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Mobilization of *Bacteroides* Plasmids by *Bacteroides* Conjugal Elements

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A 4.2-kilobase cryptic *Bacteroides* plasmid, pB8-51, is found in several colonic *Bacteroides* species. To determine whether pB8-51 is mobilized by any of the known *Bacteroides* conjugal elements, we constructed an *Escherichia coli-Bacteroides* shuttle vector, pVAL-1, which contains pB8-51. We constructed *Bacteroides uniformis* 0061 derivatives which carry pVAL-1 and various *Bacteroides* conjugal elements. The *Bacteroides* conjugal elements tested were six conjugal tetracycline resistance (Tc^r) elements (which appear to be chromosomal), i.e., Tc^r ERL, Tc^r V479, Tc^r Em^r ERL, Tc^r Em^r 12256, Tc^r Em^r DOT, and Tc^r Em^r CEST, and the conjugal erythromycin resistance (Em^r) plasmid pBF4. These Tc^r conjugal elements have not been extensively characterized, except for Tc^r ERL. All six Tc^r elements tested mobilized pVAL-1 at high frequency (10^{-3} to 10^{-5}) from one *Bacteroides* strain to another or from a *Bacteroides* strain to *E. coli*. Pregrowth of the donors (containing one of the Tc^r elements and pVAL-1) in 1 μg of tetracycline per ml enhanced the transfer of pVAL-1 by 20- to 10,000-fold, depending on which Tc^r element was present in the donor. An Em^s derivative of pBF4 (pBF4ΔE2) mobilized pVAL-1 from one *Bacteroides* strain to another at a frequency of 10^{-4} but did not mobilize pVAL-1 from a *Bacteroides* strain to *E. coli* as efficiently. Thus the Tc^r conjugal elements and pBF4 recognize a mobilization region on pB8-51.

Colonic *Bacteroides* species contain many small cryptic plasmids in the 3- to 6-kilobase (kb) size range (9; T. D. Mays, Ph.D. thesis, Virginia Polytechnic Institute, Blacksburg, 1978). These plasmids have been assigned to three classes on the basis of Southern hybridization: class 1 (2.7 kb), class 2 (4 to 6 kb), and class 3 (5.6 kb) (1). The first two classes are the most prevalent. pB8-51, a 4.2-kb (class 2) cryptic *Bacteroides* plasmid, has been found in several colonic *Bacteroides* species (Mays, Ph.D. thesis; N. B. Shoemaker, unpublished results). One possible explanation for the ubiquity of pB8-51 is that some *Bacteroides* conjugal element(s) can mobilize it.

There are two known types of conjugal elements in *Bacteroides*: (i) large self-mobilizing plasmids, such as pBF4 (17, 18) and pBI136 (16), which carry a gene that codes for resistance to clindamycin and erythromycin (Em^r), and (ii) conjugal tetracycline resistance (Tc^r) elements which may be chromosomal (7). All of the Tc^r conjugal elements were independently isolated from clinical *Bacteroides* species from different regions of the world, except for Tc^r ERL and Tc^r Em^r ERL (Table 1). The conjugal Tc^r elements can be divided into two types: elements which transfer only Tc^r and elements which cotransfer Tc^r and Em^r (Tc^r Em^r elements). The conjugal elements also differ with respect to the effect on transfer frequency of pregrowth in tetracycline. Privitera et al. (12), Smith et al. (17), and Shoemaker et al. (14), have reported that elements which transfer Tc^r alone transfer it at a higher frequency when pregrown in tetracycline. This appears not to be the case with at least one of the Tc^r Em^r elements (10), but there has been no systematic study of Tc enhancement of transfer of other Tc^r Em^r elements. Since none of the Tc^r or Tc^r Em^r elements have been cloned, it is not known whether the different conjugal elements are related.

Previously, Shoemaker et al. (14) showed that a Tc^r element originally found in *B. fragilis* ERL (Tc^r ERL) recognizes a mobilization region on pBFTM10, an Em^r *Bacteroides* plasmid that is not self-mobilizing. The Tc^r ERL element mobilizes pDP1, a chimeric plasmid which contains pBFTM10, from a *Bacteroides* species to *Escherichia coli* and from one *Bacteroides* strain to another at frequencies of 10^{-5} to 10^{-6}. Shoemaker et al. (14) also found that pEG920, a chimeric plasmid which contains pB8-51, appears to be mobilized by the Tc^r ERL element, but at a frequency close to the level of detection (10^{-8}).

