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Construction and Characterization of a *Bacteroides thetaiotaomicron recA* Mutant: Transfer of *Bacteroides* Integrated Conjugative Elements Is RecA Independent

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We report the construction and analysis of a *Bacteroides thetaiotaomicron recA* disruption mutant and an investigation of whether RecA is required for excision and integration of *Bacteroides* mobile DNA elements. The *recA* mutant was deficient in homologous recombination and was more sensitive than the wild-type strain to DNA-damaging agents. The *recA* mutant was also more sensitive to oxygen than the wild type, indicating that repair of DNA contributes to the aerotolerance of *B. thetaiotaomicron*. Many *Bacteroides* clinical isolates carry self-transmissible chromosomal elements known as conjugative transposons. These conjugative transposons can also excise and mobilize in *trans* a family of unlinked integrated elements called nonreplicating *Bacteroides* units (NBUs). The results of a previous study had raised the possibility that RecA plays a role in excision of *Bacteroides* conjugative transposons, but this hypothesis could not be tested in *Bacteroides* spp. because no RecA-deficient *Bacteroides* strain was available. We report here that the excision and integration of the *Bacteroides* conjugative transposons, as well as NBU1 and Tn*4351*, were unaffected by the absence of RecA activity.

Many strains of *Bacteroides* contain large (>60-kb) integrated self-transmissible elements called conjugative transposons (21, 22). During transfer, these conjugative transposons excise from the chromosome to form a circular transfer intermediate that is transferred by conjugation to a recipient and integrates into the recipient genome. The *Bacteroides* conjugative transposons not only transfer themselves but also can stimulate the excision and circularization of unlinked integrated elements called nonreplicating *Bacteroides* units (NBUs). The NBU circular form is then mobilized by the conjugative transposon into the recipient, where it integrates into the chromosome (28–30). A growing body of evidence suggests that dissemination of antibiotic resistance genes within the *Bacteroides* phylogenetic group is being mediated by conjugative transposons and the elements they mobilize (2, 20–22).

Little is known about the mechanism of excision and integration of the *Bacteroides* conjugative transposons. Previous sequence analysis of integrated and excised forms of the cryptic *Bacteroides* conjugative transposon XBU4422 revealed a 14-bp segment close to one end of the element that was identical to a similarly sized segment adjacent to the insertion site (1, 27). Also, the two ends of the element contained 23-bp imperfect inverted repeats. Although these regions of sequence similarity seemed too small for homologous recombination to be involved in excision or integration, one of our earlier findings suggested the possibility that homologous recombination was involved in excision of XBU4422, at least in *Escherichia coli* (1, 27). XBU4422 had been trapped on a *Bacteroides-E. coli* shuttle plasmid, pEG920, to form the chimera XBU4422::pEG920. This chimera could transfer itself from *Bacteroides uniformis* to an isogenic *B. uniformis* strain, and the chimera was recovered intact in the recipients. When the chimera was transferred from *B. uniformis* to *E. coli* (RecA$^+$), however, XBU4422 was lost and only pEG920 was recovered in the recipient. If the *E. coli* recipient was RecA$^-$, no pEG920-containing transconjugants were recovered. One possible explanation for these observations was that RecA was required for excision of XBU4422, which was toxic to *E. coli* recipients, and that only recipients that supported the excision of XBU4422 could survive. Another reason for thinking that RecA might have a role in transfer of the conjugative transposons was the recent report that transfer of the *Vibrio cholerae* SXT element, which may be a conjugative transposon, decreased dramatically if the donor or recipient was RecA deficient (36).

The question whether NBU excision or integration required RecA was also unresolved. The joined ends of NBU1, the best studied of the NBUs, have a 14-bp sequence that aligns with an identical 14-bp chromosomal target site (30). During integration, the crossover occurs within this segment. We have shown that NBU1 integrates in *E. coli* and that integration occurs in a RecA$^-$ *E. coli* strain. However, integration in *E. coli* was nearly random (29), unlike integration in *Bacteroides* spp., which was highly site specific (28). Thus, the integration process in *E. coli* could have differed in some respects from the integration process in *Bacteroides* spp. Also, integrated NBU1 could not excise from the *E. coli* chromosome, so that the dependence of excision on RecA could not be tested in this genetic background.

In this paper, we describe the construction of a RecA$^-$ strain of *B. thetaiotaomicron* and show that integration and excision of the conjugative transposons and NBU1 are independent of RecA. Also, we show that transposition of the compound, nonconjugative *Bacteroides* transposon Tn*4351*, is independent of homologous recombination. Tn*4351* has been widely used for mutagenesis of *Bacteroides*, *Prevotella*, and *Cytophaga* spp. and has been assumed to be a transposon, but the fact that its integration was RecA independent had not been shown previously.

