have recently been described to account for the *en bloc* transfer of chloramphenicol and Tc resistance in *Streptococcus pneumoniae* (15) and for transfer of the Tc resistance transposon Tn*916* in *Streptococcus faecalis* (3). Moreover, these results bear a striking resemblance to three other transfer systems recently described for gastrointestinal tract anaerobic bacteria. In two independent studies, conjugative, plasmid-free, *en bloc* transfer of Cc and Tc resistance has been observed in clinical isolates of *B. fragilis* (10, 18). Transferable Tc resistance in *Clostridium difficile* also appears to occur in the absence of detectable plasmid DNA (16). These unusual transfer systems are clearly of evolutionary and clinical importance but will require further study before their true significance can be evaluated.

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LITERATURE CITED

1. **Finegold, S. M.** 1977. Anaerobic bacteria in human disease. Academic Press, Inc., New York.
2. **Finegold, S. M., H. R. Atteberg, and V. L. Sutter.** 1974. Effect of diet on human fecal flora: comparison of Japanese and American diets. Am. J. Clin. Nutr. **27**:1456–1469.
3. **Franke, A. E. and D. B. Clewell.** 1981. Evidence for a chromosome-borne resistance transposon (Tn*916*) in *Streptococcus faecalis* that is capable of "conjugal" transfer in the absence of a conjugative plasmid. J. Bacteriol. **145**:494–502.
4. **Guerry, P., D. J. LeBlanc, and S. Falkow.** 1973. General method for the isolation of plasmid deoxyribonucleic acid. J. Bacteriol. **116**:1064–1066.
5. **Hansen, J. B., and R. H. Olsen.** 1978. Isolation of large bacterial plasmids and characterization of the p2 incompatibility group plasmids pMG1 and pMG5. J. Bacteriol. **135**:227–238.
6. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual. Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg, Va.
7. **Macrina, F. L., D. J. Kopecko, K. R. Jones, D. J. Ayers, and S. M. McCowen.** 1978. A multiple plasmid-containing *Escherichia coli* strain: convenient source of size reference plasmid molecules. Plasmid **1**:417–420.
8. **Macrina, F. L., G. G. Weatherly, and R. Curtiss III.** 1974. R6K plasmid replication: influence of chromosomal genotype in minicell-producing strains of *Escherichia coli* K-12. J. Bacteriol. **120**:1387–1400.
9. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **3**:208–218.
10. **Mays, T. D., C. J. Smith, R. A. Welch, C. Delfini, and F. L. Macrina.** 1982. Novel antibiotic resistance transfer in *Bacteroides*. Antimicrob. Agents Chemother. **21**:110–118.
11. **Meyers, J. A., D. Sanchez, L. P. Elwell, and S. Falkow.** 1976. Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. J. Bacteriol. **127**:1529–1537.
12. **Privitera, G., A. Dublanchet, and M. Sebald.** 1979. Transfer of multiple antibiotic resistance between subspecies of *Bacteroides fragilis*. J. Infect. Dis. **139**:83–87.

13. **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.
14. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
15. **Shoemaker, N. B., M. D. Smith, and W. R. Guild.** 1980. DNase-resistant transfer of chromosomal Cat and Tet insertions by filter mating in pneumococcus. Plasmid **3**:80–87.
16. **Smith, C. J., S. M. Markowitz, and F. L. Macrina.** 1981. Transferable tetracycline Resistance in *Clostridium difficile*. Antimicrob. Agents Chemother. **19**:997–1003.
17. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
18. **Tally, F. P., M. J. Schimell, G. R. Carson, and M. H. Malamy.** 1981. Chromosomal and plasmid mediated transfer of clindamycin resistance in *Bacteroides fragilis*, p. 51–59. *In* S. Levy, R. C. Clowes, and E. Koenig (ed.), Molecular biology, pathogenicity and ecology of bacterial plasmids. Plenum Publishing Corp., New York.
19. **Tally, F. P., D. R. Snydman, S. L. Gorbach, and M. H. Malamy.** 1979. Plasmid-mediated, transferable resistance to clindamycin and erythromycin in *Bacteroides fragilis*. J. Infect. Dis. **139**:83–87.
20. **Thayer, R. E.** 1979. An improved method for detecting foreign DNA in plasmids of *Escherichia coli*. Anal. Biochem. **98**:60–63.
21. **Tinnell, W., and F. L. Macrina.** 1976. Extrachromosomal elements in a variety of strains representing the *Bacteroides fragilis* group of organisms. Infect. Immun. **14**:955–964.
22. **Varel, V. H., and M. P. Bryant.** 1974. Nutritional features of *Bacteroides fragilis* subsp. *fragilis*. Appl. Microbiol. **18**:251–257.
23. **Welch, R. A., K. R. Jones, and F. L. Macrina.** 1979. Transferable lincosamide-macrolide resistance in *Bacteroides*. Plasmid **2**:261–268.
24. **Welch, R. A., and F. L. Macrina.** 1980. Deletion analysis of a transferable *Bacteroides* R plasmid, p. 137–144. *In* C. Stuttard and K. Rozee (ed.), Plasmids and transposons. Academic Press, Inc., New York.
25. **Welch, R. A., and F. L. Macrina.** 1981. Physical characterization of the *Bacteroides* R plasmid pBF4. J. Bacteriol. **145**:867–872.