Attempted Transfer of Antibiotic Resistance between *Bacteroides* and *Escherichia coli*

By V. E. DEL BENE, MARY ROGERS
AND W. E. FARRAR, JR

*Infectious Diseases and Immunology Division,
Department of Medicine,
Medical University of South Carolina, Charleston,
South Carolina 29401, U.S.A.*

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SUMMARY

Transfer of antibiotic resistance between *Bacteroides* organisms and *E. coli* in mixed culture under optimal anaerobic conditions was attempted. Donor strains used were *E. coli* with R factors of a number of compatibility groups and *Bacteroides* with unusually high antibiotic resistance. Recipient strains included *E. coli* strains with characteristics favouring conjugal transfer. Although control experiments verified that conjugal transfer of derepressed R factors could occur at a high frequency between *E. coli* in an anaerobic environment, transfer of antibiotic resistance between *E. coli* and *Bacteroides* was never demonstrated.

INTRODUCTION

It might be anticipated that the abundant multi-resistant *Bacteroides* in the endogenous flora of man serve as a reservoir for transferable drug resistance factors (R factors). *Bacteroides* organisms are among the most abundant bacteria present in the human colon (Gorbach *et al.*, 1967). These organisms are usually resistant to numerous antimicrobial agents, including the β -lactam antibiotics, tetracycline, and the aminoglycosides (Del Bene & Farrar, 1972). In the colon they are in intimate contact with facultative Gram-negative rods like *Escherichia coli* in which the occurrence and importance of transferable drug resistance has been well documented (Richmond, 1973). Anaerobic conditions are no impediment to conjugal transfer of either chromosomal material between *E. coli* at high frequency (Stallions & Curtiss, 1972) or of R factors at low frequencies (Moodie & Woods, 1973). Limited attempts at demonstrating R factor transfer between *E. coli* and *Bacteroides* have been unsuccessful (Anderson & Sykes, 1973). Evidence that strains of *Bacteroides* harbour extrachromosomal DNA has further suggested the possibility that these anaerobic organisms may participate in conjugal transfer of resistance determinants, although some plasmid-bearing *Bacteroides* are not resistant to antimicrobial agents (Stiffler, Keller & Traub, 1974).

Our study was primarily designed to determine whether conjugal transfer of drug resistance could be demonstrated *in vitro* between strains of *E. coli* and strains of the genus *Bacteroides*. In addition, the effect of an anaerobic environment on the frequency of transfer of derepressed R factors between strains of *E. coli* was studied.

Table 1. Characteristics of bacterial strains

Donor strains	Com- patibility group	Resistance profile*	Reference
<i>Escherichia coli</i>			
RS2 (R1-19) <i>drd</i> fi^+	FII	Cm, Amp, Sm, Su, Km	(LeBlanc & Falkow, 1973)
W1485 (R192-7) <i>drd</i> fi^+	FII	Cm, Tc, Sm, Su	(Datta & Hedges, 1972)
22 (R100-1) <i>drd</i> fi^+	FII	Cm, Tc, Sm, Su	(LeBlanc & Falkow, 1973)
W3110 (S-a) fi^-	W	Cm, Sm, Su, Km	(Datta & Hedges, 1972)
J53 (R27) fi^-	H	Tc	(Datta & Olarte, 1974)
J53 (R46) fi^-	N	Tc	(Datta & Hedges, 1972)
J53 (RP4) fi^-	P	Tc, Amp, Km	(Datta & Hedges, 1972)
W3110 (Galvez) fi^-	O, Ix	Cm, Tc, Sm, Su	(Thorne & Farrar, 1974)
<i>Bacteroides clostridiiformis</i>			
8035TA		Cm, Tc, Amp, P, Ceph	
<i>B. fragilis</i>			
C20-22		Tc, P, Ceph, Nx	
C11-16		Tc, P, Ceph, Nx	
Recipient strains			
<i>E. coli</i>			
W1485 F^-		Nx	
W1485 3a F^- Col ^R		Nx	
CSH27 F^- <i>trp</i> , <i>thi</i>		Nx	
K12-04 r^{III} m^{III} , <i>met</i>		Nx	
<i>B. fragilis</i>			
B571, 114C, 209, 108B		Nx	
<i>B. melaninogenicus</i>			
4201		Gm	

* Amp, ampicillin; Ceph, cephalothin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Nx, nalidixic acid; P, penicillin; Tc, tetracycline; Su, sulphonamides.

