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Transferable Resistance to Cefoxitin in *Bacteroides thetaiotaomicron*

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Cefoxitin resistance, but not resistance to clindamycin, erythromycin, lincomycin, or tetracycline, was transferred by a conjugation-like process from *Bacteroides thetaiotaomicron* UN101, a clinical isolate harboring four kinds of plasmids, to other *Bacteroides* species. Of a sample of 20 cefoxitin-resistant transconjugants, 8 contained all 4 plasmids, 10 contained 1 to 3 plasmids, and 2 contained no plasmids.

Although the transfer of resistance to several antibiotics has recently been demonstrated in *Bacteroides* species (6, 7, 9, 12), there have been no reports of transferable resistance to β-lactam antibiotics in this genus. In this paper, we report on a conjugation-like transfer of cefoxitin resistance from a strain of *B. thetaiotaomicron* to other species of *Bacteroides*.

Four strains of *Bacteroides* were used: *B. thetaiotaomicron* UN101, a multiply antibiotic-resistant isolate from a blood culture at the University of Nebraska Hospital; *B. fragilis* 638 *rfm*, a rifampin- (6) and chloramphenicol-resistant isolate; *B. fragilis* V479-1, harboring plasmid pBF4, which determines resistance to clindamycin (13); and *B. uniformis* V528, a rifampin-resistant isolate (13). Routine growth of bacteria has been previously described (9).

Minimal inhibitory concentrations of antibiotics were determined essentially by the agar dilution method for anaerobic bacteria (11), except that the plates were incubated in an anaerobic chamber instead of anaerobic jars.

Four methods (2, 4, 5, 9) were used to isolate and detect plasmids. Analysis of plasmids was by agarose gel electrophoresis as described previously (9).

Curing experiments were performed by diluting overnight cultures 1:20 in fresh supplemented brain heart infusion broth containing 5 μM ethidium bromide, incubating for 24 h anaerobically at 37°C, and plating dilutions on an antibiotic-free medium. Individual colonies were then tested for antibiotic resistance. Transferability of antibiotic resistance markers was tested by the filter-mating technique as described previously (9).

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B. thetaiotaomicron UN101 was found to be resistant to six antibiotics, but susceptible to chloramphenicol and rifampin. The minimal inhibitory concentration for each antibiotic was as follows (in micrograms per milliliter): cefoxitin, 128; chloramphenicol, 2; clindamycin, >32; erythromycin, >32; lincomycin, >32; penicillin G, >256; rifampin, 0.5; and tetracycline, 8.

Filter-mating experiments showed that only cefoxitin resistance was transferred from UN101 to 638 *rfm*. Selection for the cefoxitin-resistant transconjugants was made on supplemented brain heart infusion agar plates containing 16 μg of cefoxitin per ml and 24 μg of rifampin per ml. No spontaneous cefoxitin- or rifampin-resistant mutants were observed when the parent strains were plated on the selective medium. The frequency of transfer of cefoxitin resistance was approximately 10^{-6} per donor input. The cefoxitin-resistant, rifampin-resistant transcipients were similar to the 638 *rfm* parent in response to the other antibiotics, i.e., resistant to chloramphenicol, but susceptible to clindamycin, erythromycin, lincomycin, and tetracycline. Transfer of cefoxitin resistance did not take place when a broth-mating technique (10) was used.

Since we (9) and others (7) have demonstrated a tetracycline-inducible gene transfer system in *Bacteroides* species, we also studied the transferability of resistance markers in the presence of tetracycline (2 μg/ml). No transfer of tetracycline resistance or clindamycin resistance was observed, and the frequency of transfer of cefoxitin resistance was not affected by tetracycline.