However, the restriction site in pB8-51 (*SstI*) which was used to construct pEG920 could have interrupted the pB8-51 mobilization region, as evidenced by Shoemaker et al. (14). R751, a conjugal IncPβ plasmid, mobilizes chimeric plasmids containing pB8-51 from *E. coli* to *E. coli* at frequencies of 10^{-6} (pEG920) and 10^{-3} (pEG-3 and pCG21) depending on the restriction site used on pB8-51. If the same mobilization region on pB8-51 is recognized by both the Tc^r ERL element and R751, mobilization of pEG920 by Tc^r ERL would be lower. In the case of pEG-3 and pCG21, it was not possible to check whether they could be mobilized by the Tc^r ERL element. The restriction sites used on pB8-51 for these particular constructs interrupt regions which are necessary for *Bacteroides* replication.

In this study, we have constructed a chimeric plasmid containing pB8-51, in which the mobilization region that is recognized by R751 is fully functional. This plasmid was used to determine if any of the *Bacteroides* Tc^r and Tc^r Em^r conjugal elements or the conjugal plasmid pBF4 could mobilize pB8-51 at a high frequency.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains, plasmids, and conjugal elements used in this study are listed in Table 1.

Construction of pVAL-1. The construction of pVAL-1 is shown in Fig. 1. pB8-51 contains at least eight *TaqI* restriction sites. Partial *TaqI* digests of pB8-51 were mixed with *ClaI* digests of pTB1 and ligated with T4 DNA ligase (5). *E.*

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TABLE 1. Strains and plasmids used in this study

Bacterial strain or plasmid	Relevant phenotype[a]	Source or reference
E. coli strains		
HB101	RecA Str^r Gen^s	H. Boyer, 8
EM24NR	RecA Str^r Gen^s Nal^r Rif^r	J. Cronan, 14
Bacteroides clinical isolates		
B. fragilis V479	Tc^r Em^r (pBF4)	17
B. fragilis CEST	Tc^r Em^r	T. England, Mercy Hospital, Urbana, Ill.
B. fragilis ERL	Tc^r Em^r	T. England
B. thetaiotaomicron DOT-1	Tc^r Em^r	T. England
B. fragilis 12256	Tc^r Em^r	VPI Anaerobe Laboratory, Blacksburg, Va. (also designated V503)
B. uniformis strains		
0061[b]	Tc^s Em^s Gen^r	14
BU1001	Tc^s Em^s Gen^r Rif^r	14
BU1100[c]	Tc^s Em^s Tpm^r Thy^- Rif^s Gen^r	14
Plasmids		
pBR328	Tc^r Ap^r Cm^r Mob^-	14
pTB1	Tc^r Ap^r Cm^s Em^r Mob^-	*Eco*RI fragment of Tn*4351* containing the *Bacteroides* Em^r-Cc^r gene cloned into the *Eco*RI site of pBR328 (E. Guthrie)
R751	IncPβ Tp^r Tra^+	14
pB8-51	4.2-kb cryptic element	14
pVAL-1	Tc^r Ap^r Cm^s Em^r Mob^+ Rep^+	This study
pDP1	$*Tc^r$ Em^r Mob^+ Rep^+	D. Guiney, 14
pE5-2	$*Tc^r$ Em^r	15
pBF4ΔE2	Em^s Tra^+	Spontaneous Em^s derivative of pBF4 in which all of Tn*4351* was lost except one insertion sequence element (Shoemaker, unpublished)

[a] Abbreviations used for antibiotic resistances (r) or sensitivities (s): Ap, ampicillin; Cm, chloramphenicol; Em, erythromycin; Gen, gentamicin sulfate; Nal, nalidixic acid; Rif, rifampin, Str, streptomycin; Tc or *Tc, tetracycline; Tpm or Tp, trimethoprim. Concentrations used to select for antibiotic resistances are given in the appropriate table or in Materials and Methods. Other abbreviations used: Mob, ability to be mobilized by a conjugative element; Rep, ability to replicate in *Bacteroides* ssp.; Tra, ability to self-transfer; Inc, plasmid incompatibility group; Rec, *E. coli* recombination mutation. Em^r is only expressed in *Bacteroides* strains, and the conjugal *Bacteroides* Tc^r gene is not known to be expressed in *E. coli*. The pBR328 Tc^r and the Tc^r ($*Tc^r$) genes on Tn*4351* are only expressed in *E. coli*. The $*Tc^r$ gene is only expressed in aerobically grown *E. coli*, unlike the pBR328 Tc^r gene.

[b] Except for the spontaneous chromosomal resistance mutation, BU1100 (Thy^- Tpm^r) and BU1001 (Rif^r) are otherwise isogenic derivatives of *B. uniformis* 0061.

[c] The trimethoprim resistance of *B. uniformis* BU1100 is due to mutation to the thymidine requirement (Thy^- Tpm^r).

coli HB101, containing R751, was transformed with each ligation mixture as described by Lederberg and Cohen (6), and Tc^r transformants were selected. Groups of approximately 30 to 50 transformants from each *Taq*I digest were pooled in 0.2-ml Luria broth (LB)-tetracycline (10 μg/ml) cultures. LB-tetracycline medium (5 ml) was inoculated with each group of transformants. These cultures were incubated at 37°C until the optical density (at 600 nm) was 0.1 to 0.2 and were used in matings as donors, with *B. uniformis* BU1001 as recipient (see below).

