Detection of Conjugal Transfer Systems in Oral, Black-Pigmented *Bacteroides* spp.

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Oral, black-pigmented *Bacteroides* spp. are important pathogens in oral anaerobic infections and dental disease. We detected conjugation systems in isolates of *Bacteroides denticola* and *Bacteroides intermedius* that transferred tetracycline resistance (Tet') and penicillin resistance to *Bacteroides buccae* and to *Bacteroides fragilis*, an intestinal *Bacteroides* species. A cloned Tet' gene from *B. fragilis* hybridized to the transferable Tet' locus in the oral strains, indicating that genetic exchange occurs between these two groups of anaerobes.

Black-pigmented *Bacteroides* spp. are important inhabitants of the human oral cavity and constitute a major component of the normal anaerobic gram-negative mouth flora. These organisms are frequently encountered in anaerobic infections of the head, neck, and respiratory tract (4). In addition, certain species of black-pigmented *Bacteroides* spp., notably, *Bacteroides gingivalis* and *Bacteroides intermedius*, have been implicated in the pathogenesis of periodontal disease (7, 13). Genetic studies of these organisms have not been reported, and most of the genetics research concerning anaerobic bacteria has been done with intestinal *Bacteroides* strains (the *Bacteroides fragilis* group) and *Clostridium* spp. (8, 11). Black-pigmented *Bacteroides* spp. are not closely related to *B. fragilis* by DNA homology, and the extent to which the genetics of *B. fragilis* can be applied to the black-pigmented strains is unknown. Studies in *B. fragilis* have discovered a novel gene transfer system resembling conjugation that operates in the absence of detectable plasmids and transfers resistance to tetracycline (Tet') and, in some strains, resistance to clindamycin as well (8, 11). Transferable Tet' loci from intestinal *Bacteroides* strains have recently been cloned in *Escherichia coli* and reintroduced into *Bacteroides* spp. (12; D. G. Guiney, P. Hasegawa, K. Bouic, and B. Matthews, Mol. Microbiol., in press). The development of widespread resistance to tetracycline and penicillin in black-pigmented *Bacteroides* spp. (2, 3) prompted us to examine these organisms for the presence of antibiotic resistance transfer systems similar to those seen in intestinal *Bacteroides* spp.

*Bacteroides denticola* 10553 is a clinical isolate that was obtained from the Microbiology Laboratory, University of California, San Diego Medical Center, and identified by standard tests. Strain 10553 produced pigment on blood agar, was bile sensitive, was resistant to tetracycline (MIC, 20 μg/ml) and penicillin (MIC, 10 μg/ml), and produced a penicillinase that was detected by the Cephanase disk test. Strain 10553 does not contain any plasmid DNA detectable by the technique of Currier and Nester (5; data not shown). To investigate the ability of *B. denticola* 10553 to transfer antibiotic resistance, we mated this strain with an antibiotic-sensitive isolate of the nonpigmented species *Bacteroides buccae*, designated 8062 (MIC of tetracycline or penicillin, less than 0.5 μg/ml). A spontaneous rifampin-resistant (Rif') mutant of strain 8062 was selected by stepwise growth on increasing concentrations of rifampin up to 80 μg/ml. All conjugation procedures were done on nonselective plates as described previously for *B. fragilis* (6), and the results are shown in Table 1. The transfer of the Tet' locus from *B. denticola* to *B. buccae* was readily detected, and the transfer frequency was not affected by growing the donor in the presence or absence of tetracycline. Tet' transconjugants were confirmed to be *B. buccae* by their lack of pigment on blood agar and their ability to ferment salicin, as *B. denticola* 10553 does not ferment salicin. Next, we studied the host range of the strain 10553 transfer system in a mating with the intestinal *Bacteroides* strain, *B. fragilis* 638, a spontaneous Rif' isolate that is frequently used as a recipient in *Bacteroides* genetic studies (6, 10). Table 1 shows that strain 10553 transferred the Tet' locus to *B. fragilis* 638 at a 100-fold lower frequency than it did to *B. buccae* and that the transfer was not affected by tetracycline pretreatment. Strain 638 Tet' transconjugants were verified by their lack of pigment and their ability to grow on bile. Furthermore, a Tet' 638 transconjugant was capable of retransferring the Tet' locus to a ciprofloxacin-resistant (Cp') mutant of *B. fragilis* 638 (Table 1), indicating that a functional conjugation system is transferred with the Tet' gene.