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TABLE 1. Bacterial strains used in this study

Strain	Relevant characteristic(s)[a]	Description and/or reference
E. coli strains		
E. coli HB101	*recA* Str^r	23
E. coli DH5α MCR	*recA*	GIBCO/BRL
E. coli S17-1	*recA* Str^r Tp^r	RP4 integrated in the chromosome (32)
E. coli S17-1 *pir*	*recA* Str^r Tp^r	*E. coli* S17-1 with chromosomal copy of *pir* gene encoding II protein required for R6Kγ plasmid replication origin (15)
E. coli S17-1 ΩNW17	*recA* Str^r Tp^r Amp^r	*E. coli* S17-1 with chromosomal insertion of modified NBU1 (this study)
E. coli EM24	*recA* Str^r	*recA* derivative of LE392 (25)
E. coli MC4100	$recA^+$ Str^r	31
Bacteroides strains		
B. thetaiotaomicron 5482		Virginia Polytechnic Institute type strain
B. thetaiotaomicron 5482 *recA*	*recA* Cm^r	*recA* derivative of *B. thetaiotaomicron* 5482 (this study)
B. thetaiotaomicron 4001	Rif^r	Spontaneous Rif^r mutant of *B. thetaiotaomicron* 5482
B. thetaiotaomicron 4001 *recA*	*recA* Rif^r Cm^r	Spontaneous Rif^r mutant of *B. thetaiotaomicron* 5482 *recA* (this study)
B. thetaiotaomicron 4100	Thy^- Tp^r	*B. thetaiotaomicron* 5482 spontaneous Thy^- mutant which also results in Tp^r
B. thetaiotaomicron 4104	Thy^- Tp^r Tc^r	*B. thetaiotaomicron* 4100 Tc^r ERL transconjugant from *B. fragilis* ERL
B. thetaiotaomicron 4104 ΩNW17	Thy^- Tp^r Tc^r Em^r	*B. thetaiotaomicron* 4104 with engineered Em^r NBU1
B. thetaiotaomicron 4107	Thy^- Tp^r Tc^r Em^r	*B. thetaiotaomicron* 4100 Tc^r Em^r DOT transconjugant from *B. thetaiotaomicron* DOT
B. thetaiotaomicron 4108	Thy^- Tp^r Tc^r Em^r	*B. thetaiotaomicron* 4100 Tc^r Em^r 12256 transconjugant from *B. fragilis* 12256
B. thetaiotaomicron 4109	Thy^- Tp^r Tc^r Em^r	*B. thetaiotaomicron* 4100 Tc^r Em^r 7853 transconjugant from *B. thetaiotaomicron* 7853
B. uniformis 1104	Thy^- Tp^r Tc^r	*B. uniformis* 0061 spontaneous Thy^- Tp^r mutant (BU1100) which is a Tc^r ERL transconjugant from *B. fragilis* ERL

[a] Abbreviations: Ap^r, ampicillin resistant; Cm^r, chloramphenicol resistant; Em^r, erythromycin resistant; Kan^r, kanamycin resistant; Rif^r, rifampin resistant; Tc^r, tetracycline resistant; Tp^r, trimethoprim resistant; Str^r, streptomycin resistant; Thy^-, thymidine auxotroph; Mob^+, mobilizable; Rep, replication.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains and plasmids used in this study are listed in Tables 1 and 2, respectively. *Bacteroides* strains were grown in prereduced Trypticase-yeast extract-glucose (TYG), brain heart infusion (BHI) broth (3.7% BHI, 0.5% yeast extract, 1 μg of vitamin K per ml, 8 μg of $CaCl_2$ per ml, 0.6 μg of hematin-histidine per ml), or agar medium in a BBL Microbiology Systems GasPak jar system (8, 26). *E. coli* strains were grown in Luria-Bertani broth.

DNA amplification, cloning, and analysis. All DNA manipulations were performed as described previously (23, 24). Southern blot analysis was performed by the Renaissance nonradioactive detection method as provided by the manufacturer (DuPont NEN). A 433-bp internal region of the *recA* gene from *Bacteroides thetaiotaomicron* 5482 genomic DNA was amplified with primers designed from the known *recA* sequence of *Bacteroides fragilis* I (GenBank accession no. M6309) (7). The primers used were RAP1 (5′GAAATTTATGGTCC GGAATCATC3′) and RAP2 (5′CCGGATTACCGAACATTACACC3′). A 2-μl sample of genomic DNA was mixed with 200 ng of each primer in 100 μl of reaction buffer (1× PCR buffer [GIBCO/BRL], 1.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphate mixture) and amplified with *Taq* polymerase. Amplification was preceded by denaturation for 5 min at 95°C followed by the addition of polymerase and 25 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min. The PCR product was cloned with the TA cloning vector pCRII as specified by the manufacturer (Invitrogen). DNA sequencing was performed with Applied Biosystems model 373A version 2.0.1S dye terminator sequencing system at the University of Illinois Biotechnology Facility.