Plasmids were isolated from *E. coli* and *Bacteroides* strains by the Ish-Horowitz modification of the method of Birnboim and Doly (8).

Growth conditions and mating experiments. *Bacteroides* strains were grown in prereduced Trypticase (BBL Microbiology Systems, Cockeysville, Md.)-yeast extract-glucose (TYG) broth or on TYG agar plates under an atmosphere of CO_2 and N_2, as described previously (15). *E. coli* strains were grown in LB or on LB or TYG agar plates.

Matings were done by the filter mating procedure as described previously (15). Matings in which both the donor and the recipient were *E. coli* strains were done aerobically at 37°C for 18 to 24 h, with a donor-to-recipient ratio of 2:1. In addition to the plasmid being tested for transfer, the *E. coli* donor carried R751, a broad-host-range self-mobilizing plasmid (2, 11). Transfer frequencies of plasmids from *E. coli* HB101 to *E. coli* EM24NR were determined by plating dilutions of the mating mixture on LB agar plates containing both an antibiotic that selected for a plasmid marker, usually tetracycline (10 μg/ml), and nalidixic acid (50 μg/ml) to select for the recipient, *E. coli* EM24NR. Transfer frequency is defined as the number of transconjugants divided by the total number of recipients in the mating mixture at the end of the mating period.

When *E. coli* HB101 was the donor and *B. uniformis* BU1001 was the recipient, the mating filters were incubated aerobically (13). R751 was the mobilizing plasmid. The donor-to-recipient ratio was 1:1. The mating mixtures were plated on TYG agar which contained gentamicin sulfate (200 μg/ml), to select against *E. coli*, and erythromycin (10 μg/ml), to select *Bacteroides* transconjugants which contained pVAL-1. Plates were incubated anaerobically. Gentamicin sulfate is effective against *E. coli* strains, including those that are resistant to other aminoglycosides, such as streptomycin, but does not inhibit the growth of *Bacteroides* spp.

When the various *B. uniformis* strains containing one of the conjugal elements, Tc^r or Tc^r Em^r, or pBF4ΔE2 and pVAL-1 or pDP1 were used as donors and *E. coli* HB101 was the recipient, the mating filters were incubated anaerobically (GasPak jar; BBL) at 37°C for 18 to 24 h as described previously (15). The donor-to-recipient ratio was 2:1. In

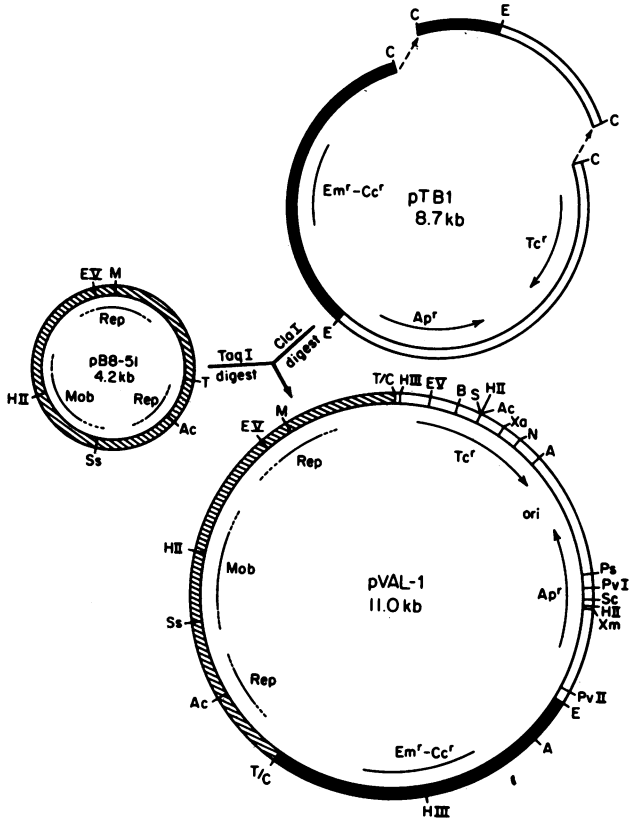

FIG. 1. Construction of pVAL-1 containing pB8-51. Symbols: ▬, DNA from pBF4 containing the Em^r gene expressed in *Bacteroides* strains; ▨, pB8-51 DNA; ▭, pBR328 DNA. Restriction sites: C, *Cla*I; E, *Eco*RI; M, *Mbo*I; T, *Taq*I; Ac, *Acc*I; Ss, *Sst*I; HII, *Hin*cII; HIII, *Hin*dIII; EV, *Eco*RV; B, *Bam*HI; S, *Sal*I; Xa, *Xma*III; N, *Nru*I; A, *Ava*I; Ps, *Pst*I; PvI, *Pvu*I; Sc, *Sca*I; Xm, *Xmn*I; PvII, *Pvu*II. ori, Origin; other abbreviations are defined in Table 1.

