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Presence of Chromosomal Elements Resembling the Composite Structure Tn*3701* in Streptococci

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Tn*3701*, carried by *Streptococcus pyogenes* A454, is the first chromosomal composite element to be described; it contains in its central region Tn*3703*, a transposon similar to Tn*916*. A comparison by DNA-DNA hybridization of Tn*3701* with Ω(*cat-tet*) and Tn*3951*, carried by *Streptococcus pneumoniae* BM6001 and by *Streptococcus agalactiae* B109, respectively, revealed that the two latter structures are also Tn*3701*-like composite elements. The DNAs of 27 other antibiotic-resistant group A, B, C, and G streptococci and of *S. pneumoniae* BM4200 showed sequence homologies to Tn*3701* (14 strains, including BM4200), to the regions of Tn*3701* outside of Tn*3703* (5 strains), and to Tn*916* alone (8 strains). The DNAs of five strains did not detectably hybridize with any probe. The *tetM* gene was identified in most chromosomal genetic elements coding for tetracycline-minocycline resistance. Since Tn*3701*-like elements are widely disseminated among antibiotic-resistant streptococci (47% of the 34 strains studied), we propose that Tn*3701* be considered the prototype of chromosomal composite elements.

Over the past 15 years, 111 multiply antibiotic-resistant strains of group A, B, C, and G streptococci (3, 6, 25, 28, 29, 41), *Streptococcus bovis* (28), *Streptococcus pneumoniae* (7, 15, 41), *Streptococcus mitis* (28), *Streptococcus anginosus* (*milleri*) (D. Clermont and T. Horaud, submitted for publication), and *Aerococcus viridans* (8) have been examined in our laboratory for conjugative transfer of resistance markers and plasmid content. Resistance determinants carried by these strains include those for chloramphenicol (Cm^r), erythromycin (Em^r) (macrolide-lincosamide-streptogramin B phenotype), kanamycin (Km^r; high-level resistance), streptomycin (Sm^r; high-level resistance), and tetracycline-minocycline (Tc^r-Mn^r). Antibiotic resistance determinants in streptococci and aerococci appear to be frequently located on the chromosome. Indeed, in 99 strains (90%), no R plasmids have been detected; few strains harbor cryptic plasmids (6, 8). The chromosomal antibiotic resistance markers of about 30% of the strains transfer by conjugation at a low frequency (10^{-4} to 10^{-9} transconjugants per donor cell) into various streptococcal and enterococcal recipients (6, 7, 26, 28, 29; Clermont and Horaud, submitted).

Until now, two types of mobile chromosomal elements carrying antibiotic resistance markers have been described in streptococci and enterococci. Chromosomal conjugative transposons encoding Tc^r-Mn^r, which range in size from 15.0 to 25.3 kilobases (kb), have been found in *Enterococcus faecalis* (10, 11, 19, 39; T. Horaud, F. Delbos, and G. de Cespédès, submitted for publication), *Streptococcus sanguis* (18), and *S. pneumoniae* (9). One of these elements, Tn*1545*, encodes (in addition to Tc^r-Mn^r) Em^r and Km^r (9). Tn*916* (13) is the prototype of this class of mobile genetic elements. Chromosomal conjugative elements larger than 50.0 kb and coding for multiple antibiotic resistance are carried by *Streptococcus agalactiae* (group B) (31), *S. pneumoniae* (48), and *Streptococcus pyogenes* (group A) (23, 33); these elements are designated Tn*3951*, Ω(*cat-tet*), and Tn*3701*, respectively. Molecular analysis of Tn*3701* revealed it to be a composite structure: it includes in its central region a Tn*916*-like transposon, designated Tn*3703*; the antibiotic resistance determinants Em^r and Tc^r-Mn^r of A454, the host of Tn*3701*, were localized on Tn*3703* (33).

One of the purposes of the present study was to compare Tn*3701* with two other chromosomal conjugative elements encoding multiple antibiotic resistance, Tn*3951* (31) and Ω(*cat-tet*) (48), and to determine whether they are composite genetic elements. Additionally, we wished to investigate the extent to which chromosomal genetic elements similar to Tn*3701* and Tn*916* are disseminated among antibiotic-resistant streptococci.

MATERIALS AND METHODS

Bacterial strains and plasmids. The plasmids used in this study are listed in Tables 1 and 2. The streptococcal strains used in this study were antibiotic-resistant clinical isolates. Four of these strains carry conjugative chromosomal elements: *S. pyogenes* A454 (26, 34) contains Tn*3701* (33) encoding Em^r Tc^r-Mn^r, *S. agalactiae* B109 (26, 27) contains Tn*3951* (31) encoding Cm^r Em^r Tc^r-Mn^r, *S. pneumoniae* BM6001 (7, 15, 45) contains the element Ω(*cat-tet*) (48) encoding Cm^r Tc^r-Mn^r, and *S. pneumoniae* BM4200 (7) contains Tn*1545* (9) encoding Em^r Km^r Tc^r-Mn^r. BM4200 is also resistant to chloramphenicol, penicillins, and trimethoprim (7, 41). BM5207 (26) and BM5234 (29) are Cm^r Em^r Tc^r-Mn^r transconjugants obtained from the mating of B109 with *E. faecalis* and *S. sanguis* recipient strains, respectively. BM5320 (7), BM5324 (7), and BM5329 (this study) are Cm^r Em^r Km^r Tc^r-Mn^r transconjugants obtained from the mating of BM4200 with *E. faecalis*, *S. pneumoniae*, and *S. sanguis* recipients, respectively. Twenty-three clinical isolates of streptococci of groups A, B, C, and G as well as three strains, BM6502 (strain 14) (3), BM6103 (strain 16) (29), and BM6106 (strain 17) (29), which were obtained by curing their R plasmids from the original wild-type strains C87 (3), B96 (25), and B97 (27), respectively, are presented in Table 3. Five other streptococci were also studied: four of group A (A453 [28], A456 [6], A472 [6], and BM6402 [5]) and one of group B (B126 [41]) which carry nonconjugative chromosomally Cm^r Em^r Km^r Sm^r, Cm^r Em^r, Em^r, Tc^r-Mn^r,

