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Natural Occurrence of Structures in Oral Streptococci and Enterococci with DNA Homology to Tn*916*

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Seventeen oral streptococci and 18 enterococci were tested for the presence of DNA sequences homologous to the conjugative transposon Tn*916* encoding tetracycline resistance. All the strains were resistant to tetracyclines, including minocycline, and most of them were resistant to other antibiotics. Tn*916*-like structures, identified by hybridization of *Hin*cII-digested DNA, were found on the chromosomes of 11 oral streptococci and four enterococci and on two plasmids, pIP1549 and pIP1440, one harbored by an *Enterococcus hirae* strain and the other harbored by an *Enterococcus faecalis* strain. Sequences homologous to Tn*916*, only some of which corresponded to its internal *Hin*cII structure (Tn*916*-modified elements), were chromosomally located in three oral streptococci and two enterococci and were plasmid borne in pIP614 harbored by an *E. faecalis* strain. Nine enterococci and three oral streptococci carried either the Tet M or the Tet O determinant chromosomally, but they carried no other sequences homologous to Tn*916*.

Conjugative transposons are genetic elements that are found in streptococci and enterococci, that can transfer by conjugation from the chromosome of the donor strain to the chromosome of the recipient strain, and that can also transpose to a hemolysin or a cryptic plasmid. Tn*916* is the first conjugative transposon to be discovered (9). It carries the Tet M determinant (3), which encodes resistance to tetracyclines, including minocycline (Tc^r-Mn^r), and has an internal structure defined by *Hin*cII digestion; the sizes of internal *Hin*cII fragments are 5.5, 4.8, 1.6, 1.1, and 0.4 kb, corresponding to the fragments A, B, F, G, and H, respectively, of the Tn*916* restriction map (24). The Tet M determinant is located on the 4.8-kb fragment.

Chromosomal conjugative transposons resembling Tn*916* have so far been found in *Enterococcus faecalis* (Tn*918* [8], Tn*920* [22], and Tn*3702* [15]), *Enterococcus faecium* (Tn*5031*, Tn*5032*, and Tn*5033* [12]), *Streptococcus sanguis* (Tn*919* [11]), and *Streptococcus pneumoniae* (Tn*1545* [5]). In certain cases, these transposons constitute a part of composite chromosomal elements; for example, Tn*3703* is the central part of Tn*3701*, which is found in *Streptococcus pyogenes* A454 (18).

Conjugative and nonconjugative Tn*916*-like chromosomal elements, identified on the basis of their *Hin*cII internal structures, have been reported to exist in group A, B, C, and G streptococci (19), *Streptococcus anginosus* (7), *E. faecalis* (23), *E. faecium* (1), *Aerococcus viridans* (4), and *Clostridium difficile* (14). Tn*916*-like transposons have occasionally been found on conjugative and nonconjugative plasmids in *E. faecalis* (6, 23) and *E. faecium* (1, 12).

The aim of the present study was to trace the dispersion of genetic elements related to Tn*916* in oral streptococci and enterococci and to screen for the existence of elements presenting homology with Tn*916* but having an internal structure different from that of Tn*916*.

MATERIALS AND METHODS

The bacterial strains used in this study (see Tables 1 and 2) were clinical isolates identified as described previously (16, 26). The oral streptococci were isolated from blood, cerebrospinal and pleural fluids, teeth, sinuses, and vaginas between 1979 and 1988. The enterococci were isolated from blood and pus between 1960 and 1987. The two plasmids encoding Tc^r-Mn^r, pIP614 and pIP1440, were originally harbored by *E. faecalis* BM6201 (10) and D397 (23), respectively (Table 2); in this study they were isolated from *E. faecalis* transconjugants.