most experiments, the various *B. uniformis* donors carrying pVAL-1 and the Tc^r or Tc^r Em^r elements were grown in medium containing tetracycline (1 μg/ml) and erythromycin (10 μg/ml) before mating. In experiments to test the effect of tetracycline on the transfer frequency of pVAL-1, the donors were also grown in medium containing erythromycin but no tetracycline. The donor which contained pBF4ΔE2 and pVAL-1 was grown in erythromycin (10 μg/ml).

E. coli transconjugants were detected by plating dilutions of the mating mixture on LB plates containing tetracycline (10 μg/ml) to select *E. coli* transconjugants and incubating the plates aerobically to select against *Bacteroides* spp. All transconjugants were streaked for purity at least twice, because *Bacteroides* strains will grow in coculture with aerobically grown *E. coli*.

For matings between *B. uniformis* strains (donor strain containing one of the conjugal elements and pVAL-1 or pDP1), the mating filters were incubated anaerobically in GasPak jars at 37°C for 18 to 24 h. The donor-to-recipient ratio was 2:1. The mating mixtures were plated on TYG agar containing trimethoprim (100 μg/ml) and thymidine (100 μg/ml), to select against *B. uniformis* BU1001 donors, or rifampin (20 μg/ml), to select against *B. uniformis* BU1100 donors. Erythromycin (10 μg/ml) was used to select for transfer of pVAL-1. Tetracycline (3 μg/ml) was used to select for transfer of Tc^r or Tc^r Em^r conjugal elements. BU1100 transconjugants were streaked on TYG plates containing thymidine (100 μg/ml) and rifampin (20 μg/ml) to confirm that they were BU1100 (Rif^s) transconjugants and not spontaneous (Thy$^-$) Tpm^r mutations of the various donor BU1001 derivatives (Rif^r). *B. uniformis* BU1001 transconjugants were streaked on TYG plates containing thymidine (100 μg/ml) and trimethoprim (100 μg/ml) to confirm they were BU1001 Tpm^s transconjugants and not spontaneous Rif^r mutations of the various donor BU1100 derivatives (Thy$^-$ Tpm^r).

Colony hybridization was used to confirm that transconjugants contained pVAL-1. The procedure was done as described by Grunstein and Hogness (3). ^{32}P-labeled pBR328 was used as the probe.

Construction of *Bacteroides* donor strains. Derivatives of *B. uniformis* BU1001 or BU1100 containing a conjugal Tc^r or Tc^r Em^r element and pVAL-1 or pDP1 were constructed by mobilizing the conjugal Tc^r or Tc^r Em^r elements into the *B. uniformis* strain which contained pVAL-1 or pDP1 and selecting for Tc^r transconjugants. The transconjugants were screened for plasmid DNA to confirm that they carried pVAL-1 or pDP1. The *B. uniformis* strain containing the conjugal plasmid pBF4ΔE2 and pVAL-1 was constructed by mobilizing pVAL-1 into BU1001(pBF4ΔE2) (*E. coli* to *B. uniformis*) and selecting for Em^r transconjugants. The transconjugants were screened for plasmid DNA to confirm that the *B. uniformis* strain contained both plasmids.

Stability. *E. coli* HB101 containing pVAL-1 was grown overnight in LB-tetracycline (10 μg/ml) medium. The culture was then diluted 1:1000 and grown to stationary phase under no selection at 37°C. This step was repeated. After the cultures reached stationary phase, the cultures were diluted and plated on LB agar. Colonies were picked onto LB agar with or without tetracycline. *B. uniformis* BU1001 containing pVAL-1 was grown overnight in chop-meat medium containing erythromycin (10 μg/ml). The culture (0.1 ml) was then transferred to 10 ml of TYG broth and grown overnight under no selection. This step was repeated twice. Dilutions of each overnight TYG broth culture were plated on TYG agar. Colonies were picked onto TYG agar with or without erythromycin (10 μg/ml). Em^s colonies were screened for plasmid DNA.