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TABLE 1. Probes for DNA-DNA hybridization

Probe[a]	Description	Reference
pIP1145[b]	9.6-kb *Eco*RI internal fragment of Tn*3701* cloned into pUC8[c]	33
pIP1147[b]	4.9-kb *Eco*RI internal fragment of Tn*3701* cloned into pUC8[c]	33
pIP1148[b]	4.4-kb *Eco*RI internal fragment of Tn*3701* cloned into pUC8[c]	33
pIP1149[b]	4.0-kb *Eco*RI internal fragment of Tn*3701* cloned into pUC8[c]	33
27.0-kb *Eco*RI fragment of pIP1116[b]	Similar to the 27.0-kb *Eco*RI internal fragment of Tn*3701*[c]	33
pAM170 (Tn*916* probe)[b]	*Eco*RI-D::Tn*916* fragment of pAD1 cloned into pGL101	20
pAM170LT[d]	pAM170 without Tn*916*	20
pAT101 (*tetM* probe)	0.85-kb *Hin*dIII-*Cla*I intragenic fragment of *tetM* cloned into pUC8	37
pUOA4 (*tetO* probe)	*tetO* cloned into pUC8	47
1.3-kb *Kpn*I-*Hpa*I fragment of pAD2 (*erm* probe)	Internal Tn*917* fragment containing *ermB* gene	44
0.7-kb *Mbo*II fragment of pC194 (*cat* probe)	Intragenic fragment of the *cat* gene	24

[a] Plasmids were isolated from *Escherichia coli*, except pIP1116 and pAD2, which were isolated from *E. faecalis*, and pC194, which was isolated from *Staphylococcus aureus*.
[b] None of the probes hybridized with the chromosomal DNA of antibiotic-sensitive streptococci of groups A, B, C, and G, *S. pneumoniae*, or *S. sanguis* (33).
[c] The five *Eco*RI fragments of the 50.0-kb internal region of Tn*3701* are represented in Fig. 1.
[d] No sequence homology was detected between pAM170LT and any of the strains which carried sequences homologous to the Tn*916* probe.

and Cm^r markers, respectively. BM6402 was obtained by curing pIP955 from A449 (5). In addition, we used four transconjugants obtained from the mating of A458 (strain 2), G44 (strain 6), A459 (strain 11), and G41 (strain 12) with group B *Streptococcus* recipient strains BM5154 (Cm^r Km^r Sm^r) (28), BM5106 (Cm^r Em^r) (26), BM5153 (Em^r Tc^r-Mn^r) (29), and BM5105 (Em^r Tc^r-Mn^r) (26), respectively. Except for A449, which was isolated in Japan in 1976, all streptococci and the two *S. pneumoniae* strains were isolated in France between 1975 and 1984.

Media and antibiotics. Media and growth conditions for streptococcal and *E. faecalis* strains have been described previously (25, 27). *Escherichia coli* strains were grown in LB medium (16). Ampicillin (100 μg/ml) and tetracycline (4 μg/ml) were used for the maintenance of *Escherichia coli* plasmids.

DNA isolation. Chromosomal DNA was isolated from *E. faecalis*, from streptococci of groups A, B, C, and G, and from *S. sanguis* and *S. pneumoniae* as described previously (33), except that the lysates were extracted with hexadecyltrimethylammonium bromide (International Biotechnologies, Inc., New Haven, Conn.) before the extraction with phenol-chloroform as described by Ausubel et al. (1).

Plasmids were isolated from *E. faecalis* and streptococcal strains by ultracentrifugation in dye-buoyant density gradients as we described previously (33). Plasmid DNAs from *Escherichia coli* strains were isolated as described by Birnboim and Doly (2), and DNA from *Staphylococcus aureus* was isolated by the technique of Novick and Bouanchaud (40).

DNA analysis. The restriction endonucleases used were *Bgl*II, *Eco*RI, *Hin*cII, *Hin*dIII, *Hpa*I, *Kpn*I, *Sac*I (*Sst*I) (Amersham International, Little Chalfont, England), and *Mbo*II (International Biotechnologies). Electrophoresis of digested DNA was done on 0.7% agarose gels. Bacteriophage λ DNA, double-digested by *Eco*RI and *Hin*dIII, and a 1-kb DNA ladder (Bethesda Research Laboratories, Inc., Cockeysville, Md.) were used as molecular size markers. Plasmid DNA restriction fragments were isolated from 0.7 or 1% low-melting-point agarose gels by the technique described in the Multiprime DNA-labeling kit (Amersham International).