Construction of a *recA* disruption mutant of *B. thetaiotaomicron*. A new *Bacteroides* insertional vector was constructed by cloning an 800-bp *Eco*RI-*Sst*I fragment from pCQW1 (4), which contains the *oriT* of the IncP plasmid RK2, into the *Xmn*I site of pACYC184, which does not replicate in *Bacteroides* species. The chloramphenicol resistance (Cm^r) gene of pACYC184 was removed by digestion with *Sca*I and *Pvu*II and replaced with a blunted 2.5-kb *Pst*I-*Kpn*I fragment containing the Cm^r gene from pFD308 (33). The resulting 6.5-kb insertional vector was called pNLY2.

TABLE 2. Plasmids used in this study

Plasmid	Relevant characteristics[a]	Description and/or reference
pCR II	Ap^r Kan^r	TA cloning vector (Invitrogen Corp.)
pNLY2	Cm^r Tc^r Mob^+	This study
pRAI49	Cm^r Mob^+	*B. thetaiotaomicron recA* insertional vector (this study)
pBT-2	Kan^r (Tc^r) Mob^+	*Bacteroides* insertional vector (34)
pBT-2 ARR	Kan^r (Tc^r) Mob^+	pBT-2 containing 2.5-kb region of homology to the *B. thetaiotaomicron* 5482 *susC* gene (this study)
pNJR5	Kan^r (Em^r) Mob^+	*E. coli-Bacteroides* shuttle vector (24)
pDP1	Tc^r (Em^r) Mob^+	*E. coli-Bacteroides* shuttle vector (13)
pEP185.2	Cm^r Mob^+	*pir*-requiring R6Kγ *oriV* replicon containing RP4 *oriT* and Cm^r from pACYC184 (17)
pVOH1	Ap^r Cm^r Tc^r (Em^r)	pBR328 with 6.2-kb *Sal*I fragment containing Tn*4351* (9)
pEP4351	Cm^r Tc^r (Em^r) Mob^+	6.2-kb *Sal*I fragment from pVOH1 cloned into *Sal*I of pEP185.2 (this study)
R751	Tp^r	Self-transmissible IncPβ plasmid (16)
R751::Tn*4351*Ω4	Tc^r (Em^r) Tra^+	Tn*4351* insertion in R751 (25)
R751::Tn*4351*Ω8	Tc^r (Em^r) Tra^+	Tn*4351* insertion in R751 (25)
pNLS21	Tc^r (Em^r) Mob^+	*E. coli-Bacteroides* shuttle plasmid using R751 *oriT* (this study)
pNW17	Ap^r Mob^+ Rep^+ (Em^r Mob^+ Rep^-)	Insertion vector pNV19 (28) containing Mob^- NBU1 (this study)

[a] Abbreviations are the same as those in Table 1. *Bacteroides* phenotypes are shown in parentheses. *E. coli* phenotypes are shown without parentheses.

TABLE 3. Homologous recombination in *B. thetaiotaomicron* wild-type and *recA* strains

Donor	Recipient	Frequency[a]
E. coli S17-1(pBT-2:ARR)	*B. thetaiotaomicron* 5482	3×10^{-7}–4×10^{-7}
	B. thetaiotaomicron 5482 *recA*	$<1 \times 10^{-9}$
E. coli S17-1(pNJR5)	*B. thetaiotaomicron* 5482	5×10^{-5}–1×10^{-4}
	B. thetaiotaomicron 5482 *recA*	8×10^{-5}–2×10^{-4}

[a] Number of transconjugants per recipient.

The *B. thetaiotaomicron* 5482 *recA* PCR product cloned in pCRII was subcloned with *Hin*dIII and *Eco*RV into the same sites in pNLY2, disrupting the pACYC184 tetracycline resistance gene. The resulting 7-kb plasmid was called pRAI49. This plasmid was transformed into *E. coli* S17-1 with selection for chloramphenicol resistance. The resulting strain was used as a donor in matings with *B. thetaiotaomicron* 5482.

A 1:1 ratio of *E. coli* S17-1 pRAI49 donors and *B. thetaiotaomicron* 5482 recipients was mixed, and matings were performed on TYG plates using the filter mating procedure described previously (24). Matings were incubated aerobically overnight at 37°C and then plated on TYG plates containing gentamicin (200 μg/ml) and chloramphenicol (15 μg/ml) to obtain *B. thetaiotaomicron* 5482 transconjugants that contained a chromosomal insertion of pRAI49 in the *recA* gene.