RESULTS

Construction of pVAL-1. The construction of pVAL-1 is shown in Fig. 1. It was necessary to use a partial *Taq*I digest of pB8-51 to construct this plasmid because the four known unique restriction sites (*Mbo*I, *Acc*I, *Hin*cII, and *Sst*I) interrupt either the replication region on pB8-51 or the mobilization region that is recognized by R751 (14). pTB1 contains a marker (Em^r) which is expressed in *Bacteroides* species, but it is not mobilized by R751 and does not contain a *Bacteroides* replication origin. A chimeric plasmid containing pTB1 and the intact replication and mobilization regions of pB8-51 should be mobilizable by R751 and be maintained in *Bacteroides* species. To select for the desired chimeric plasmid, we mated pools of *E. coli* transformants with *B. uniformis* BU1001 and selected for Em^r transconjugants. The resultant Em^r *B. uniformis* BU1001 transconjugants were screened for plasmid DNA. The majority of plasmids in the transconjugants (18 of 22 which were characterized) were identical in size and restriction enzyme pattern to pVAL-1 (data not shown). The other four plasmids were either larger than pVAL-1 or unstable.

TABLE 2. Transfer of pVAL-1 between *B. uniformis* strains

B. uniformis BU1001 donor content	Em^r transfer frequency[a]	No. of transconjugants with phenotype:		Estimated pVAL-1 transfer frequency
		Em^r Tc^s	Em^r Tc^r	
pVAL-1	1×10^{-7}	99	0	1×10^{-7}
Tc^r ERL, pVAL-1	6×10^{-4}	174	0	6×10^{-4}
Tc^r V479, pVAL-1	1×10^{-3}	235	1 (100)[b]	1×10^{-3}
Tc^r Em^r ERL, pVAL-1	8×10^{-3}	25	507 (0)	3×10^{-4}
Tc^r Em^r 12256, pVAL-1	9×10^{-3}	213	64 (59)	8×10^{-3}
Tc^r Em^r DOT, pVAL-1	5×10^{-3}	522	87 (11)	5×10^{-3}
Tc^r Em^r CEST, pVAL-1	1×10^{-3}	191	68 (9)	7×10^{-4}
pBF4ΔE2, pVAL-1	1×10^{-4}	100	0	1×10^{-4}

[a] These results are averages of at least three experiments. Donors were pregrown in tetracycline (1 μg/ml) and erythromycin (10 μg/ml), except for BU1001 (pVAL-1).

[b] The numbers in parentheses are the percentages of Em^r Tc^r transconjugants tested which contain pVAL-1, as shown by colony hybridization with pBR328 as probe.

Stability of pVAL-1 in *E. coli* and in *B. uniformis*. pVAL-1 was very stable in *E. coli*. When *E. coli* containing pVAL-1 was grown without drug selection, there was less than 0.3% loss of Tc^r after approximately 10 and 20 generations. pVAL-1 was not as stable in *B. uniformis* BU1001. After approximately 10 generations of growth without Em^r selection, only 60% of the *Bacteroides* colonies were Em^r. At the end of three consecutive transfers (~20 generations) only 2 to 4% of the *Bacteroides* colonies were Em^r. Six Em^s *B. uniformis* colonies were screened for plasmid DNA. None contained plasmid DNA. Thus the loss of Em^r was probably due to loss of the plasmid and not simply to loss of the region that carries the Em^r gene.

Mobilization of pVAL-1 by R751. pVAL-1 was mobilized by R751 from *E. coli* to *E. coli* at a frequency of 10^{-3}. The mobilization frequency of pVAL-1 is thus comparable to that of the constructs of Shoemaker et al. (pEG-3 and pCG21; 14) in which the mobilization region of pB8-51 is fully functional. Unlike those constructs, pVAL-1 can replicate in *Bacteroides* strains. R751 mobilized pVAL-1 from *E. coli* to *Bacteroides* strains at a frequency of 10^{-4}.

Mobilization of pVAL-1 by *Bacteroides* Tc^r and Tc^r Em^r conjugal elements. The *Bacteroides* conjugal elements, Tc^r ERL and Tc^r V479, mobilized pVAL-1 between *B. uniformis* strains at frequencies of 10^{-4} and 10^{-3}, respectively. None of the Em^r transconjugants from a mating involving Tc^r ERL were Tc^r. Of 236 Em^r transconjugants involving Tc^r V479, 1 transconjugant was Tc^r (Table 2, column 3). Restriction enzyme analysis of plasmid DNA from three transconjugants showed that the plasmid carried by the transconjugants was identical to pVAL-1.

All the Tc^r Em^r elements transferred Em^r between *Bacteroides* strains at high frequencies (Table 2, column 2). Since all the Tc^r Em^r elements used in this study always cotransferred Tc^r and Em^r, transconjugants that were Em^r Tc^s should contain pVAL-1. Em^r Tc^s transconjugants were seen in all cases (Table 2, column 3). We confirmed that these transconjugants contained pVAL-1 by doing restriction digests of plasmids isolated from three transconjugants per mating and by colony hybridization of at least 20 colonies with pBR328 as a probe. pBR328 does not hybridize to total DNA of *B. uniformis* BU1001 under the same conditions.