DNA blotting and hybridization. DNA was transferred from agarose gels to nitrocellulose filters by the bidirectional method described by Smith and Summers (46). The DNA probes used in this study are described in Table 1.

Probes were prepared by labeling DNA with [α-^{32}P]dCTP (3,000 Ci/mmol; Amersham International) by the random oligonucleotide primer method described by Feinberg and Vogelstein (17). Hybridization was carried out under stringent conditions (65°C) as we reported previously (33). The radioactivity of the probe (specific activity, about 10^9 cpm/μg) loaded onto each filter corresponded to approximately 10^7 cpm.

RESULTS

Sequence homology among Tn*3701*, Ω(*cat-tet*), and Tn*3951*. DNA-DNA hybridization experiments were performed in which the chromosomal DNAs of BM6001 and B109, which

TABLE 2. Results of hybridization between Tn*3701* and different R plasmids

Plasmid (reference)	Relevant markers[a]	Species of origin (serogroup, strain designation)	Size (kb) of *Hin*dIII fragments hybridizing with: Tn*3701*-derivative probe					*erm* probe
			pIP1145	pIP1147	pIP1148	pIP1149	27.0-kb *Eco*RI fragment of pIP1116	
pEL1 (36)	Em^r Tra$^+$	*S. pyogenes* (A, 13234)	—[b]	—	—	—	3.6	3.6[c]
pIP501 (25)	Em^r Cm^r Tra$^+$	*S. agalactiae* (B, B96)	—	—	—	—	4.8	4.8
pIP955 (5)	Em^r Cm^r Tra$^-$	*S. pyogenes* (A, A449)	—	—	—	—	1.8	1.8
pCO2 (35)	Em^r Cm^r Sm^r Tra$^-$	*S. agalactiae* (B, PB2)	—	—	—	—	4.6	4.6
pAMβ1 (14)	Em^r Tra$^+$	*E. faecalis* (D, S5)	—	—	—	—	3.4	3.4[d]

[a] For abbreviations of antibiotic resistance markers, see Table 3, footnote *b*; Tra$^+$, conjugative transfer; Tra$^-$, nonconjugative transfer.
[b] —, No homology detected.
[c] This result was obtained by Golubkov et al. (21).
[d] This result was obtained by LeBlanc and Lee (32).