All other matings that used wild-type or *recA Bacteroides* recipients were performed on BHI plates, and the mixtures were incubated anaerobically overnight at 37°C. The antibiotic concentrations used for selection were as follows: ampicillin, 100 μg/ml; chloramphenicol, 15 μg/ml; erythromycin, 10 μg/ml; kanamycin, 100 μg/ml; rifampin, 10 μg/ml; tetracycline, 10 μg/ml in *E. coli* and 3 μg/ml in *Bacteroides*; trimethoprim, 100 μg/ml; streptomycin, 300 μg/ml.

Effect of the *recA* disruption on integration and excision of conjugative transposons and NBU1. Matings to examine the influence of RecA on insertion of the chromosomal conjugative elements were performed with Thy$^-$ *Bacteroides* donors and Rif^r wild-type and *recA* recipients. Transconjugants were selected on BHI medium containing rifampin to select for Rif^r recipients, plus either erythromycin or tetracycline to select for element transconjugants. NBU1 does not carry any known antibiotic resistances. To monitor NBU1 excision, transfer, and integration, a *mob* derivative of NBU1 was cloned into the *Bacteroides* insertional vector pNV19 (28) to produce pNW17. The pNV19 vector provides selectable markers for *E. coli* (Ap^r) and for *Bacteroides* (Em^r). It also provides a mobilization region that functions in *E. coli* and *Bacteroides*. The pNW17 construct replicates in *E. coli*, but not in *Bacteroides*, where the NBU1 portion provides integration and excision functions.

Matings to test the influence of RecA on excision were performed with the element-containing *B. thetaiotaomicron recA* strains obtained from integration experiments as donors. The recipient for these matings was *B. thetaiotaomicron* 4100 (Tp^r Thy$^-$). Recipient selection took advantage of the trimethoprim resistance of the recipient, as well as the poor growth on TYG medium and sensitivity to the DNA-damaging agent methyl methanesulfonate (MMS) of the *recA* donor strain. Transconjugants were selected on TYG medium containing thymidine, trimethoprim, and MMS (0.01%), plus either erythromycin or tetracycline. For all matings, frequencies are reported as the number of transconjugants per recipient and are the range of values obtained from at least two separate mating frequency determinations.

Sensitivity to DNA-damaging agents. Tubes (10 ml) of prereduced BHI broth containing increasing concentrations of MMS or metronidazole were inoculated with 50 μl of a wild-type or *recA* strain, which had been grown in BHI to a final optical density at 600 nm (OD_{600}) of 0.6. The final OD_{600} attained by each culture was recorded after incubation at 37°C for 24 h.

Enzyme assays. Cell extracts were prepared for superoxide dismutase (SOD) assays by growing 500-ml cultures of *B. thetaiotaomicron* wild-type and *recA* strains in prereduced TYG broth to an OD_{600} of 0.25. For anaerobically maintained cultures, chloramphenicol (30 μg/ml) was added prior to harvesting to prevent SOD production during exposure of the cells to oxygen during processing. For aerated cells, cells that had reached the same OD were transferred to a 2-liter flask and shaken in air for 1 h at 37°C before being harvested. The cells were harvested by centrifugation, resuspended in 50 mM potassium phosphate–0.1 mM EDTA (pH 7.8), and disrupted by three passages through a chilled French pressure cell at 14,000 lb/in^2. The disrupted cell suspension was cleared by centrifugation at 19,000 × *g* for 20 min and dialyzed against the same buffer overnight at 4°C. SOD assays were performed by the xanthine oxidase-cytochrome *c* method (14). The total protein content of cell extracts was determined by a Coomassie blue stain-based assay (Pierce).

Nucleotide sequence accession number. The DNA sequence of the *B. thetaiotaomicron* 5482 *recA* gene internal region has been deposited in GenBank (accession no. U63514).

RESULTS AND DISCUSSION

Characteristics of a *Bacteroides recA* mutant. The *recA* gene from *B. fragilis* has been cloned previously by complementation of an *E. coli recA* mutant (6, 7), but a *B. fragilis recA* mutant had not been constructed. For our purposes, *B. fragilis* was not a good species for studying the excision and integration of *Bacteroides* conjugative transposons, for two reasons. First, transfer into and out of *B. fragilis* by conjugative transposons generally occurs at 100- to 1,000-fold-lower frequencies than those attainable with *B. thetaiotaomicron* 5482. Second, many *Bacteroides* isolates already contain conjugative transposons, some of which are cryptic. The presence of such native elements might interfere with investigations of the excision and integration of known conjugative transposons by providing