Agarose gel electrophoretic analysis demonstrated the presence of four plasmids in UN101 (Fig. 1). These plasmids, designated pBY10, pBY11, pBY12, and pBY13, had molecular masses of approximately 5.1, 2.9, 2.2, and 2.1 megadaltons, respectively. Digestion of these

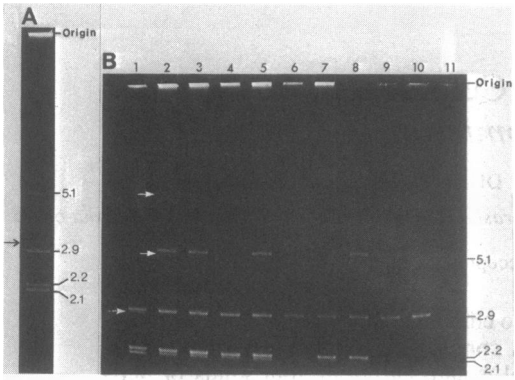

FIG. 1. Electrophoretic analysis of plasmid DNA from (A) donor strain *B. thetaiotaomicron* UN101 and (B) cefoxitin-resistant transconjugants after mating UN101 with the recipient strain *B. fragilis* 638 *rfm*. (A) and (B) represent separate electrophoretic runs. Approximate molecular masses were determined by direct comparison of the plasmids in UN101 and the plasmids in the transconjugants with the following standard plasmids: pSC101, 5.6 megadaltons (Md); RSF1030, 5.5 Md; ColE1, 4.2 Md; and pBR322, 2.6 Md. The four plasmids from UN101 are as follows: pBY10, 5.1 Md; pBY11, 2.9 Md, pBY12, 2.2 Md; and pBY13, 2.1 Md. The 11 representative cefoxitin-resistant transconjugant clones shown in (B) harbored the following plasmids: pBY10, pBY11, pBY12, and pBY13 (lanes 2, 3, 5, 6, and 8); pBY11, pBY12, and pBY13 (lanes 1, 4, and 7); pBY11 (lanes 9 and 10); and no plasmids (lane 11). Open circular forms are designated by arrows.

plasmids under the recommended conditions with restriction endonuclease AluI (Bethesda Research Laboratories, Gaithersburg, Md.) and deliberate nicking with ethidium bromide and light (8) (data not shown) confirmed that (i) four plasmids were present and (ii) the minor plasmid bands represented open circular (i.e., nicked) forms of the plasmids.

Twenty randomly chosen clones of cefoxitin-resistant transconjugants were also screened for plasmids. With respect to plasmid content, as determined by all four plasmid-screening procedures, five transconjugant types were detected among the 20 clones: 8 clones contained all four plasmids; 5 contained pBY11, pBY12, and pBY13; 3 contained pBY11; 2 contained pBY12 and pBY13; and 2 contained no detectable plasmids. Electropherograms of plasmids from 11 of the 20 clones are shown in Fig. 1B.

Representative clones of each of the five transconjugant types and the recipient strain 638 *rfm* were indole negative, arabinose negative, trehalose negative, and rhamnose negative. The donor strain UN101 was indole positive, arabinose positive, trehalose positive, and rhamnose positive. In addition, all of the cefoxitin-resistant transcipients were susceptible to clindamycin, erythromycin, lincomycin, and tetracycline, suggesting that none of the four plasmids carried the genes coding for resistance to these antibiotics.

Serial transmissibility of cefoxitin resistance was tested by mating UN101 with *B. uniformis* V528 (a rifampin-resistant, chloramphenicol- and cefoxitin-susceptible recipient) and selecting for rifampin- and cefoxitin-resistant transconjugants. The transfer of cefoxitin resistance occurred at a frequency of 10^{-7} per donor input. Resistant progeny with none or various combinations of the four plasmids were then mated with the recipient strain 638 *rfm* (rifampin and chloramphenicol resistant), and selection was made for progeny resistant to both cefoxitin and chloramphenicol. No such doubly antibiotic-resistant transcipients were detected.

Ethidium bromide was used in an attempt to cure the antibiotic resistance markers in UN101. Examination of 250 clones after growth of UN101 in the presence of ethidium bromide did not reveal any cefoxitin-, clindamycin-, or tetracycline-susceptible derivatives. *B. fragilis* V479-1, which harbors plasmid pBF4 coding for resistance to clindamycin (14), was used as a positive control and was found to produce clindamycin-susceptible derivatives after growth in the presence of ethidium bromide.