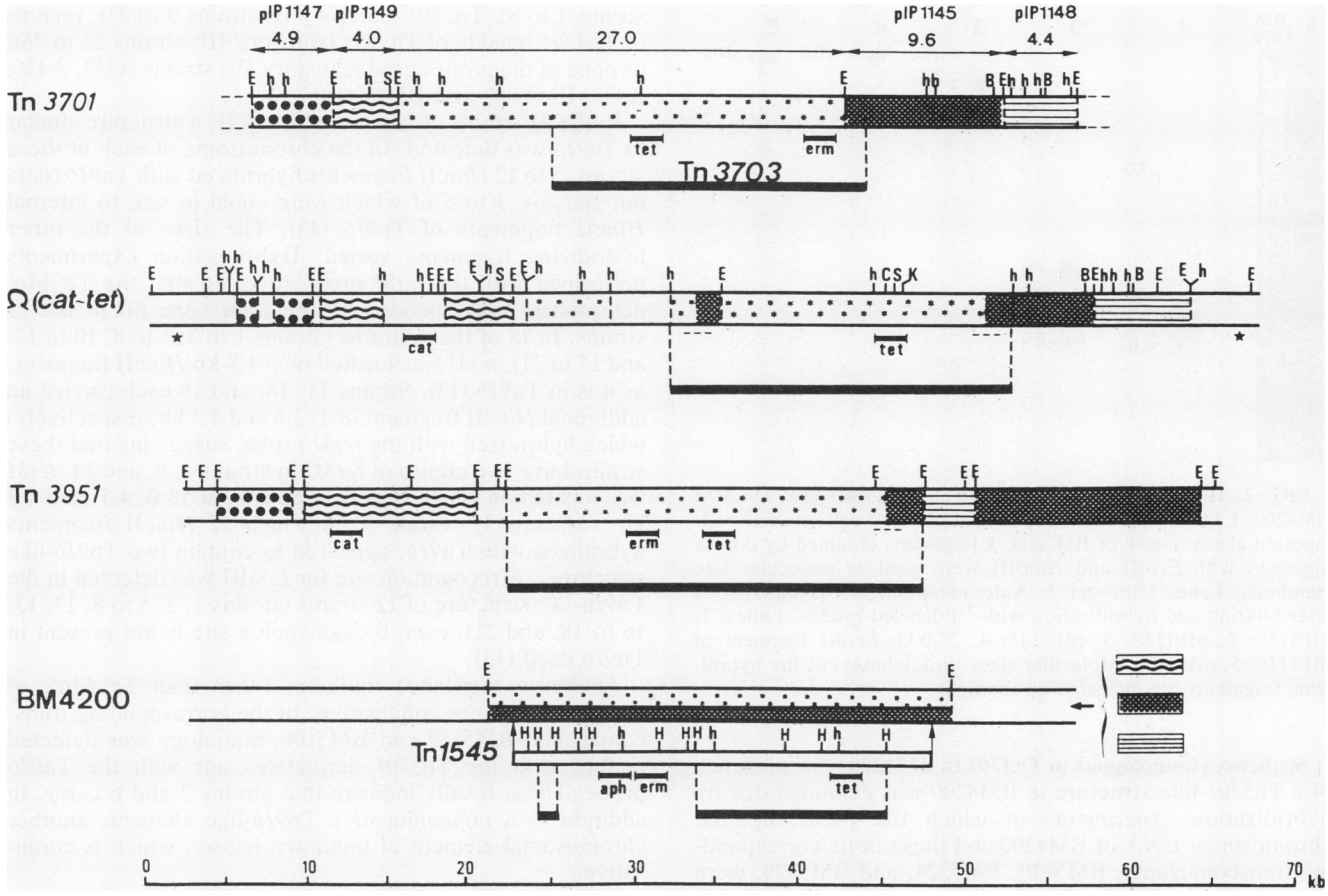

FIG. 1. Hybridization between Tn*3701* and chromosomal DNAs of BM6001, B109, and BM4200. Recognition sites for restriction endonucleases in Tn*3701*, Ω(*cat-tet*), Tn*3951*, and Tn*1545* are localized as reported previously by Le Bouguénec et al. (33), Vijayakumar et al. (48), Inamine and Burdett (31), and Caillaud et al. (9), respectively: *Bgl*II (B), *Cla*I (C), *Eco*RI (E), *Hin*cII (H), *Hin*dIII (h), *Kpn*I (K), and *Sst*I (S). Two additional *Bgl*II sites and one *Sst*I site are positioned on Tn*3701*. The sizes and sites of the five *Eco*RI fragments of Tn*3701* used as probes (Table 1) are indicated above the map. The locations of the extremities of Ω(*cat-tet*) are indicated by stars. In the maps of Ω(*cat-tet*) and Tn*3951* and in the chromosomal DNA of BM4200, the regions of homology with Tn*3701*-derivative probes are indicated by the same symbol as in the Tn*3701* map. Sequence homology with pIP1145, pIP1148, and pIP1149, which could not be positioned on BM4200, is indicated on the right side. Homology symbols: , pIP1147; , pIP1149; , 27.0-kb *Eco*RI fragment of Tn*3701*; , pIP1145; , pIP1148; ---, regions in which homology with pIP1145 and the 27.0-kb *Eco*RI fragment of Tn*3701* was not precisely delimited. In the four maps, the homology with Tn*916* (—), which corresponds to Tn*3703* in Tn*3701* (33), is indicated. Localization of *aph*, *cat*, *erm*, and *tet* ( ) is also indicated.

carry Ω(*cat-tet*) (48) and Tn*3951* (31), respectively, were probed with the five Tn*3701*-derivative probes and the Tn*916* probe (Table 1) (Fig. 1). In order to test the contiguity of the hybridizing fragments, the chromosomal DNA of BM6001 and B109 was digested by *Eco*RI, *Hin*cII, *Hin*dIII, and *Eco*RI-*Hin*dIII. In each chromosome, sequences homologous to Tn*3701* were localized on restriction fragments with sizes that corresponded to the fragments present in the published maps of the chromosomal conjugative elements (31, 48). The deduced locations on Ω(*cat-tet*) and Tn*3951* of the regions of homology with Tn*3701* are presented in Fig. 1.

Between Ω(*cat-tet*) and Tn*3701* we observed not only homology but also a similarity of the restriction maps. Like Tn*3701*, Ω(*cat-tet*) contains sequences homologous to Tn*916* in its central region (Fig. 1). The seven *Hin*cII fragments homologous to Tn*916* (data not shown) could not be precisely localized, since the *Hin*cII restriction map of Ω(*cat-tet*) is not published (48). The presence among the seven hybridizing fragments of four fragments equivalent in size to four of the five *Hin*cII internal fragments of Tn*916* (43) indicates that this region has a structure resembling that of Tn*916*. Moreover, one *Kpn*I site and one *Sst*I site appear to be similarly positioned in Tn*916* (13) and in the Tn*916*-like structure present in Ω(*cat-tet*) which, nevertheless, differed from Tn*916* by the presence of one *Cla*I site and one *Eco*RI site (48). The Tn*916*-like structure in Ω(*cat-tet*) appeared to have an orientation opposite to that of Tn*3703* within Tn*3701* (33).

The comparison of Tn*3701* with Tn*3951* was less precise than that with Ω(*cat-tet*), since only the *Eco*RI restriction map has been published for Tn*3951* (31). B109 carried seven *Hin*cII fragments homologous to Tn*916* (data not shown). Among these, four fragments were equal in size to four of the five *Hin*cII internal fragments of Tn*916* (13). A *Hin*cII chromosomal fragment of about 5.0 kb, which appears to be a doublet in B109, was not detected on the chromosomes of two transconjugants, BM5207 and BM5234. These results indicate that, like Tn*3701* and Ω(*cat-tet*), Tn*3951* also carries a Tn*916*-like structure; it is possible that one of the 5.0-kb *Hin*cII hybridizing fragments detected in B109 corresponds to a second element, encoding only Tc^r-Mn^r, as was previously suggested by Inamine and Burdett (30).

