Transfer of Conjugal Elements in Oral Black-Pigmented *Bacteroides* (*Prevotella*) spp. Involves DNA Rearrangements

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Conjugal genetic elements in isolates of oral black-pigmented *Bacteroides denticola* (*Prevotella denticola*) and *B. intermedius* (*P. intermedia*) transfer tetracycline and penicillin resistance in the absence of plasmids. Transverse alternating-field electrophoresis of restricted chromosomal DNAs from transconjugants revealed rearrangements indicating that transfer and insertion can involve more than one 60-kb copy of the elements and occurs at strongly preferred sites in the recipient chromosome.

Conjugal elements that transfer resistance to tetracycline (Tc^r) and penicillin together have recently been described in *Bacteroides denticola* and *B. intermedius* (3). These black-pigmented organisms, also designated *Prevotella denticola* and *P. intermedia*, respectively, in recent taxonomy, constitute major components of the oral anaerobic flora and are also important pathogens in diseases of the head, neck, and respiratory tract (2, 11). The Tc^r locus in these oral organisms is homologous to the Tc^r genes of intestinal *Bacteroides* spp. (3). In intestinal *Bacteroides* spp., all known Tc^r loci have been located on the chromosome, often in association with conjugal elements greater than 50 kb in size that transfer from the donor chromosome into the recipient chromosome (1, 4, 8, 9). These conjugal factors, designated tetracycline resistance transfer elements, are frequently induced by pretreatment with tetracycline and may transfer erythromycin-clindamycin resistance as well. Cryptic conjugal elements capable of transposition into plasmids have also been described and possess certain properties of conjugal transposons (1, 5, 6, 10). However, transposition of the Tc^r transfer elements into plasmids has not been seen, and the mechanisms of conjugal transfer and insertion in the recipient remain obscure. Recent evidence indicates that more than one site is used in the recipient but insertion is not random (1).

The conjugal factors discovered in *B. denticola* and *B. intermedius* are similar to the intestinal *Bacteroides* Tc^r elements in the property of transferring from the donor chromosome to the recipient chromosome (3). The oral *Bacteroides* elements are distinct in not requiring tetracycline induction and in cotransferring penicillin rather than clindamycin resistance. In this study, we used transverse alternating-field electrophoresis (TAFE) to examine the transfer of these elements and the locations of insertions in the recipients.

Transfer of the Tc^r elements from *B. denticola* 10553 and *B. intermedius* M87-1738 to *B. buccae* 8062 was accomplished as described previously (3). In each case, five independent Tc^r transconjugants were saved from separate mating experiments for use in the TAFE analysis. These transconjugants, as well as the donor and recipient strains,

were embedded in low-melting-point agarose, lysed with proteinase K-sodium dodecyl sulfate, washed, and digested overnight with the appropriate restriction enzyme (4). The digested plugs were inserted into a 1% agarose gel and subjected to TAFE in a Geneline apparatus (Beckman Instruments, Inc., Fullerton, Calif.). The gels were stained with ethidium bromide, photographed, and then transferred by Southern blotting to nitrocellulose. The membranes were probed with a biotin dATP-labeled 7.8-kb *KpnI-HincII* fragment from pPH7 containing the Tc^r locus from *B. fragilis* 1126 (4).

Figure 1A shows a *PacI* digest of total cellular DNA from the donor, *B. denticola* 10553 (lane 7), the recipient, *B. buccae* 8062 (lane 6), and five independent transconjugants selected for transfer of Tc^r (lanes 1 to 5). In recipients 8062-1 and 8062-5, the 360-kb *PacI* band of 8062 has been replaced by a larger band at 410 kb. The accompanying hybridization (Fig. 1B) shows that this 410-kb band contains the Tc^r locus. In three other recipients, no change in the 360-kb band was seen, but there was hybridization with a 180-kb band (lanes 2, 3, and 4), which was also present in the 8062-1 transconjugant. An additional change in 8062-1 is a size increase in the large 480-kb *PacI* band (lane 1). Since the Tc^r probe does not hybridize with this band (Fig. 1B), this size increase reflects a DNA rearrangement that does not contain the Tc^r gene. Figure 1B also shows that the Tc^r locus in the donor is located on a 220-kb *PacI* fragment. This experiment provides strong confirmation of the preliminary findings that transfer of the Tc^r elements involves DNA recombination events (3). Since two *PacI* fragments hybridized in lane 1, the results for 8062-1 suggest that more than one copy of the Tc^r gene can be transferred and integrated in the recipient. The pattern in Fig. 1B indicates that two insertion sites can be used. Finally, the change in fragment size shows that the transferred DNA must be at least 50 kb long.