To demonstrate the presence of a conjugation system in another black-pigmented species, we used *B. intermedius* M87-1738, a Tet' and penicillin-resistant strain obtained from J. Slots (University of Pennsylvania, Philadelphia). This *B. intermedius* strain transferred the Tet' locus at about a 10-fold higher frequency than *B. denticola* 10553 did (Table 1). Next, we tested 25 of the *B. buccae* Tet' transconjugants from matings with both *B. denticola* and *B. intermedius* for penicillin resistance, and all grew on plates containing 20 μg of penicillin per ml and produced penicillinase. These results suggest that transfer of penicillin resistance may be linked to Tet' in both *B. denticola* and *B. intermedius*.

Gene transfer by *B. denticola* has the properties of a conjugation system rather than those of transformation or transduction. Transfer was resistant to DNase treatment, and no transfer was seen with a sterile filtrate of the donor culture (Table 1). These results are similar to those obtained with the Tet' transfer systems of intestinal *Bacteroides* spp. (8, 11).

The similarities between the Tet' transfer systems of black-pigmented and intestinal *Bacteroides* spp. prompted us to examine these loci for DNA homology. We used the cloned Tet' region from *B. fragilis* 1126 (Guiney et al., in
TABLE 1. Transfer of tetracycline resistance by Bacteroides strains

<table>
<thead>
<tr>
<th>Step</th>
<th>Donor</th>
<th>Recipient</th>
<th>Pretreatment with tetracycline</th>
<th>Tet' transfer frequencya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfer by B. denticola 10553</td>
<td>B. denticola</td>
<td>B. buccae 8062 Rif'</td>
<td>+</td>
<td>2 × 10⁻⁵</td>
</tr>
<tr>
<td></td>
<td>B. denticola</td>
<td>B. buccae 8062 Rif'</td>
<td>+</td>
<td>1 × 10⁻⁵</td>
</tr>
<tr>
<td></td>
<td>B. fragilis 638 Rif'</td>
<td>-</td>
<td>3 × 10⁻⁷</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B. fragilis 638 Rif'</td>
<td>+</td>
<td>4 × 10⁻⁷</td>
<td></td>
</tr>
<tr>
<td>Retransfer from B. fragilis 638 Tet'</td>
<td>B. fragilis 638 Tet'</td>
<td>B. fragilis 638 Cp'</td>
<td>+</td>
<td>2 × 10⁻⁷</td>
</tr>
<tr>
<td>Transfer by B. intermedius M87-1738</td>
<td>B. intermedius</td>
<td>B. buccae 8062 Rif'</td>
<td>+</td>
<td>3 × 10⁻⁴</td>
</tr>
<tr>
<td>Transfer with DNase treatment and by a culture filtrate</td>
<td>B. denticola</td>
<td>B. buccae 8062 Rif'</td>
<td>+</td>
<td>0.2 × 10⁻⁵</td>
</tr>
<tr>
<td></td>
<td>B. denticola</td>
<td>B. buccae 8062 Rif'</td>
<td>+</td>
<td>0.8 × 10⁻⁵</td>
</tr>
<tr>
<td></td>
<td>B. denticola (sterile filtrate)</td>
<td>B. buccae 8062 Rif'</td>
<td>+</td>
<td>&lt;10⁻⁹</td>
</tr>
</tbody>
</table>

a Transfer frequency is the number of Tet' transconjugants divided by the number of viable donors at the end of mating. The recipients were present in a two- to threefold excess. Following mating, serial dilutions of cells were plated onto BHI-HC medium (6) containing (i) tetracycline (1 µg/ml) and rifampin (80 µg/ml) to detect transconjugants, (ii) tetracycline (1 µg/ml) only to enumerate the donors, and (iii) rifampin (80 µg/ml) only to verify an excess of viable recipients.