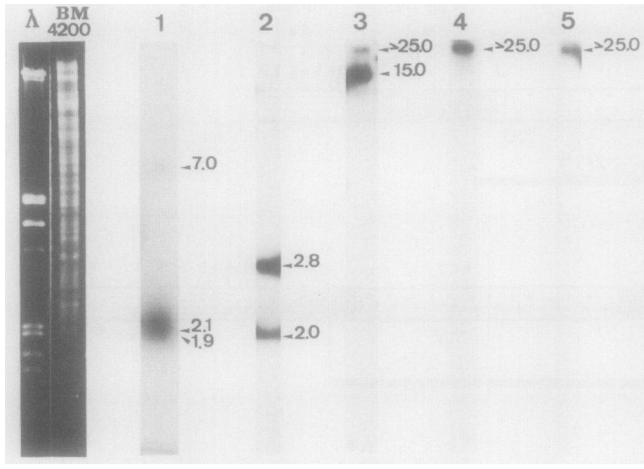

FIG. 2. Homology between Tn*3701* and chromosomal DNA of BM4200. Lanes λ and BM4200, Electrophoretic pattern of *Eco*RI-digested chromosome of BM4200. λ fragments obtained by double digestion with *Eco*RI and *Hin*dIII were used as molecular size standards. Lanes 1 through 5, Autoradiograms of the same DNA after blotting and hybridization with ^{32}P-labeled probes. Lanes: 1, pIP1149; 2, pIP1148; 3, pIP1145; 4, 27.0-kb *Eco*RI fragment of pIP1116; 5, pAM170. Molecular sizes (in kilobases) of the hybridizing fragments are indicated on the right.

Sequences homologous to Tn*3701* in BM4200. The presence of a Tn*3701*-like structure in BM4200 was demonstrated by hybridization experiments in which the *Eco*RI-digested chromosomal DNA of BM4200 and those of its corresponding transconjugants, BM5320, BM5324, and BM5329, were probed with the five Tn*3701*-derivative probes and the Tn*916* probe. Homology was detected on each chromosome with all the probes except pIP1147 (Fig. 1 and 2). With each probe, the hybridizing fragments had the same size in both the transconjugants (not shown) and in the wild-type strain, BM4200. A total of seven *Eco*RI fragments of BM4200 hybridized with Tn*3701*, whereas Tn*1545*, carried by BM4200, did not contain recognition sites for *Eco*RI.

Hybridization of *Hin*dIII- and *Hin*cII-digested chromosomal DNA of BM4200 with the Tn*916* probe showed homology between Tn*1545* and Tn*916*. Tn*1545* is cleaved at three sites by *Hin*dIII (9); we localized sequences homologous to Tn*916* on four *Hin*dIII chromosomal fragments of BM4200. Among these, two fragments of 7.5 and 5.3 kb were the same size as two of the *Hin*dIII internal fragments of Tn*1545* (9) (Fig. 1). Moreover, we detected six *Hin*cII fragments on the chromosome of BM4200 which were homologous to Tn*916*. Four of these fragments (5.5, 4.0, 2.2, and 1.1 kb) were equal in size to the internal *Hin*cII fragments of Tn*1545*. Three of the hybridizing fragments are contiguous on the map and are separated from the fourth by a region in which the *aph* and *erm* genes are localized (9) (Fig. 1). These results indicate that Tn*1545* does not carry sequences homologous to the regions of Tn*3701* outside of Tn*3703* which are, in return, detected in BM4200.

Sequence homology between Tn*3701* and Tn*916* and 31 antibiotic-resistant streptococci. The chromosomal DNA of the strains listed in Table 3 and those of A453, A456, A472, BM6402, and B126 were digested by *Eco*RI and *Hin*cII in order to detect and localize, by DNA-DNA hybridization, sequences homologous to Tn*3701* and Tn*916*. The strains were divided into four categories according to whether they carried sequences homologous to Tn*916* alone (category I; strains 1 to 8), Tn*3701* (category II; strains 9 to 21), regions of Tn*3701* outside of Tn*3703* (category III; strains 22 to 26), or none of the probes used (category IV; strains A453, A456, A472, BM6402, and B126) (Table 3).

In the 21 strains of categories I and II, a structure similar to Tn*916* was detected. In the chromosome of each of these strains, 5 to 12 *Hin*cII fragments hybridized with Tn*916* (data not shown), 3 to 5 of which were equal in size to internal *Hin*cII fragments of Tn*916* (43). The sizes of the other hybridizing fragments varied. Hybridization experiments performed with the *tetM* probe revealed that the Tc^r-Mn^r determinant was encoded by the *tetM* gene (4) in the 21 strains. In 18 of these strains (strains 1 to 3, 5 to 8, 10 to 13, and 15 to 21), *tetM* was located on a 4.8-kb *Hin*cII fragment, as it is in Tn*916* (13). Strains 11, 18, and 19 each carried an additional *Hin*cII fragment (8.1, 3.6 and 4.5 kb, respectively) which hybridized with the *tetM* probe, suggesting that these strains have two copies of *tetM*. In strains 4, 9, and 14, *tetM* was located on single *Hin*cII fragments of 15.0, 4.7, and 4.3 kb, respectively. Strain 5, in which 12 *Hin*cII fragments hybridized with Tn*916*, appeared to contain two Tn*916*-like structures. A recognition site for *Eco*RI was detected in the Tn*916-like* structure of 12 strains (strains 1, 2, 5 to 8, 12, 13, 16 to 18, and 21), even though such a site is not present in Tn*916* itself (13).