DNA isolation, digestion by restriction enzymes (*Hin*cII, *Hin*dIII, and *Eco*RI), agarose gel electrophoresis, DNA blotting, DNA-DNA hybridization (under stringent conditions), and labeling of the probes with [α-^{32}P]dCTP were done as reported earlier (18). The probes used in the hybridization experiments were the *Escherichia coli* recombinant plasmids pAM170 (Tn*916* probe) (13), pAT101 (Tet M probe) (21), and pU0A4 (Tet O probe) (25) and the 1.1-kb *Hha*I fragment of pMV158 (Tet L probe) (3). pAM170LT, which is pAM170 without Tn*916* (13), was used as a negative control.

The nomenclature for the tetracycline resistance determinants used in this study is that proposed by Levy et al. (20).

Mating experiments were performed as described previously (7) by using *E. faecalis* JH2-2 (17) as the recipient and tetracycline (4 μg/ml) as the selective agent. Counterselection of donor strains was done with rifampin (100 μg/ml) and fusidic acid (25 μg/ml).

RESULTS

Hybridization with Tn*916*. Chromosomal *Hin*cII Tn*916*-like structures (Tables 1 and 2) were found in almost all the oral streptococci examined here, but they were found only in some enterococci, such as *Enterococcus avium* D330, *Enterococcus durans* D469 and D470, and *Enterococcus hirae* 976/79. Plasmids pIP1549 and pIP1440, harbored by *E. hirae* D468 (this study) and *E. faecalis* D397 (23), respectively, also carried Tn*916*-like structures (Fig. 1, lanes 1 and 2).

Elements homologous to Tn*916* but having a different *Hin*cII structure (Tables 1 and 2) were detected on the chromosomes of *Streptococcus mitis* MT308, *Streptococcus oralis* OR1 and OR2, *E. durans* D281, and *E. hirae* 1709/79 (Fig. 2, lanes 2 to 6) and on the plasmid pIP614 (Fig. 1C, lane 2). The main modification observed in all these structures

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TABLE 1. Sequence homology between Tn*916* and antibiotic-resistant oral streptococci

Bacterial strain	Antibiotic resistance marker[a]	Size (kb) of *Hin*cII chromosomal fragments that hybridized with:	
		Tn*916*[b]	Tet M
S. mitis			
MT305	Em^r Km^r Pc^r Tc^r–Mn^r	5.5, 4.8, 3.1, 2.6, 2.4, 1.6, 1.1, 0.4	4.8
MT306	Em^r Km^r Pc^r Tc^r–Mn^r	5.5, 4.8, 3.1, 1.6, 1.1, 0.4	4.8
MT307	Em^r Tc^r–Mn^r	5.5, 4.6, 3.5, 2.2, 1.6, 1.1, 0.4	4.6
MT308[c]	Em^r Pc^r Tc^r–Mn^r	6.4, 5.5, 4.0, 2.8, 2.4, 1.3, 1.1, 0.4	6.4
MT309	Em^r Km^r Pc^r Sm^r Tc^r–Mn^r	5.5, 4.8, 3.5, 1.6, 1.1, 0.4	4.8
MT310	Tc^r–Mn^r	5.5, 4.8, 3.8, 1.6, 1.1	4.8
MT311	Pc^r Sm^r Tc^r–Mn^r	6.1	6.1
MT312	Em^r Pc^r Tc^r–Mn^r	7.1, 5.5, 4.8, 1.7, 1.6, 1.4, 1.1	4.8
MT313	Em^r Pc^r Tc^r–Mn^r	7.3, 5.5, 4.8, 3.5, 1.6, 1.1	4.8
S. oralis			
OR1[c]	Em^r Pc^r Sm^r Tc^r–Mn^r	8.0, 5.5, 4.0, 2.8, 2.4, 1.7, 1.3, 1.1	8.0
OR2[c]	Em^r Tc^r–Mn^r	18.0, 11.2, 6.3, 2.8, 2.4, 1.3, 1.1, 0.94, 0.4	18.0, 11.2
OR3	Tc^r–Mn^r	6.8, 5.5, 4.8, 2.8, 1.6, 1.1, 0.4	4.8
OR4	Em^r Km^r Sm^r Tc^r–Mn^r	5.5, 4.8, 4.0, 1.6, 1.1, 0.4	4.8
OR5	Em^r Km^r Pc^r Tc^r–Mn^r	6.2, 5.5, 4.8, 4.2, 1.6, 1.1, 0.4	4.8
OR6	Tc^r–Mn^r	5.5, 4.8, 4.4, 4.2, 3.4, 1.6, 1.1, 0.4	4.8
OR7	Pc^r Tc^r–Mn^r	—[d]	—
OR8	Em^r Tc^r–Mn^r	—	—