To define the Tc^r locus insertions in the recipient further, whole-cell DNA was digested with *SfiI*. The resulting TAFE gel and hybridization are shown in Fig. 2A and B. Figure 2A, lane 6, shows the *SfiI* digestion pattern of the recipient 8062. The remarkable feature is a doublet band at 160 kb, well above the remainder of the *SfiI* bands, which all run smaller than 100 kb. In the transconjugants shown in lanes 1 to 4, this doublet has increased in size to 240 kb (lane 1) and 220 kb (lanes 2 to 4). The hybridization in Fig. 2B showed that

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FIG. 1. Ethidium bromide-stained TAFE gel (A) and Southern blot (B) of *PacI*-digested DNAs from matings using *B. denticola* 10553 as the donor. Lanes: 1 to 5, independent *B. buccae* 8062 Tc^r transconjugants designated 8062-1 through 8062-5; 6, *B. buccae* 8062; 7, *B. denticola* 10553. The gel was run for 30 min at 140 mA with a 4-s switch time and then for 20 h with a 25-s switch time. Lane M contained lambda concatemer molecular weight markers.

the Tc^r -encoding gene was present in each of these new doublet bands. In the transconjugant shown in lane 5 (Fig. 2), no change in the 160-kb doublet was seen, but hybridization to a 90-kb band, also present in the 8062-1 transconjugant (lane 1), was demonstrated. This experiment confirmed the findings from the *PacI* digestion but also showed that particular targets for insertion exist in the *SfiI* doublet fragments present in strain 8062. The simplest interpretation of these results is that duplicate insertions of the Tc^r element occur in each *SfiI* fragment. The larger size of the doublet in 8062-1 was consistent with the *PacI* digest result and suggested that additional DNA was also inserted in this transconjugant. Another possibility is that the *SfiI* fragments in strain 8062 represent alternate conformations of a chromosomal segment that undergoes a reversible DNA rearrangement, such as inversion. In this case, only one insertion at this site could give rise to the two hybridizing bands in different transconjugants if the rearrangement rapidly

FIG. 2. Ethidium bromide-stained TAFE gel (A) and Southern blot (B) of *SfiI*-digested DNAs from matings using *B. denticola* 10553 as the donor. The lanes contained DNAs from the same strains as in Fig. 1, and identical gel conditions were used.

FIG. 3. Ethidium bromide-stained TAFE gel (A) and Southern blot (B) of *SfiI*-digested DNAs from matings using *B. intermedius* M87-1738 as the donor. Lanes: 1 to 5, independent *B. buccae* 8062 Tc^r transconjugants; 6, *B. buccae* 8062. *B. intermedius* M87-1738 DNA was not digested in the TAFE gel plugs and therefore is not shown. The gel was run for 30 min at 140 mA with a 4-s pulse time and then for 20 h with a 15-s pulse time. Lane M contained lambda concatemer molecular weight markers.

reached equilibrium. However, the 90-kb hybridizing band (Fig. 2B, lanes 1 and 5) indicates that at least one other insertion site can also be used.

Transfer of Tc^r from *B. intermedius* M87-1738 was also analyzed by *SfiI* digestion and TAFE as shown in Fig. 3. Figure 3, lane 6, shows the digestion pattern of the recipient, 8062, and confirms the presence of two bands, 170 and 160 kb long. In this mating, all of the transconjugants (lanes 1 through 5) showed identical hybridizing bands at 230 and 220 kb, indicating that these transfer events resulted in insertions only in the two large *SfiI* fragments of strain 8062. No hybridization in the 90-kb region was seen. The increased resolution of this gel allowed us to determine that each of the large *SfiI* bands was increased by 60 kb in the transconjugants. This result is in reasonable agreement with the 50-kb increase in size of the *PacI* fragments seen in Fig. 1A.