METHODS

Bacterial strains. The characteristics of strains used as donors and recipients in this study are shown in Table 1. Organisms used as donors include *Escherichia coli* containing R factors of both fi^+ and fi^- types (Watanabe *et al.*, 1964), *Bacteroides clostridiiformis* and *Bacteroides fragilis*. The *E. coli* strains with their well-characterized R factors were selected because they either represented a spectrum of compatibility groups with broad host ranges (Datta & Hedges, 1972), or transferred at high frequency. The *B. clostridiiformis* strain was chosen because it has significant resistance to ampicillin (2500 µg/ml), penicillin (2048 µg/ml) and cephalothin (78 µg/ml), which is mediated by a β -lactamase, and because it also has significant resistance to tetracycline (50 µg/ml) and chloramphenicol (12.5 µg/ml). The latter resistance is associated with chloramphenicol acetyltransferase activity. *Bacteroides fragilis* strains which were used as donors were resistant to tetracycline (100 µg/ml) and penicillin (50 µg/ml), the latter associated with production of a β -lactamase (unpublished results).

Recipient organisms included strains of *Escherichia coli*, *Bacteroides fragilis*, and *Bacteroides melaninogenicus*. These strains are relatively sensitive to β -lactam antibiotics as well as to tetracycline and chloramphenicol. *Escherichia coli* CSH27 F^- is a plasmid-free recipient, and K12-04 does not possess the K12 system of restriction and modification.

The anaerobic bacteria were identified and characterized according to the methods described by Holdeman & Moore (1973). These included biochemical reactions and gas chromatographic analysis of the short chain fatty acid end-products of these bacteria.

Escherichia coli strains were verified by triple-sugar-iron reactions and by colonial appearance on MacConkey's medium. The levels of susceptibility to antimicrobial agents were determined by the agar plate dilution technique utilizing a replicating apparatus (Steers, Foltz & Graves, 1959). Twofold dilutions of the antibiotic were made in Schaedler's agar (BBL) which had been modified by the addition of L-cysteine (0.05 g/l). An inoculum of 10^4 organisms was delivered to the surface of the plates containing antibiotic by means of the replicator in an anaerobic environment. The levels of susceptibility of anaerobic organisms to each antibiotic were determined after an incubation period of 24 h.

Transfer experiments. Equal amounts (about 10^9 cells/ml) of donor and recipient strains in the exponential phase of growth were mixed in Schaedler's broth containing L-cysteine (0.05 g/l) and were allowed to incubate at 37 °C in an anaerobic glove box for 18 h (1 h for frequency studies, Table 2). The incubation mixtures (0.3 ml; 3×10^8 cells) were spread on agar plates containing one antibiotic to which the donor was resistant. As detailed in Table 3, the donor strains were eliminated from the mating mixture by either nalidixic acid or gentamicin or, when the donor was an anaerobic organism, by selection for transconjugants in an aerobic environment. In 30 experiments the donor and recipient strains were brought together by means of filtration on to a Millipore filter membrane (Matney & Achenbach, 1962) which was then incubated upon Schaedler's enriched agar for 18 h. Selection of transconjugants from these membrane matings was accomplished by resuspending the 18 h mating mixture from the membrane filter in modified Schaedler's broth, followed by incubation for 4 to 6 h before plating the entire culture on antibiotic-containing selection plates (donor resistance markers were individually selected on plates containing one antibiotic). Colonies which appeared after 18 h incubation on the antibiotic-containing selection plates were then examined for drug resistance markers by the disc susceptibility test and their genus and species were re-identified. Each mating mixture was approximately examined to verify viability of donor and recipient strains.