Antibiotic resistance markers, other than Tc^r-Mn^r, of strains 2 and 6 are conjugative. In the corresponding transconjugants, BM5154 and BM5106, homology was detected neither with the Tn*3701* derivatives nor with the Tn*916* probe. These results indicate that strains 2 and 6 carry, in addition to a nonconjugative Tn*916*-like element, another chromosomal element of unknown nature, which is conjugative.

In the chromosomal DNAs of the strains of category II, in addition to the Tn*916*-like structure (see above), sequences homologous to the regions of Tn*3701* outside of Tn*3703* were also detected (Table 3). In these strains, the hybridizing fragments were different in size and number from those detected in the wild-type strain A454, which carries Tn*3701*, indicating that in each of these strains the regions of homology with Tn*3701* had a structural organization different from that of Tn*3701* (Fig. 1). However, in strain 10, four hybridizing fragments (4.9, 4.0, 4.4, and 9.6 kb) had the same sizes as those in A454 (33), suggesting that the element carried by this strain, although nonconjugative, is more closely related to Tn*3701* than are the elements carried by the other strains. For six strains (strains 11, 12, 14, and 16 to 18), the results indicated that sequences homologous to Tn*916* and to the regions of Tn*3701* outside of Tn*3703* are clustered. Indeed, four probes, pIP1148, pIP1145, the 27.0-kb *Eco*RI fragment of pIP1116, and Tn*916*, all hybridized with a single *Eco*RI fragment in strains 14 (28.0 kb), 16, 17, and 18 (18.5 kb). That the hybridization profile, whatever the probe, in transconjugants BM5153 and BM5105 was identical with that in wild-type strains 11 and 12 favors the existence of composite elements in these strains.

The antibiotic resistance markers of six strains of categories I and II appeared to be linked to sequences of Tn*916* or of Tn*3701*. Indeed, in strains 3 and 8, the same *Eco*RI fragment (27.0 and >30.0 kb, respectively) hybridized with both Tn*916* and the *erm* probe. In strain 9, the same *Eco*RI fragment (>30.0 kb) also hybridized with these two probes, while another *Eco*RI fragment (7.0 kb) hybridized with both pIP1149 and the *cat* probe. Moreover, in strains 10, 19, and 20, the Em^r determinant was located on >30.0-, 25.0-, and >30.0-kb *Eco*RI fragments, respectively. These fragments

TABLE 3. Hybridization results between Tn*3701* and Tn*916* and the chromosome of different streptococci

Bacterial strain[a]		Antibiotic resistance markers[b]	Size (kb)[c] of *Eco*RI fragment hybridizing with:					
			Tn*3701*-derivative probe					
Category and no.	Designation (serogroup, reference)		pIP1147	pIP1149	pIP1148	pIP1145	27.0-kb *Eco*RI fragment of pIP1116	Tn*916*
I								
1	A471 (A, 5)	Tc^r Mn^r	—[d]	—	—	5.6	18.0	18.0, 5.6
2	A458 (A, 28)	Cm^r Km^r Sm^r Tc^r Mn^r	—	—	—	4.2	18.0	18.0, 4.2
3	B127 (B, 41)	Cm^r Em^r Tc^r Mn^r	—	—	—	27.0[e]	27.0[e]	27.0[e]
4	C88 (C, 5)	Em^r Tc^r Mn^r	—	—	—	>30.0	>30.0	>30.0
5	G42 (G, 26)	Em^r Km^r Sm^r Tc^r Mn^r	—	—	—	5.5, 5.1	>30.0, 27.0	>30.0, 27.0, 5.5, 5.1
6	G44 (G, 26)	Cm^r Em^r Tc^r Mn^r	—	—	—	7.0	19.5	19.5, 7.0
7	G49 (G, 3)	[Cm^r Em^r] Tc^r Mn^r	—	—	—	4.4	>30.0	>30.0, 4.4
8	G50 (G, 5)	Em^r Tc^r Mn^r	—	—	—	4.4	>30.0[e]	>30.0[e], 4.4
II								
9	A451 (A, 5)	Cm^r Em^r Tc^r Mn^r	5.8	7.0, 2.8, 2.6	3.7, 2.3	>30.0[e], 17.0	>30.0[e], 9.8	>30.0[e]
10	A470 (A, 5)	Em^r Tc^r Mn^r	4.9	4.0	4.4	>30.0[e], 25.0, 9.6	>30.0[e], 25.0	25.0
11	A459 (A, 20)	Em^r Tc^r Mn^r	7.0	4.0	7.2, 4.5, 2.2	25.0, 13.0	25.0	25.0
12	G41 (G, 26)	Em^r Tc^r Mn^r	7.8	4.0	4.5, 2.2	13.0, 6.1	19.0	19.0, 6.1
13	G43 (G, 5)	Em^r Km^r Tc^r Mn^r	—	5.3	5.1, 3.8, 3.1	22.0, 15.0, 7.2, 3.7	27.0	27.0, 15.0
14	BM6502 (C, 3)	Tc^r Mn^r	7.2	—	28.0	28.0	28.0	28.0
15	B119 (B, 26)	Tc^r Mn^r	10.0	—	7.6, 7.2	25.0	25.0	25.0
16	BM6103 (B, 29)	Tc^r Mn^r	8.5	—	18.5	18.5, 4.8	18.5	18.5, 4.8
17	BM6106 (B, 29)	Tc^r Mn^r	8.5	—	18.5	18.5, 4.8	18.5	18.5, 4.8
18	B121 (B, 26)	Em^r Tc^r Mn^r	—	—	18.5	18.5, 4.8, 3.2	>30.0, 18.5, 9.5	18.5, 9.6, 3.2
19	A455 (A, this study)	Em^r Tc^r Mn^r	—	—	—	>30.0, 25.0[e]	>30.0, 25.0[e]	>30.0
20	A457 (A, 5)	Em^r Tc^r Mn^r	—	—	—	>30.0[e], 28.0	>30.0[e], 28.0	28.0
21	G52 (G, 28)	Cm^r Em^r Km^r Sm^r Tc^r Mn^r	—	—	—	>30.0, 10.0, 4.2	>30.0, 8.6, 4.2	>30.0, 10.0, 4.2
III								
22	B122 (B, 28)	Em^r Km^r Sm^r Tc^r Mn^r[f]	—	—	—	10.0, 4.8	10.0	10.0
23	G45 (G, 5)	Em^r Tc^r Mn^r	9.0	—	25.0	25.0, 12.0	12.0	12.0
24	G47 (G, 5)	Em^r Tc^r Mn^r	9.0	—	25.0	25.0, 12.0	12.0	12.0
25	B117 (B, 26)	Cm^r Tc^r Mn^r	8.0	—	18.5	18.5, 4.8	—	—
26	B125 (B, 41)	Cm^r Tc^r Mn^r	8.0	—	18.5	18.5, 4.8	—	—