[a] Abbreviations: Em^r, erythromycin resistance (as well as resistance to other macrolides, lincosamides, and streptogramin B); Km^r, high-level kanamycin resistance; Mn^r, minocycline resistance; Pc^r, penicillin resistance; Sm^r, high-level streptomycin resistance; Tc^r, tetracycline resistance.

[b] Fragments of 5.5, 4.8, 1.6, 1.1, and 0.4 kb are equivalent in size to the internal *Hin*cII fragments of Tn*916* (9); the 0.4-kb fragment was not detected in some strains. This fragment could be truly missing or difficult to detect, as in the case in Tn*916*. The other hybridizing fragments could be junctions between the element and the chromosome.

[c] The structures carried by these strains were considered to be Tn*916*-modified elements.

[d] —, no homology detected wtih Tn*916* or Tet M. In each of these strains sequence homology with the Tet O probe was detected on a 1.8-kb *Hin*cII chromosomal fragment.

was manifested by the lack of a 4.8-kb *Hin*cII fragment, which is present in Tn*916* (9) and in most Tn*916*-like structures (1, 4, 7, 9, 14, 19, 23) (this study). Moreover, only one or two fragments that hybridized with Tn*916* were of the same size (5.5, 1.6, or 1.1 kb) as the internal *Hin*cII fragments of Tn*916*; the other hybridizing fragments were of different sizes. We designated these structures as being Tn*916* modified.

The chromosomal DNAs of the strains carrying Tn*916*-modified structures were also digested with *Hin*dIII or *Eco*RI and were probed with Tn*916*. Two hybridizing *Hin*dIII fragments were detected in MT308 and OR1, as in Tn*916* (9) and in the Tn*916*-like structure of the control strain. Four hybridizing *Hin*dIII fragments were detected in D281, 1709/79, and OR2, suggesting either the existence of two copies of the structures or the presence of several *Hin*dIII sites (Fig. 3A). An *Eco*RI site was present in the Tn*916*-modified elements carried by MT308 and OR2 as well as in the control strain carrying a Tn*916*-like structure but not in the other Tn*916*-modified structures (Fig. 3B).

Hybridization with Tet M, Tet O, and Tet L. In this study, in most of the strains studied that carried Tn*916*-like elements, the Tet M determinant was located on a 4.8-kb *Hin*cII fragment, as it is in Tn*916* (9), and more rarely, Tet M was located on a slightly smaller fragment (4.3 or 4.6 kb) (Tables 1 and 2 and Fig. 1A and B, lanes 2), as in other Tn*916*-like transposons (9, 19). The Tet M determinant was situated in Tn*916*-modified structures on *Hin*cII fragments with sizes larger than 6.0 kb (Tables 1 and 2; Fig. 2, lanes 2 to 6; and Fig. 1C, lane 2). The location of Tet M in Tn*916*-modified structures, determined by using *Hin*dIII- or *Eco*RI-digested DNA, is shown in Fig. 3A and B. Two copies of Tet M were detected on the chromosome of OR2, as shown by hybridization experiments with *Hin*dIII- or *Hin*cII-digested DNA.

In *S. mitis* MT311, as well as in eight enterococci, hybridization with Tn*916* was limited to a single *Hin*cII fragment which was identical in size to that which hybridized with the Tet M probe. The *Hin*cII fragments carrying Tet M in the different strains varied in size (Tables 1 and 2). We considered that these strains carry the Tet M determinant but not the rest of Tn*916*.