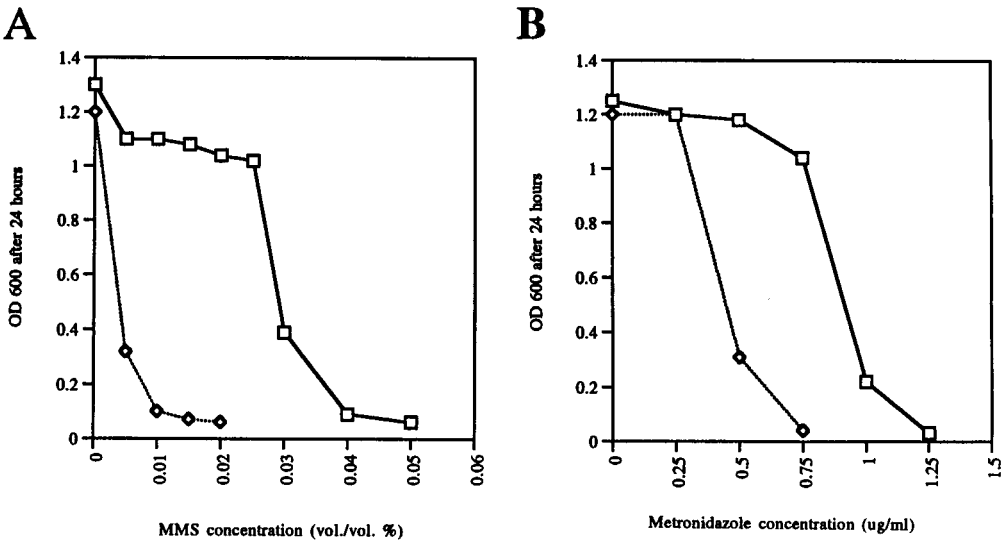


FIG. 1. Resistance of *B. thetaiotaomicron* 5482 wild-type (□) and *recA* (◇) strains to the DNA-damaging agents MMS (A) and metronidazole (B).

TABLE 4. Transfer frequency of conjugal elements into *B. thetaiotaomicron* wild-type and *recA* strains

Donor	Recipient	Element transferred	Frequency[a]
B. thetaiotaomicron 4107	*B. thetaiotaomicron* 4001	DOT	3×10^{-8}–6×10^{-8}
	B. thetaiotaomicron 4001 *recA*	DOT	5×10^{-8}–7×10^{-8}
B. thetaiotaomicron 4108	*B. thetaiotaomicron* 4001	12256	2×10^{-7}–9×10^{-7}
	B. thetaiotaomicron 4001 *recA*	12256	1×10^{-6}–7×10^{-6}
B. thetaiotaomicron 4109	*B. thetaiotaomicron* 4001	7853	3×10^{-6}–6×10^{-6}
	B. thetaiotaomicron 4001 *recA*	7853	4×10^{-6}–9×10^{-6}
B. thetaiotaomicron 4104 ΩNW17	*B. thetaiotaomicron* 4001	ERL	3×10^{-7}–4×10^{-6}
	B. thetaiotaomicron 4001 *recA*	ERL	5×10^{-6}–1×10^{-5}
	B. thetaiotaomicron 4001	NBU1	4×10^{-6}–2×10^{-5}
	B. thetaiotaomicron 4001 *recA*	NBU1	4×10^{-5}–9×10^{-5}
B. uniformis 1104 pDP1	*B. thetaiotaomicron* 4001	ERL	2×10^{-6}–4×10^{-6}
	B. thetaiotaomicron 4001 *recA*	ERL	5×10^{-6}–3×10^{-5}
	B. thetaiotaomicron 4001	pDP1	1×10^{-6}–4×10^{-6}
	B. thetaiotaomicron 4001 *recA*	pDP1	7×10^{-6}–2×10^{-5}

[a] Number of transconjugants per recipient.

functions in *trans*. *B. thetaiotaomicron* 5482 appears to be free of this type of element and has thus become a preferred strain for working on conjugative transposons.

Attempts to use an internal portion of the *recA* gene from *B. fragilis* to disrupt the *recA* gene of *B. thetaiotaomicron* were unsuccessful, raising the possibility that the *recA* genes in these two species differed too much at the DNA sequence level for efficient homologous recombination to occur. These two species have overall DNA homology of only 45% (11), and so it was conceivable that there was appreciable sequence variation even in conserved genes such as *recA*. Accordingly, a 433-bp internal region of the *recA* gene from *B. thetaiotaomicron* 5482 was amplified by PCR and cloned. There was 80% nucleotide sequence identity between the cloned *B. thetaiotaomicron* 5482 sequence and the corresponding *B. fragilis* I *recA* sequence. This level of *recA* sequence divergence is consistent with the genetic distances already known to exist between different *Bacteroides* species (10). The *recA* internal region from *B. thetaiotaomicron* 5482 was used to construct a single-crossover insertional disruption in the *recA* gene. Disruption of the *recA* gene was confirmed by Southern blot analysis with the labeled *recA* amplification product as a probe (data not shown).