[a] These strains are plasmid free, except strain 2 (A458) and strain 7 (G49), which harbor pIP968, a nonconjugative cryptic plasmid (28), and pIP920, encoding Cm^r Em^r (3), respectively.

[b] Cm^r, Chloramphenicol resistance; Em^r, erythromycin resistance (as well as resistance to macrolides, lincosamides, and streptogramin B); Km^r, high-level kanamycin resistance; Mn^r, minocycline resistance; Sm^r, high-level streptomycin resistance; Tc^r, tetracycline resistance. Conjugative markers are underscored, and plasmid-borne markers are boxed.

[c] The sizes of fragments of >30.0 kb were not precisely calculated.

[d] —, No homology detected.

[e] Fragment hybridizing with the *erm* probe.

[f] The antibiotic resistance markers of this strain transferred separately: Em^r Km^r Sm^r and Tc^r-Mn^r (28).

also hybridized with pIP1145 and the 27.0-kb *Eco*RI fragment of pIP1116 but not with Tn*916*, indicating that in these three strains, the Em^r determinant may be associated with the regions of Tn*3701* outside of Tn*3703*.

In the strains of category III (Table 3), hybridization was detected with several of the Tn*3701*-derivative probes. In strains 22, 23, and 24, sequences homologous to Tn*916* were found on *Eco*RI chromosomal fragments of 10.0, 12.0, and 12.0 kb, respectively. When the chromosomes of these strains were digested by *Hin*cII and probed with Tn*916*, only one hybridizing fragment was found in each strain (9.0, 5.9, and 5.9 kb, respectively). The same hybridization was obtained when the *tetM* gene (Table 1) was used as the probe. Therefore, strains 22, 23, and 24 carry *tetM* but lack other sequences of Tn*916*. In strains 25 and 26, in which no sequences homologous to Tn*916* were found, homology with the *tetO* gene was detected on a 21.0-kb *Eco*RI chromosomal fragment which did not hybridize with the Tn*3701*-derivative probes.

Category IV consisted of strains (A453, A456, A472, BM6402, and B126) in which no homology to any of the probes derived from Tn*3701* or to Tn*916* was detected. None of these strains transfers its antibiotic resistance markers by conjugation (5, 6, 28, 41). Resistance to tetracycline-minocycline of BM6402 was encoded by the *tetO* gene, localized on a 1.8-kb *Hin*cII chromosomal fragment.

Hybridization between Tn*3701* and different R plasmids. The plasmids listed in Table 2 were digested by *Hin*dIII and probed by Tn*3701* derivatives and the *erm* gene (Table 1). Homology was detected on the same *Hin*dIII plasmid DNA fragment with both the 27.0-kb *Eco*RI fragment of pIP1116 (which contains Em^r Tc^r-Mn^r markers [33]) and the *erm* gene (Table 2). No homology was revealed with pIP1145, pIP1147, pIP1148, and pIP1149.

DISCUSSION

The aim of this study was to investigate the frequency of occurrence of genetic elements such as Tn*3701* (33) and Tn*916* (13) among streptococci (34 strains examined).