Sequences homologous to the Tet O probe were detected on *Hin*cII fragments of 1.8 kb in OR7, OR8, and *Enterococcus gallinarum* D417. No homology with the Tet M probe was found in these strains.

Homology with the Tet L probe was found on 1.1-kb *Hin*cII fragments of pIP1549 and pIP1440.

Conjugative transfer of Tn*916*-like and Tn*916*-modified elements. No detectable transfer into JH2-2 of the Tc^r marker in the 17 oral streptococci ($<10^{-8}$ transconjugants per donor cell) or in *E. avium* D330, *E. durans* D281, or *E. hirae* 1709/79 ($<10^{-9}$) was obtained. Tetracycline-resistant transconjugants were detected at a low frequency of transfer (about 3×10^{-8}) from *E. durans* D469 and D470 and *E. hirae* 976/79 into JH2-2. pIP1549, harbored by *E. hirae* D468, transferred at low frequency (2×10^{-8}), while pIP614 (10) and pIP1440 (23) transferred into JH2-2 at a high frequency (3×10^{-2} and 5×10^{-2}, respectively).

DISCUSSION

Chromosomal Tn*916*-like elements were the predominant genetic structures associated with the Tet M resistance determinant in the oral streptococci examined in the present study, as they were in the *S. anginosus* strains studied previously (7). While such chromosomal elements are also prevalent in *E. faecalis* (9, 23) and *E. faecium* (1, 12), they were detected in only 4 of the 16 enterococci belonging to

TABLE 2. Sequence homology between Tn916 and antibiotic-resistant enterococci

Bacterial strain or plasmid	Antibiotic resistance marker[a]	Size (kb) of *Hin*cII chromosomal or plasmid fragments that hybridized with:	
		Tn916[b]	Tet M
E. avium			
D330	Tc^r $-Mn^r$	5.5, 4.8, 1.8, 1.6, 1.1	4.8
D373	Tc^r $-Mn^r$	10.2	10.2
D387	Tc^r $-Mn^r$	10.2	10.2
D390	Tc^r $-Mn^r$	10.2	10.2
W33[c]	Tc^r $-Mn^r$	10.2	10.2
K461[c]	Tc^r $-Mn^r$	10.6	10.6
E. casseliflavius			
D196	Tc^r $-Mn^r$	2.6	2.6
D235	Tc^r $-Mn^r$	3.4	3.4
E. durans			
D281[d]	Cm^r Em^r Tc^r $-Mn^r$	7.0, 5.5, 5.4, 2.8, 2.4, 1.6	7.0
D469	Em^r Km^r Pc^r Sm^r Tc^r $-Mn^r$	5.5, 4.8, 3.2, 1.9, 1.6, 1.1	4.8
D470	Tc^r $-Mn^r$	5.5, 4.8, 1.6, 1.1	4.8
E. gallinarum			
D417	Tc^r $-Mn^r$	—[e]	—
W32[c]	Tc^r $-Mn^r$	6.5	6.5
E. hirae			
1709/79[d,f]	Em^r Pc^r Sm^r Tc^r $-Mn^r$	7.0, 5.4, 2.8, 2.4, 1.6	7.0
976/79[f]	Em^r Tc^r $-Mn^r$	5.5, 4.8, 3.9, 3.6, 1.6, 1.1, 0.4	4.8
pIPI549[g]	Tc^r $-Mn^r$	8.0, 5.5, 4.3, 1.1, 0.4[h]	4.3
E. faecalis			
pIP614[d,g]	Tc^r $-Mn^r$	7.0, 3.8, 2.8, 2.4, 1.6	7.0
pIP1440[g]	Sm^r Tc^r $-Mn^r$	5.5, 4.3, 1.6, 1.1, 0.4[h]	4.3