The *recA* disruption was stable in the absence of selection. When the *recA* strain was transferred daily for 7 days to a fresh tube of BHI medium without chloramphenicol and then plated on medium with and without chloramphenicol, the counts were the same (data not shown).

Increased oxygen sensitivity of the *recA* disruption mutant. An unexpected phenotype of the *recA* strain was poor survival under aerobic conditions. Wild-type *B. thetaiotaomicron* is normally quite aerotolerant. The bacteria can be plated aerobically, and colonies on agar medium can survive exposure to oxygen for several days. The *recA* strain exhibited poor growth on TYG agar medium if the plates had first been exposed to oxygen and would not withstand aerobic exposure routinely tolerated by the wild-type strain, such as spread plating and storing plates on the bench top. One possible source of toxicity from oxygen-exposed TYG medium could be the cysteine and

TABLE 5. Transfer frequency of conjugal elements out of *B. thetaiotaomicron recA*

Donor	Recipient	Element transferred	Frequency[a]
B. thetaiotaomicron 4007 *recA*	*B. thetaiotaomicron* 4100	DOT	2×10^{-7}–3×10^{-6}
B. thetaiotaomicron 4008 *recA*	*B. thetaiotaomicron* 4100	12256	1×10^{-6}–2×10^{-6}
B. thetaiotaomicron 4009 *recA*	*B. thetaiotaomicron* 4100	7853	4×10^{-5}–1×10^{-4}
B. thetaiotaomicron 4004 ΩNW17 *recA*	*B. thetaiotaomicron* 4100	ERL	2×10^{-6}–2×10^{-4}
		NBU1	3×10^{-6}–8×10^{-5}
B. thetaiotaomicron 4004 pDP1 *recA*	*B. thetaiotaomicron* 4100	ERL	4×10^{-5}–1×10^{-4}
		pDP1	5×10^{-5}–1×10^{-4}

[a] Number of transconjugants per recipient.

TABLE 6. Tn*4351* transposition in *B. thetaiotaomicron* wild-type and *recA* strains

Donor	Recipient	Frequency[a]
E. coli HB101 R751::Tn*4351* Ω4	*B. thetaiotaomicron* 5482	1×10^{-7}–4×10^{-7}
	B. thetaiotaomicron 5482 *recA*	$<1 \times 10^{-9}$
E. coli HB101 R751::Tn*4351*Ω8	*B. thetaiotaomicron* 5482	5×10^{-8}–1×10^{-7}
	B. thetaiotaomicron 5482 *recA*	$<1 \times 10^{-9}$
E. coli S17-1 *pir* pEP4351	*B. thetaiotaomicron* 5482	1×10^{-6}–8×10^{-6}
	B. thetaiotaomicron 5482 *recA*	1×10^{-6}–4×10^{-6}
E. coli S17-1 *pir* pEP4351	*E. coli* MC4100 (*recA*$^+$)	1×10^{-8}–3×10^{-7}
	E. coli EM24 (*recA*)	1×10^{-5}–2×10^{-5}

[a] Number of transconjugants per recipient.

resazurin in this medium, which together can generate reactive oxygen species from atmospheric oxygen. The use of BHI medium with no added cysteine or resazurin, together with minimization of oxygen exposure, restored the *recA* strain to wild-type growth rates. These observations suggest that exposure of *Bacteroides* strains to air causes DNA damage but that *B. thetaiotaomicron* is aerotolerant because RecA is available to repair this damage. A similar observation of increased oxygen sensitivity of a *recA*-disrupted strain has been reported for the microaerophile *Lactococcus lactis* (3) and for an *Escherichia coli* double mutant lacking both functional *recA* and superoxide dismutase (*sod*) genes (12). Thus, the involvement of RecA in protection of bacteria from oxidative DNA damage appears to be common among a variety of bacterial genera.

Superoxide (O_2^-) has been implicated in the production of oxidative DNA damage by a variety of mechanisms (12). The concentration of superoxide within most organisms is decreased by SOD. Although SOD activity has been detected in a strain of *B. thetaiotaomicron* (18), SOD activity in *B. thetaiotaomicron* 5482 has not been tested previously. We assayed SOD specific activity in *B. thetaiotaomicron* 5482 wild-type and *recA* cells, either kept under anaerobic conditions until disruption of the cells or exposed to oxygen prior to disruption. Anaerobically maintained cultures of early-exponential-phase wild-type and *recA* strains had SOD specific activities of 0.5 and 0.6 U/mg, respectively. Cultures which were aerated for 1 h before being harvested had SOD specific activities of 1.4 U/mg (wild type) and 1.3 U/mg (mutant). These results indicate that the absence of RecA does not affect SOD levels and that oxidative damage occurs in the *recA* strain despite the presence of SOD activity. This finding supports the hypothesis that some RecA-mediated repair process is necessary to take care of the damage caused by reactive forms of oxygen that escape the protective action of SOD.