Em^r Tc^r transconjugants were detected in all matings involving the Tc^r Em^r elements (Table 2, column 3). These transconjugants could have arisen either from self-transfer of the Tc^r Em^r elements or from cotransfer of the conjugal element and pVAL-1. Cotransfer of pVAL-1 with the conjugal element could only be detected by direct screening of plasmid DNA or by colony hybridization. We determined the proportion of the Em^r Tc^r transconjugants tested which contained pVAL-1 by probing at least 40 transconjugants with pBR328. The total number of Em^r Tc^r transconjugants tested (from at least three matings) which contained pVAL-1 varied: 0 of 209 (Tc^r Em^r ERL), 29 of 49 (Tc^r Em^r 12256), 6 of 55 (Tc^r Em^r DOT), and 4 of 43 (Tc^r Em^r CEST). This indicated that some of the Tc^r Em^r elements, unlike Tc^r ERL, cotransfer pVAL-1 at a high frequency. Restriction digests of plasmid DNA from five Em^r Tc^r transconjugants from each mating confirmed the presence of pVAL-1.

The mobilization frequency of pVAL-1 (Table 2, column 5) was calculated by multiplying the frequency of Em^r transfer (Table 2, column 2) by the percentage of Em^r transconjugants which contained pVAL-1. All the Tc^r Em^r elements mobilized pVAL-1 at frequencies of 10^{-5} to 10^{-2}.

The conjugal Tc^r and Tc^r Em^r elements not only mobilized pVAL-1 between *B. uniformis* strains but also mobilized pVAL-1 at high frequency from *B. uniformis* to *E. coli* HB101 (Table 3). The frequency of pVAL-1 mobilization from *B. uniformis* to *E. coli* was consistently lower than the frequency of mobilization between *B. uniformis* strains in all cases, except when Tc^r Em^r CEST was the conjugal element in question.

Enhancement of pVAL-1 mobilization frequency by tetracycline. For all the matings described in the preceding section, the *B. uniformis* donor was pregrown in medium containing tetracycline. This was done because Shoemaker et al. (14) had previously shown that Tc^r ERL-mediated mobilization of coresident plasmids (from *Bacteroides* strain to *Bacteroides* strain or *E. coli*) was enhanced when the donor was pregrown in a subinhibitory concentration of tetracycline (1 μg/ml). However, the effect of pregrowth in

TABLE 3. Transfer of pVAL-1 from *B. uniformis* to *E. coli*[a]

B. uniformis BU1001 donor content	Frequency of pVAL-1 transfer to *E. coli*[b]
pVAL-1	3×10^{-8}
pE5-2	$<2 \times 10^{-9}$
Tc^r ERL, pVAL-1	2×10^{-5}
Tc^r V479, pVAL-1	7×10^{-4}
Tc^r Em^r ERL, pVAL-1	3×10^{-5}
Tc^r Em^r 12256, pVAL-1	8×10^{-4}
Tc^r Em^r DOT, pVAL-1	5×10^{-3}
Tc^r Em^r CEST, pVAL-1	3×10^{-3}
pBF4ΔE2, pVAL-1	2×10^{-7}

[a] Donors were grown in tetracycline (1 μg/ml) and erythromycin (10 μg/ml), except for BU1001(pVAL-1) and BU1001(pE5-2).

[b] These results are averages of at least three experiments.

the presence of tetracycline on mobilization frequencies associated with most of the other Tc^r Em^r elements had not been determined. To test the effect of pregrowth on tetracycline, we compared the frequency of pVAL-1 mobilization from *Bacteroides* strains to *E. coli* when the donors were pregrown with or without tetracycline. The enhancement of pVAL-1 mobilization was 200- to 500-fold (Tc^r ERL), 25- to 1,000-fold (Tc^r V479), 60- to 600-fold (Tc^r Em^r ERL), 20- to 25-fold (Tc^r Em^r 12256), and 600- to 1,000-fold (Tc^r Em^r DOT) when the donors were pregrown in tetracycline. Tc^r Em^r CEST had the highest level of enhancement (100- to 10,000-fold), and in this case, the range of enhancement values with different experiments was unusually large. In contrast, self-transfer of the Tc^r Em^r 12256 element between *Bacteroides* species was not enhanced by tetracycline. This agrees with an earlier work of Macrina et al. (10). However, we observed enhanced mobilization of the coresident plasmid pVAL-1 from *B. uniformis* to *E. coli* when the BU1001 (Tc^r Em^r 12256, pVAL-1) donor was pregrown in tetracycline.