b The mating plates contained 1 mg of DNase (Sigma Chemical Co., St. Louis, Mo.) per ml.

c The sterile filtrate was prepared by centrifuging 10⁶ donor cells and passing the supernatant through a 0.45-µm-pore-size filter (Millipore Corp., Bedford, Mass.).

press) as a hybridization probe to detect homologous sequences in B. denticola 10553 and in B. intermedius M87-1738. Both 10553 and M87-1738 have a 9-kilobase EcoRI band with strong homology to the probe (Fig. 1). Analysis of M87-1738 by the technique of Currier and Nester (5) revealed two large cryptic plasmids, but neither of these hybridized to the Tet' probe. Instead, the homology in strain M87-1738 was contained in the chromosomal DNA band (data not shown). By using strain 10553 as the donor, the hybridizing EcoRI band was transferred with the Tet' gene into three independent B. buccae transconjugants (Fig. 1, lanes D, E, and F). The homologous EcoRI band in the black-pigmented strains had a different size than those in B. fragilis 1126, indicating a difference in EcoRI sites in and around the homologous region. However, strong homology was seen at high stringency, indicating close conservation of the Tet' sequences. Faintly hybridizing bands were also seen in all the strains, including B. buccae. Similar faint bands have been found in tetracycline-sensitive B. fragilis strains (Guiney et al., in press), indicating that some cross-hybridizing sequences are widely distributed in Bacteroides spp.

These results demonstrate that transferable Tet' loci in oral, black-pigmented Bacteroides spp. are closely related to similar transferable genes in the intestinal B. fragilis group of organisms. In addition to strong DNA homology, the systems from oral and intestinal strains have common phenotypic properties. (i) The Tet' locus is not encoded by detectable plasmids, and (ii) gene transfer occurs by a conjugation-like process at frequencies of 10⁻⁴ to 10⁻⁷. Most of the Tet' transfer systems in B. fragilis, including strain 1126, are enhanced by pretreatment with tetracycline (9, 10; Guiney et al., in press), but transfer by B. denticola 10553 is not affected. These results indicate that the control mechanisms regulating the transfer of the Tet' genes of B. fragilis 1126 and B. denticola 10553 are different, despite the close homology between their Tet' loci.

The host range of the B. denticola conjugation system extends to nonpigmented oral B. buccae and to intestinal B. fragilis isolates. The presence of these Tet' transfer systems accounts for the widespread development of Tet' in Bacteroides spp. The cotransfer of Tet' and penicillin resistance in black-pigmented strains is particularly significant, since these organisms were originally considered to be sensitive to penicillin, an antibiotic that is commonly used to treat anaerobic infections that originate in the human oral cavity. However, a dramatic increase in penicillin resistance has been seen in these strains (3). Our results document the existence of genetic transfer between oral and intestinal Bacteroides spp. and indicate important similarities between the genetic systems of these two groups of anaerobes.

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We thank J. Slots for sending isolates of black-pigmented Bacteroides spp.

LITERATURE CITED


FIG. 1. Southern hybridization between the Tet' gene from B. fragilis 1126 and whole-cell DNA from Bacteroides strains. The probe was a 7.8-kilobase KpnI-HincII fragment from pPH7 (Guiney et al., in press) and was labeled with biotin-dATP and hybridized under high-stringency conditions as described previously (1; Guiney et al., in press). Lane A, B. intermedius M87-1738; lane B, B. fragilis 1126; lane C, B. denticola 10553; lanes D, E, and F, B. buccae 8062 Tet' transconjugants from a mating with strain 10553; lane G, B. buccae 8062.