[a] Cm^r, chloramphenicol resistance. For the other abbreviations, see footnote *a* of Table 1.
[b] See footnote *b* of Table 1.
[c] These strains were provided by Bridge and Sneath (2).
[d] The elements carried by these strains as well as by pIP614 are considered to be Tn916-modified elements.
[e] See footnote *d* of Table 1.
[f] From the Centers for Disease Control, Atlanta, Ga.
[g] pIP1549 is a conjugative plasmid harbored by *E. hirae* D468 (this study); pIP614 and pIP1440 are harbored by BM6201 (10) and D397 (23), respectively.
[h] pIP1549 and pIP1440 carried the Tet L determinant on a 1.1-kb *Hin*cII fragment.

five other species studied here. More rarely, chromosomal or plasmid-borne elements presenting homology with Tn916 but differing from it in structure (Tn916 modified) were detected in some of the strains studied here. In each of the modified structures, the Tet M determinant was present, although it is carried by *Hin*cII fragments that differ in size from that of the Tet M-carrying fragment (4.8 kb) of Tn916 (9).

The same Tn916-modified structure observed in strains MT308 and OR1 has been found in three *S. anginosus* strains which are, like MT308 and OR1, resistant to penicillins (7). However, this type of modification was not found, as it was in *S. anginosus*, in all the penicillin-resistant oral streptococci studied here.

It is also interesting that Tn916-modified structures with very similar, if not identical, *Hin*cII profiles were detected on the chromosomes or plasmids of several enterococcal strains, i.e., *E. faecium* D297 (1), *E. durans* D281 (Fig. 2, lane 2), *E. hirae* 1709/79 (Fig. 2, lane 3), and pIP614 harbored by *E. faecalis* BM6201 (10) (Fig. 1C, lane 2), which were isolated in 1967 (France), 1962 (France), 1979 (United States), and 1972 (France), respectively. These results suggest that perhaps the Tn916-modified structures are as old as Tn916-like elements.

Several questions arise with regard to the evolution of these genetic elements. First, which of the two types of elements is ancestral? Second, which of them arose by molecular rearrangement of the other? Deletions in an ancestral Tn916-modified element may have given rise to the elements that closely resemble Tn916; conversely, the accretion of additional DNA sequences on an original Tn916-like element may have resulted in the modified structures we observed. Third, why are the Tn916-like elements so prevalent in comparison with the rarity of Tn916-modified structures? This suggests either that the former are ancestral or that they have a greater capacity to become disseminated.

To date, only six enterococcal resistance plasmids have been found to encode Tc^r-Mn^r and to carry genetic elements which share DNA homology with Tn916: pCF10 carrying Tn925 (6), pIP614 (10; this study), and pIP1440 (23; this study) in *E. faecalis*; a large plasmid (12) and pIP1534 (1) in *E. faecium*; and pIP1549 (this study) in *E. hirae*. pIP1440, pIP1534, and pIP1549 carry Tet L in addition to Tet M. Four of the six plasmids transfer by conjugation. Such plasmids may have been formed by the transposition of Tn916-like or Tn916-modified genetic elements from the chromosome either onto a plasmid that already carried the Tet L determinant or onto a resident cryptic plasmid. Transposition onto a cryptic plasmid has so far been reported for the chromosomal conjugative transposons Tn918 (8) and Tn3702 (15).

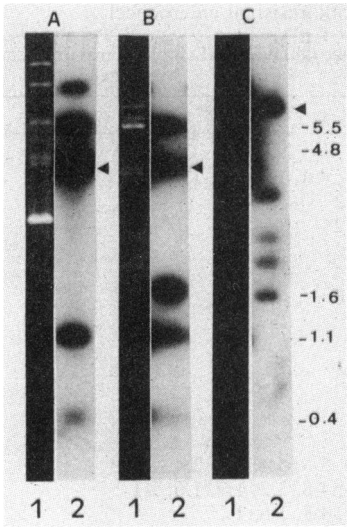

FIG. 1. Hybridization of plasmid DNA with Tn*916* and Tet M probes. Lanes: 1, electrophoretic pattern of *Hin*cII-digested DNA; 2, the same DNA as that in lanes 1 that was transferred to a nitrocellulose filter and probed with α-^{32}P-labeled pAM170. (A) pIP1549, (B) pIP1440, (C) pIP614. The molecular sizes (in kilobases) of Tn*916* internal *Hin*cII fragments are given on the right. Molecular sizes of plasmid *Hin*cII fragments are given in Table 2. Arrowheads indicate the location of Tet M, which was determined by hybridization with pAT101.