To confirm the size of the transferred Tc^r element further, the DNAs from the strains shown in Fig. 3 were digested with *NotI* and run on a TAFE gel. As shown in Fig. 4, new hybridizing *NotI* bands were seen in each transconjugant (lanes 1 through 5) at 260 kb. Close inspection of the recipient, strain 8062 (lane 6) revealed a 200-kb *NotI* fragment that was not present in the transconjugants and that was likely to represent the target of the insertion event. The size increase of 60 kb seen with the *NotI* digest is consistent with the estimates from the *PacI* and *SfiI* analysis.

These studies demonstrated that transfer of Tc^r elements in these *Bacteroides* strains occurs by insertion of DNA from the donor into the recipient. For both transfer elements, preferred insertion sites were demonstrated in the recipient, *B. buccae* 8062, by using separate transconjugants, each selected from an independent mating experiment. Five of five matings involving the *B. intermedius* element and four of five matings of the *B. denticola* element resulted in insertions into the two large *SfiI* fragments of strain 8062. The structural basis for these two fragments, which demonstrate a paucity of *SfiI* sites compared with the rest of the *B. buccae* chromosome, is not known. No plasmid DNA was detected in 8062 by conventional tech-

FIG. 4. Ethidium bromide-stained TAFE gel (A) and Southern blot (B) of *NotI*-digested DNAs from matings using *B. intermedius* M87-1738 as the donor. The lanes contained DNAs from the same strains as in Fig. 3, and identical gel conditions were used.

niques or by analysis of undigested whole-cell DNA in selected transconjugants by TAFE and Southern blotting using the Tc^r probe. However, the possibility of the existence of a very large plasmid, approaching the size of the chromosome, cannot be excluded. The large *SfiI* fragments could represent DNA segments with an evolutionary origin different from that of the bulk of the *B. buccae* chromosome and might share related sequences that act as the target sites for the Tc^r transfer elements. Alternatively, the two fragments could be generated by a rapidly reversible DNA rearrangement process, such as inversion. According to this model, the incoming transfer element would insert in one conformation but the culture would rapidly reach equilibrium between the two alternate conformations, generating the two *SfiI* bands seen to hybridize to the Tc^r probe. Most chromosomal inversions occur at a relatively low frequency, leading to predominance of one conformational form in cultures derived from a single cell. *B. fragilis* plasmid pBF4 contains an invertible DNA segment that is influenced by the presence of conjugal Tc^r elements, such that the ratio of the two forms is dramatically altered (7). However, the constant and nearly equimolar ratios of the two *SfiI* fragments of 8062 in all of the strains examined dictates that the putative DNA rearrangement event would have to occur at a high frequency with an equilibrium ratio close to unity, and no effect of the Tc^r element on the relative intensities of the bands was seen.

The results obtained with three independent restriction enzymes indicate that each Tc^r transfer element is approximately 60 kb long. Despite the uncertainty in the interpreta-

tion of the *SfiI* digests, transfer of more than one copy of the *B. denticola* element was documented in at least one case (Fig. 1 and 2, lanes A). These results are most consistent with a model in which more than one copy of the 60-kb conjugal Tc^r elements can be transferred, with insertion occurring at strongly preferred sites in the recipient. Other DNA rearrangements, not containing sequences detected by the Tc^r probe, were occasionally seen in the transconjugants (new bands in Fig. 3A, lane 5, and Fig. 4A, lane 4). This mechanism in oral, black-pigmented *Bacteroides* strains differs from the transfer of the conjugal Tc^r elements in intestinal *Bacteroides* strains, in which multiple (but nonrandom) insertion sites were found and integration of more than one copy of the element was not reported (1).

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