RESULTS

The frequencies at which R factor transfer between *E. coli* strains occurred in aerobic and anaerobic environments are compared in Table 2. The derepressed R factors, R192-7, R1-19 and R100-1, were transferred at a high frequency in air and only minor differences

Table 2. *Comparison of the frequency of R factor transfer between E. coli in an aerobic and an anaerobic environment*

Donors and recipients were incubated in mixed culture for 1 h at 37 °C.
Recipient strain for all matings was W1485 3a.

Donor strain	Transfer frequency*	
	Aerobic	Anaerobic
W1485 (R192-7)	1.4×10^{-1} 9.2×10^{-1}	1.5×10^{-1} 1.9×10^{-1}
RS2 (R1-19)	1.8×10^{-1}	8.0×10^{-2}
22 (R100-1)	2.5×10^{-2}	8.6×10^{-2}
W3110 (S-a)	4.3×10^{-4}	NC
J53 (R46)	1.4×10^{-5}	NC
J53 (RP4)	4.3×10^{-5}	4.4×10^{-6}
J53 (R27)	1.0×10^{-7}	1.3×10^{-8}

NC, Transfer occurred, frequency not calculated. * Transfer frequency = transconjugants/donors.

Table 3. Summary of conjugation experiments conducted under anaerobic conditions

Experiment		Donor	Recipient	Mating conditions*	Donor eliminated by†	Recipient eliminated by‡	Transfer
Type	No.						
A	4	RS2 (R1-19)	<i>B. fragilis</i> B571	B	Nx	Cm	—
	1	RS2 (R1-19)	<i>B. fragilis</i> 114C	B	Nx	Cm	—
	1	RS2 (R1-19)	<i>B. melaninogenicus</i> 4201	B	Gm	Cm	—
	1	W1485 (R192-7)	<i>B. fragilis</i> B571	F	Nx	Tc, Cm	—
	4	22 (R100-1)	<i>B. fragilis</i> 209, B571, 114C, 108B	F	Nx	Tc, Cm	—
B	1	J53 (RP4)	<i>B. fragilis</i> B571	F	Nx	Tc	—
	1	J53 (R27)	<i>B. fragilis</i> B571	F	Nx	Tc	—
	1	J53 (R46)	<i>B. fragilis</i> B571	F	Nx	Tc	—
	1	W3110 (S-a)	<i>B. fragilis</i> B571	F	Nx	Cm	—
	1	W3110 (Galvez)	<i>B. fragilis</i> B571	F	Nx	Cm	—
C	3	<i>B. clostridioformis</i>	<i>E. coli</i> 1485 F-	F	Air	Tc, Cm, Amp	—
	3	<i>B. clostridioformis</i>	<i>E. coli</i> 1485 3a	F	Air	Tc, Cm, Amp	—
	6	<i>B. clostridioformis</i>	<i>E. coli</i> CSH27	F	Air	Tc, Cm, Amp	—
	6	<i>B. clostridioformis</i>	<i>E. coli</i> K12-04	F	Air	Tc, Cm, Amp	—
	1	<i>B. clostridioformis</i>	<i>B. melaninogenicus</i> 4201	F	Gm	Tc, Cm, Amp	—
	2	<i>B. fragilis</i>	<i>E. coli</i> 1485 F-	F	Air	Tc	—
	4	<i>B. fragilis</i>	<i>E. coli</i> K12-04	F	Air	Tc	—

* B, co-incubation in broth; F, co-incubation on Millipore filter.

† Abbreviations as in Table 1.

‡ Each antibiotic used separately to eliminate recipient. Abbreviations as in Table 1.

were found when the 1 h transfer frequency was determined simultaneously in the anaerobic glove box. The R factors RP4, S-a, R46 and R27 transfer 100 to 100000-fold less frequently than the derepressed R factors (R192-7, R1-19 and R100-1), and transfer of these R factors also occurred in the anaerobic environment.

In 41 experiments, we attempted to demonstrate the transmission of antibiotic resistance between facultative and anaerobic Gram-negative bacteria (Table 3), but no evidence of transfer was obtained. Under precisely the same conditions of anaerobiosis we were able to demonstrate transfer between facultative Gram-negative bacteria.