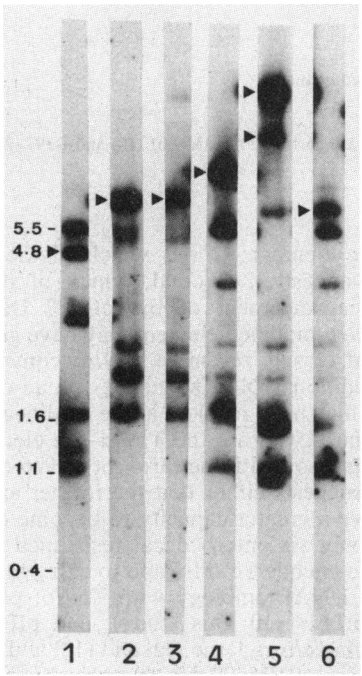

FIG. 2. Hybridization of *Hin*cII-digested chromosomal DNA with Tn*916* and Tet M probes (autoradiogram). The strains shown carried Tn*916*-modified structures. Lanes: 1, Tn*916*-like element (control); 2, D281; 3, 1709/79; 4, OR1; 5, OR2; 6, MT308. Molecular sizes (in kilobases) corresponding to internal *Hin*cII fragments of Tn*916* in the control strain are given on the left. Molecular sizes of Tn*916*-modified structures are given in Tables 1 and 2. The location of Tet M, which was determined by hybridization with pAT101, is indicated by arrowheads.

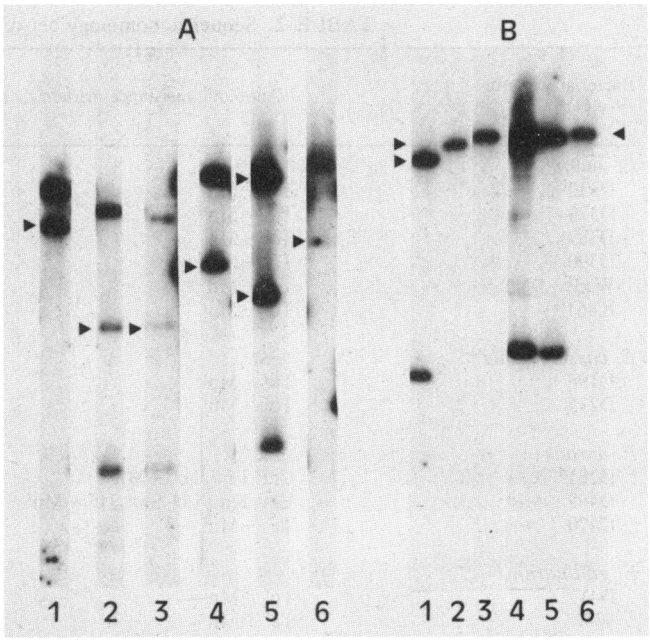

FIG. 3. Hybridization of *Hin*dIII (A)- and *Eco*RI (B)-digested chromosomal DNA of strains carrying Tn*916*-modified structures with Tn*916* and Tet M probes (autoradiograms). For lanes and symbols, see the legend to Fig. 2.

A number of the strains examined here, as well as other streptococci (19) and enterococci (1), carried the Tet M determinant, but apparently did not carry any other sequences of Tn*916*. The vehicle or mechanism by which Tet M enters these strains remains to be discovered.

Finally, it should be pointed out that the division into Tn*916*-like and Tn*916*-modified elements is arbitrary. While it serves as a useful means of characterizing the genetic elements that carry Tet M, there are elements that are more or less modified and that have various degrees of divergence from Tn*916*. Further characterization of these elements will allow them to be classified on a functional rather than a structural basis.

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