RecA is required in *Bacteroides* for homologous recombination and repair of DNA damage. The frequency of homologous recombination events in the wild-type and *recA* strains was tested with the insertional vector pBT-2:ARR, which contained a 2.5-kb fragment of the *B. thetaiotaomicron* chromosomal gene *susC*. The *susC* gene product is involved in utilization of starch and maltoheptaose (19). The pBT-2:ARR construct was mobilized from *E. coli* S17-1 into the *B. thetaiotaomicron* wild-type and *recA* strains. Insertion of pBT-2:ARR was efficient in the wild-type strain, while insertion in the *recA* strain was not detected (Table 3). To confirm that the lack of insertion events was due to failure of the suicide vector to integrate into the chromosome of the *recA* mutant by homologous recombination and not to some defect in conjugation caused by disruption of *recA*, the *E. coli-Bacteroides* shuttle plasmid pNJR5 (24) was also mobilized from *E. coli* S17-1 into wild-type and *recA* strains. This shuttle plasmid transferred into the wild-type and *recA* strains at equivalent frequencies (Table 3).

The sensitivity of wild-type and *recA* strains to DNA-damaging agents was also evaluated by using the DNA alkylating agent MMS and the nitroimidazole drug metronidazole, which also causes DNA damage (35). Both agents exhibited greater activity against the *recA* strain than against the wild-type strain (Fig. 1).

Insertion and excision of *Bacteroides* conjugative transposons and NBU1 are RecA independent. To determine whether insertion of the *Bacteroides* integrated conjugal elements was influenced by RecA, the transfer frequencies of four conjugative transposons (Tc^r ERL, Tc^r Em^r DOT, Tc^r Em^r 12256, and Tc^r Em^r 7853) and of NBU1 were determined. Several different conjugative transposons were tested because although Tc^r ERL and Tc^r Em^r DOT appear to have the same ends, which are closely related to the ends of XBU4422, the ends of Tc^r Em^r 12256 and Tc^r Em^r 7853 are different enough not to cross-hybridize with the ends of XBU4422 on Southern blots. Thus, Tc^r Em^r 12256 and Tc^r Em^r 7853 could have different integration and excision requirements from those of Tc^r ERL and Tc^r Em^r DOT. The frequencies with which NBU1 and the four conjugative transposons transferred into the *B. thetaiotaomicron* wild-type and *recA* strains were essentially the same (Table 4). Therefore, insertion of the *Bacteroides* chromosomal conjugal elements is RecA independent. NBU1 is normally site specific in *Bacteroides* (28). To determine if NBU1 site specificity was affected in the *recA* strain, 10 NBU1-containing *recA* transconjugants were subjected to Southern blot analysis. All 10 NBU1 insertions were in the primary target site (data not shown). Therefore, absence of RecA in the recipient did not affect the site specificity of NBU1.

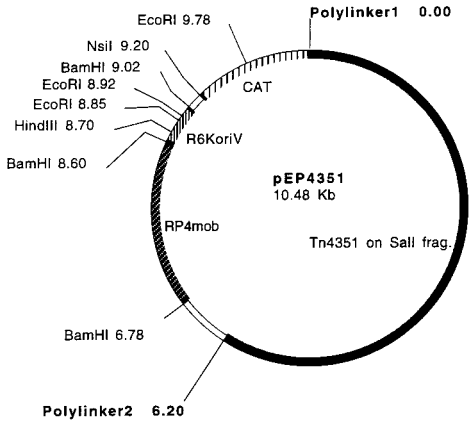
To examine the influence of RecA on excision, each of the element-containing *B. thetaiotaomicron recA* transconjugants obtained from integration experiments was used as the donor in matings with *B. thetaiotaomicron* 4100 (Tp^r Thy$^-$). Excision of the *Bacteroides* conjugative transposons and NBU1 was RecA independent (Table 5). Curiously, the transfer frequency for each element was somewhat higher out of the *recA* mutant. The RecA independence of the *Bacteroides* conjugative transposons is similar to that reported for the Tn*916* family of gram-positive conjugative transposons (5). One apparent exception to this *recA* independence of transmissible integrated elements is provided by the SXT element from *V. cholerae* O139 (36). For the SXT element, inactivation of *recA* in *E. coli* or *V. cholerae* recipients decreased the frequency of SXT ele-

ment transmission 20- to 50-fold. Deletion of *recA* from the *V. cholerae* O139 donor strain abolished transfer of the SXT element (36). If the SXT element is in fact a conjugative transposon, it presumably excises and integrates differently from either the Tn*916* or *Bacteroides*-type conjugative transposons.