The possibility of transfer of derepressed fi^+ R factors between *E. coli* and various *Bacteroides* recipients was examined in 11 experiments (type A, Table 3). In six of these experiments the mating was attempted in broth culture and in five the filter membrane technique was utilized. We were not able to demonstrate transfer of any of the three fi^+ R factors by *E. coli* donor strains when a *Bacteroides* was used as recipient but in simultaneous experiments, in which a sensitive *E. coli* strain was used as recipient, transfer always occurred.

Donor *E. coli* strains which harboured fi^- R factors of different compatibility groups (P, H, N, W, O and $I\alpha$) were examined for their ability to transfer drug resistance to *B. fragilis* B571 (type B, Table 3). In each of these experiments the mating mixture was incubated on a filter membrane and the donor strain was eliminated by nalidixic acid. Again, transfer was not demonstrated except when the recipient w1485 3a was used simultaneously as a control.

We failed to demonstrate transfer between the antibiotic-resistant anaerobic donor strain of *B. clostridiiformis* and *E. coli* recipients, in 18 attempts (type C, Table 3) using the filter membrane technique. The donor strain was eliminated by aerobic incubation of the antibiotic-containing agar plates upon which the mating mixture was spread. One attempt to demonstrate transfer between *B. clostridiiformis* and *B. melaninogenicus* was unsuccessful. Two strains of *B. fragilis* failed to transfer resistance to either *E. coli* strain w1485 or K12-04 which lacks a restriction and modification system ($r^- m^-$).

DISCUSSION

An anaerobic environment was thought to be detrimental to the transfer of R factors between facultative Gram-negative organisms (Mitsuhashi, 1965). Chromosome transfer in *E. coli* can occur at high frequency in an anaerobic environment if the medium provided for the mating mixture is sufficiently enriched (Stallions & Curtiss, 1972). Moodie & Woods (1973) demonstrated that the transfer of normally repressed R factors at a low frequency was little affected by a stringent anaerobic environment. The present report confirms their findings and further demonstrates that the transfer of derepressed plasmids conferring multiple drug resistance (R factors) can occur at a high frequency under anaerobic conditions. This would suggest that high frequency R factor transfer would not be inhibited by anaerobiosis *per se* in the human colon.

The experiments presented here were designed to eliminate the likely causes of failure of conjugal transfer between two organisms. (i) The *E. coli* donor strains used possessed R factors of several compatibility groups. Some were derepressed for donor ability even in the anaerobic environment while others (i.e. the P group plasmid RP4) were previously shown to have broad host ranges. We were not able to demonstrate transfer of antibiotic resistance between these organisms and *Bacteroides fragilis* strains. Although not all compatibility groups were studied, the variety examined would indicate that the com-

patibility group may not be the limiting factor in conjugal transfer between facultative Gram-negative and anaerobic bacteria. (ii) The *E. coli* strains used in the matings with *Bacteroides* donor strains possessed certain characteristics which made them desirable as recipients. *Escherichia coli* strain CSH27 F⁺ is known to be free of plasmid DNA and *E. coli* strain K12-04 lacked the K12 restriction and modification system. (iii) The possibility of incompatibility between the anaerobic donor strains and the *E. coli* recipient organisms due to phage and/or colicin-like activity was eliminated by the demonstration of viability of the donor and recipient strain in each mating mixture. (iv) The *Bacteroides* donor strains exhibited a wide spectrum of high-level antibiotic resistance. In spite of these favourable circumstances we were unable to demonstrate transfer of antibiotic resistance from anaerobic to facultative Gram-negative bacilli in 19 attempts.

The conjugal transfer of antibiotic resistance between *E. coli* and *Bacteroides*, and between *Bacteroides* species, did not occur under anaerobic conditions which supported maximal R factor transfer between *E. coli* strains. However, it is still possible that Gram-negative anaerobic bacteria present in the large bowel of man and animals participate in interbacterial genetic transfer by means of transduction, transformation, or infrequent ($< 10^{-9}$) conjugation. It also remains possible that *Bacteroides* organisms contain resistance plasmids which could be mobilized if an appropriate sex factor became available or a suitable recipient was present.

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