The mechanism of transfer of cefoxitin resistance from UN101 to 638 *rfm* was characterized. Since DNase did not reduce the frequency of transfer of cefoxitin resistance compared with the $MgSO_4$ control (Table 1), the transfer of cefoxitin resistance was not by genetic transformation. Similarly, the transfer was probably not by transduction since the incubation of either (i) cell-free filtrates of donor cells or (ii) chloroform-treated donor cells with recipient cells did not result in the production of cefoxitin-resistant transconjugants (Table 1). Transfer of cefoxitin resistance also did not occur when the donor and recipient cells were separated by a membrane filter (Table 1). It therefore appears that the transfer of cefoxitin resistance is by a conjugation-like mechanism.

The absence of detectable plasmid DNA in some of the cefoxitin-resistant transconjugants suggests that the cefoxitin-resistance genes either are not carried on any of the four plasmids or are carried on one or more plasmids which can integrate into the chromosome of the transconjugants, as with an episome. Another possibility is that the cefoxitin resistance genes are located on conjugative transposons, as has been observed in *Streptococcus faecalis* (3). However, this is unlikely, since the progeny of the mating between UN101 and V528 did not become donors of cefoxitin resistance. The inabil-

TABLE 1. Characterization of mode of transfer of cefoxitin resistance from *B. thetaiotaomicron* UN101

Treatment	Frequency of transfer[a]
Control	4.0×10^{-6}
DNase + $MgSO_4$ in mating mix[b]	3.1×10^{-6}
$MgSO_4$ in mating mix[c]	3.0×10^{-6}
DNase + $MgSO_4$ in mating mix and mating agar plates[d]	2.9×10^{-6}
$MgSO_4$ in mating mix and mating agar plates[e]	3.1×10^{-6}
Cell-free filtrates of donor added to recipient[f]	$<10^{-9}$
Chloroform-treated donor cells added to recipient[g]	$<10^{-9}$
Donor and recipient separated by membrane filter	$<10^{-9}$

[a] Frequency of transfer was calculated as the number of transcipients per donor input. *B. fragilis* 638 *rfm* was the recipient.

[b] The donor strain in supplemented brain heart infusion broth was treated with 100 μg of DNase per ml (Sigma Chemical Co., St. Louis, Mo.) in 5 mM $MgSO_4$ for 45 min at 37°C and then mixed with the recipient strain. The cell mixture (mating mix) was then collected onto membrane filters. The filters were placed cell side up onto the surface of antibiotic-free supplemented brain heart infusion agar plates. After being incubated in an anaerobic glove box at 37°C for 48 h, the cells were suspended in 1 ml of supplemented brain heart infusion broth. Appropriate dilutions were plated onto selective media containing 16 μg of cefoxitin per ml and 24 μg of rifampin per ml.

[c] The donor cells were treated as above, but only with 5 mM $MgSO_4$.

[d] The donor cells were treated as above with DNase and $MgSO_4$, but 100 μg of DNase per ml in 5 mM $MgSO_4$ was also present in the agar medium of the mating plates.

[e] The donor cells were treated as above, but only with 5 mM $MgSO_4$, which was also present in the agar medium of the mating plates.

[f] A 0.5-ml sample of a cell-free filtrate from an overnight culture of strain UN101 was added to 0.5 ml of strain 638 *rfm* and cells were collected on membrane filters. The filter was placed on a supplemented brain heart infusion agar plate (cell side up), and an additional 0.2 ml of cell-free filtrate was added to the cells on the filter. After 48 h, the cells were plated onto a selective medium.

[g] Chloroform (0.5 ml) was mixed with 0.5 ml of suspension of donor cells. The mixture was incubated at about 23°C for 20 min. After evaporation of the residual chloroform from the removed aqueous phase, it was mixed with recipient cells. The filter-mating protocol as previously described was then followed.

ity of transconjugants to transfer cefoxitin resistance may be analogous to the observation that most transconjugants obtained after mating with *Escherichia coli* Hfr strains do not become conjugative donors (1). It is unlikely that a very large plasmid which has escaped detection is involved in the transfer process, since growth of the donor strain in the presence of ethidium bromide did not result in antibiotic-susceptible derivatives.

The demonstration of the transfer of cefoxitin resistance genes from a strain of *B. thetaiotaomicron* to two other species of *Bacteroides* suggests the possibility of widespread conjugative acquisition of cefoxitin resistance by different species of *Bacteroides*.

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