Tn*4351* transposition is RecA independent in *B. thetaiotaomicron*. The *Bacteroides* transposable element Tn*4351* has become a useful tool for mutagenesis of species in the *Bacteroides* phylogenetic group. Tn*4351* confers erythromycin resistance on *Bacteroides* and tetracycline resistance on aerobically grown *E. coli*. The broad-host-range IncPβ plasmid R751 carrying Tn*4351* (R751::Tn*4351*) can mobilize itself from *E. coli* to *Bacteroides*. In the *Bacteroides* recipient, R751 does not replicate, but Tn*4351* can integrate alone or along with R751 into the chromosome at a frequency of 10^{-5} to 10^{-7} (25). Tn*4351* integrates in *E. coli*, and integration is independent of RecA in this genetic background (9). No previous study has investigated the role of RecA in Tn*4351* transposition in *Bacteroides.*

The insertion frequencies of Tn*4351* carried on two different R751::Tn*4351* constructs (Ω4 and Ω8) were determined for the *Bacteroides recA* and wild-type strains. The insertion frequency of each construct was below the limits of detection in the *recA* recipient strain (Table 6). The failure of R751::Tn*4351* to insert in the *Bacteroides recA* strain was surprising and warranted additional experiments. To further investigate the role of RecA in Tn*4351* transposition, a new Tn*4351* insertional vector, pEP4351 (Fig. 2), was constructed based on the conditionally replicative *pir* plasmid pEP185.2 (17). This construct can be mobilized from *E. coli* S17-1 *pir* via its RP4 *oriT* into both *Bacteroides* and *E. coli*. When pEP4351 was the delivery vector, Tn*4351* integrated into both the *Bacteroides* and *E. coli* chromosomes in a RecA-independent fashion (Table 6). In *E. coli*, Tn*4351* integration in the $RecA^-$ strain was approximately 100-fold more frequent than in the $RecA^+$ strain and the pEP185.2 vector cointegrated with Tn*4351* in 54 and 85% of $RecA^+$ and $RecA^-$ transconjugants, respectively. The fact that cointegration of the plasmid occurred in the *recA* background indicates that cointegration is mediated by the transposon rather than resulting from integration of a copy of the transposon into the chromosome followed by homologous recombination of the plasmid-borne copy with the chromosomal copy.

These results demonstrate that Tn*4351* integration is RecA independent in *Bacteroides* and suggest that there is something peculiar about R751 transfer that prevented or at least lowered R751::Tn*4351* transfer to the *recA* strain. To test this hypothesis, a new *E. coli-Bacteroides* shuttle plasmid, pNLS21 (27 kb), was constructed. This vector contains a 15-kb *Pst*I fragment from R751, within which is the R751 *oriT*. The pNLS21 plasmid is mobilized from *E. coli* EM24 donors to *E. coli* or *Bacteroides* recipients by R751. The pNLS21 plasmid transferred into the *B. thetaiotaomicron recA* strain at a frequency sixfold (diparental mating) to eightfold (triparental mating) lower than that at which it transferred into the wild-type strain. Even with this drop in frequency, transfer into the *recA* strain was easily detectable at 3×10^{-6} to 9×10^{-6} transconjugants per recipient. However, if the Tn*4351* transposition event from the R751::Tn*4351* construct lowers the detected insertion frequency an additional 2 to 3 log units, the ability to detect Tn*4351* transposition in the *recA* strain would be marginal. Therefore, the negative effect on R751-mediated delivery of Tn*4351* that was seen with *Bacteroides* $RecA^-$ recipients may be partly explained by an effect on processes occurring during R751 transfer.



Polylinker1: 0.0/T7/KpnI.ApaI.DraI.XhoI.SalI
Polylinker2: 6.2/SalI.AccI.HincII.EcoRV.PstI.BglII.SmaI.SpeI.XbaI.EagI.NotI.SstII.SstI/T3

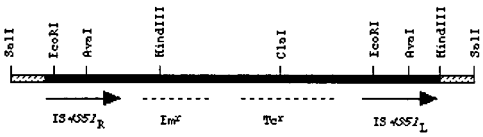


FIG. 2. Partial restriction map of the new Tn*4351* insertional vector pEP4351 and the 6.2-kb *Sal*I fragment containing Tn*4351*. The vector pEP185.2 (17), into which the 6.2-kb *Sal*I fragment was cloned, contains the origin of transfer for RP4 (RP4mob), the origin of replication of R6K (R6KoriV), and the chloramphenicol acetyltransferase (CAT) gene from pACYC184. ▨, R751 sequences; ▬, Tn*4351* sequences.

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