Mobilization of pVAL-1 independent of known conjugal elements. An unexpected finding was that pVAL-1 can be mobilized out of *B. uniformis* BU1001 to *E. coli* or to another *B. uniformis* strain (Tables 2 and 3). *B. uniformis* BU1001 does not contain any known conjugal element. Mobilization of pVAL-1 out of *B. uniformis* BU1001 was not always detected (~30%) since the level of mobilization was close to the limit of detection. The plasmids in the resultant transconjugants were identical in size and restriction enzyme pattern to pVAL-1. The possibility of natural transformation occurring during the incubation period on filters was ruled out, since transfer was not detected in experiments with pE5-2 (Table 3) and pSAL-12 (14; *E. coli* to *E. coli* mating), which together contain all the DNA sequences present on pVAL-1. Hence, the transfer of pVAL-1 is most likely due to mobilization.

Mobilization of pVAL-1 by the *Bacteroides* conjugal plasmid, pBF4. To determine whether pBF4 could mobilize pVAL-1, we used a spontaneous Em^s derivative of pBF4 (pBF4ΔE2) so that we could follow the transfer of Em^r on pVAL-1. pBF4ΔE2 mobilized pVAL-1 between *B. uniformis* strains at a frequency of 10^{-4} (Table 2, column 5). pBF4ΔE2 contains one copy of the insertion sequence element which flanks Tn*4351*. Hwa et al. (4) have shown that both insertion sequence elements of Tn*4351* can mediate cointegrate formation. Since pBF4 can transfer itself between *B. uniformis* strains at a frequency of 10^{-3} (Shoemaker, unpublished; this study, data not shown), it was necessary to determine whether Em^r transfer mediated by pBF4ΔE2 was due to mobilization, to homologous recombination, or to insertion sequence-mediated cointegrate formation. The size and *Hin*cII digest pattern of plasmids from five Em^r *B. uniformis* BU1100 transconjugants were compared with those of pVAL-1. We found that pBF4ΔE2 did not cotransfer with pVAL-1 (<20%), since none of the plasmids screened contained any extra DNA, nor were any other plasmid species present besides pVAL-1.

The frequency at which pBF4ΔE2 mobilized pVAL-1 from *B. uniformis* to *E. coli* was approximately 1,000-fold lower than to *B. uniformis* (Table 3), although the frequency was consistently 5- to 10-fold higher than the mobilization frequency of pVAL-1 by the cryptic element.

Mobilization of pDP1 by the Tc^r and Tc^r Em^r conjugal elements. Shoemaker et al. (14) had shown previously that the Tc^r ERL element can mobilize pDP1. pDP1 is a chimeric plasmid that contains a *Bacteroides* plasmid (pBFTM10) which is not related to pB8-51. We found that all the Tc^r Em^r conjugal elements which mobilized pVAL-1 could also mobilize pDP1 (Table 4). The cryptic element also mobilized pDP1 (Table 4).

TABLE 4. Frequency of conjugal transfer of pDP1 from various *B. uniformis* strains to *B. uniformis* BU1001 or to *E. coli*[a]

B. uniformis BU1100 donor content	Frequency of transfer to:	
	B. uniformis BU1001	*E. coli*
pDP1	8×10^{-9}	$<5 \times 10^{-9}$
Tc^r ERL, pDP1	2×10^{-5}	2×10^{-5}
Tc^r Em^r ERL, pDP1	7×10^{-5}	7×10^{-6}
Tc^r Em^r 12256, pDP1	1×10^{-4}	2×10^{-5}
Tc^r Em^r DOT, pDP1	2×10^{-5}	3×10^{-5}
Tc^r Em^r CEST, pDP1	1×10^{-4}	2×10^{-4}

[a] Frequency of pDP1 transfer was deduced by the frequency of Em^r transfer. Thus, the frequency of pDP1 mobilization by the Tc^r Em^r elements shown above is probably lower.

DISCUSSION

Conjugal transfer of the chimeric shuttle vector pDP1 from one *Bacteroides* strain to another or from a *Bacteroides* strain to *E. coli* has been demonstrated previously in the case of one *Bacteroides* conjugal element, Tc^r ERL (14). A construct containing pB8-51 was also tested by Shoemaker et al., but the frequency of mobilization was so low that it was not clear whether pB8-51 contained a mobilization region that is recognized by the *Bacteroides* Tc^r ERL element. We have shown that if a different site on pB8-51 is used to construct the chimeric plasmid, the Tc^r ERL element can mobilize the chimeric plasmid (pVAL-1) out of a *Bacteroides* strain at a frequency of 10^{-5} to 10^{-4}. Thus pB8-51 carries a mobilization region that is recognized by the Tc^r ERL element and permits transfer of pB8-51 at frequencies comparable with those seen with pDP1.

R751 mobilized pVAL-1 from *E. coli* to *E. coli* at a frequency that was 1,000-fold higher than that seen with pEG920. This difference is the same as that obtained when the Tc^r ERL element was the mobilizing element. Moreover, neither R751 nor the Tc^r ERL element mobilized constructs in which the *Hin*cII site of pB8-51 was used for cloning (14). These results indicate that R751 and the Tc^r ERL element recognize either the same or overlapping mobilization regions on pB8-51.

The Tc^r ERL element is not the only *Bacteroides* conjugal element that can mobilize pB8-51. All the Tc^r or Tc^r Em^r elements tested in this study mobilized pB8-51 (in pVAL-1) from one *Bacteroides* strain to another and from a *Bacteroides* strain to *E. coli* at frequencies comparable with those obtained with the Tc^r ERL element. Thus all of these elements could recognize the mobilization region on pB8-51 and could donate to *E. coli* as efficiently as to *Bacteroides* strains. pB8-51 was also mobilized by pBF4ΔE2, an Em^s derivative of the self-transmissible *Bacteroides* plasmid, pBF4. This is the first evidence for the ability of pBF4 to mobilize coresident plasmids. pBF4, unlike the Tc^r or Tc^r Em^r elements, did not donate to *E. coli* as efficiently as it did to *Bacteroides* strains.

In all cases, mobilization of pVAL-1 appeared to be due to mobilization of pB8-51 rather than to insertion sequence- or to recombination-mediated transfer. Either insertion sequence- or recombination-mediated transfer would have been characterized by 100% cotransfer of pVAL-1 and the

conjugal element. Although the conjugal elements might have been lost in matings between a *Bacteroides* strain and *E. coli*, it was possible to follow them in matings between *Bacteroides* strains. Most of the Em^r transconjugants in matings between *Bacteroides* strains contained pVAL-1 but not the mobilizing element.

The finding that all the conjugal elements tested in this study mobilized pB8-51 may explain why this plasmid is found in so many *Bacteroides* strains. An unrelated small cryptic plasmid (p12256) cotransfers with the Tc^r Em^r 12256 element (10; Shoemaker, unpublished). The fact that many naturally occurring *Bacteroides* plasmids contain mobilization regions that are recognized by *Bacteroides* conjugal elements may have clinical significance, since a cryptic plasmid could become an R plasmid by acquiring an Em^r transposon, such as Tn*4351* or the closely related Tn*4400*.

The broad host range of pVAL-1 and the ability of this plasmid to be mobilized by a variety of elements into and out of *Bacteroides* strains make this plasmid a good shuttle vector. Moreover, unlike the previously described *E. coli-Bacteroides* shuttle vectors pE5-2 and pDP1, pVAL-1 is a much better cloning vector because it contains many unique sites in the interruptible Tc^r and Ap^r genes of pBR328.

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LITERATURE CITED

1. **Callihan, D. R., F. E. Young, and V. L. Clark.** 1983. Identification of three homology classes of small cryptic plasmids in intestinal *Bacteroides* species. Plasmid **9**:17–30.
2. **Chikami, G. K., D. G. Guiney, T. J. Schmidhauser, and D. R. Helinski.** 1985. Comparison of 10 IncP plasmids: homology in the regions involved in plasmid replication. J. Bacteriol. **162**: 656–660.
3. **Grunstein, M., and D. S. Hogness.** 1975. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. USA **72**:3961–3965.
4. **Hwa, V., N. B. Shoemaker, and A. A. Salyers.** 1988. Direct repeats flanking the *Bacteroides* transposon Tn*4351* are insertion sequence elements. J. Bacteriol. **170**:449–451.
5. **King, P. V., and R. W. Blakesley.** 1986. Optimizing DNA ligations for transformation. Focus **8**:1–3.
6. **Lederberg, E. M., and S. M. Cohen.** 1974. Transformation of *Salmonella typhimurium* by plasmid deoxyribonucleic acid. J. Bacteriol. **119**:1072–1074.
7. **Malamy, M. H., and F. P. Tally.** 1981. Mechanisms of drug-resistance transfer in *Bacteroides fragilis*. J. Antimicrob. Chemother. **8**:(Suppl. D):59–75.
8. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Mays, T. D., and J. L. Johnson.** 1979. Plasmid DNA homology studies of *Bacteroides fragilis* and related species. Plasmid **2**:299.
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. **Meyer, R. J., and J. A. Shapiro.** 1980. Genetic organization of the broad-host-range IncP-1 plasmid R751. J. Bacteriol. **143**: 1362–1373.
12. **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.
13. **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.
14. **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.
15. **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.
16. **Smith, C. J., and F. L. Macrina.** 1984. Large transmissible clindamycin resistance plasmid in *Bacteroides ovatus*. J. Bacteriol. **158**:739–741.
17. **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.
18. **Welch, R. A., and F. L. Macrina.** 1981. Physical characterization of *Bacteroides fragilis* R plasmid pBF4. J. Bacteriol. **145**:867–872.