The composite conjugative element Tn*3701*, present in *S. pyogenes* A454, contains Tn*3703*, a transposon very similar in structure to Tn*916*; Em^r Tc^r-Mn^r determinants of A454 are localized on Tn*3703* (33). The chromosomal conjugative elements Ω(*cat-tet*) (48) and Tn*3951* (31) are structurally similar to Tn*3701*. We conclude that they are composite elements because they contain in their central regions a Tn*916*-like structure. The data obtained here strongly suggest that the conjugative transposon Tn*1545* (9), which appears to be related to Tn*916*, may be a part of a Tn*3701*-like conjugative composite element carried by *S. pneumoniae* BM4200. Another possibility is that BM4200 harbors two independent genetic elements: one, Tn*1545*, similar to Tn*916* and another with homology to the regions of Tn*3701* outside of Tn*3703*.

In 13 of the other 31 strains studied here (Table 3, category II), we detected sequences homologous to Tn*3701*. At least six of these strains (strains 11, 12, 14, and 16 to 18) appear to carry Tn*3701*-like chromosomal composite elements, although not all of them are conjugative. The structural organization of the elements carried by these strains is variable, indicating that different composite elements may exist. Interestingly, strains 14, 16, and 17, although resistant to only tetracycline-minocycline, carry a Tn*3701*-like element. For the other strains of category II, it was not possible to determine whether homology to Tn*916* and to the regions of Tn*3701* outside of Tn*3703* are contiguous or belong to separate structures in the same strain.

Tn*916*-like chromosomal elements have so far been identified in *E. faecalis* (10, 11, 19, 39, 42), *S. sanguis* (18), *S. anginosus* (Clermont and Horaud, submitted), *Enterococcus faecium* (F. Bentorcha, G. de Cespédès, and T. Horaud, submitted for publication), *A. viridans* (8), and *Clostridium difficile* (22). We report here that Tn*916*-like chromosomal elements are also carried by group A, B, C, and G streptococci (Table 3, category I). These elements are nonconjugative in six of the eight strains as well as in most of the *S. anginosus* strains tested. The structure of the Tn*916*-like elements which we detected in *E. faecalis*, *E. faecium*, *S. anginosus*, *A. viridans*, and now in group A, B, C, and G streptococci appears to be preserved with regard to that of Tn*916*, even though most of these elements are cleaved by *Eco*RI. The presence of a Tn*916*-like structure appears always to be associated with the *tetM* gene (4), which is usually located on a 4.8-kb *Hin*cII fragment, as it is in Tn*916* (13). This suggests that the combination of *tetM* with the transposition functions is old and stable. The results obtained here also show that the *tetM* gene is present in genetic elements bearing homology to regions of Tn*3701* outside of Tn*3703*. Three streptococcal strains, one of group A and two of group B, carry the *tetO* gene, which is not linked to sequences homologous to Tn*3701* or Tn*916*.

We have recently described the presence in A454 of an *erm* gene borne by Tn*3703* (33). Similarly, we observed that the antibiotic resistance genes, other than *cat*, of B109 (31), BM6001 (48), and BM4200 (9) are borne by the parts of their conjugative elements related to Tn*916*. These three strains appear to carry *cat* genes (homologous to that of the staphylococcal plasmid pC194 [41]) on an *Eco*RI chromosomal fragment of about 6.7 kb (31, 48; this study). This result suggests that, like the *cat* genes of BM6001 and B109, the conjugative *cat* gene of BM4200 may be carried by a Tn*3701*-like composite element present in this strain. We also observed that the *erm* gene of the most wild-type strains studied here can be associated either with the Tn*916*-like structure or with the regions homologous to the parts of Tn*3701* outside of Tn*3703*. All these data indicate that Tn*916*- or Tn*3701*-like chromosomal genetic elements can carry genes such as those encoding Cm^r, Em^r, Km^r, and Tc^r-Mn^r, the most frequently encountered antibiotic resistance determinants in streptococci and enterococci (T. Horaud and F. Delbos, unpublished data). Furthermore, our results suggest that the apparent ease with which these elements can acquire antibiotic resistance determinants might confer to streptococci a selective advantage and, since they are borne by the chromosome, a stability of the information they carry.

The origin of the genetic elements described here remains unknown. The absence of homology between Tn*3701* and the most common R plasmids harbored by streptococci of groups A and B and by *E. faecalis* suggests that Tn*3701* and related genetic elements are not chromosomally integrated plasmids which lack genes for autonomous replication. Clewell et al. (12) suggested recently that some of the properties of Tn*916* and of the staphylococcal transposon Tn*554* (38) are similar. In order to establish whether Tn*3701* is related to Tn*554*, cross-hybridization experiments were performed between the five Tn*3701* derivatives and pRN4166, which carries Tn*554*; no homology was detected (C. Le Bouguénec, unpublished data).

This report revealed the complexity and diversity of the chromosomal genetic elements encoding antibiotic resistance in streptococci. Indeed, in addition to the two categories of elements, one resembling Tn*916* and the other Tn*3701*, we observed that at least two other categories of elements may exist: one consists of elements related to the parts of Tn*3701* outside of Tn*3703*, and the other consists of elements not yet identified. The observation that the orientation of the Tn*916*-like structure detected in the composite elements Tn*3701*, Ω(*cat-tet*), and Tn*3951* is variable suggests that these elements could result from the insertion of a Tn*916*-like conjugative transposon into preexisting genetic elements, like those found in category III, giving rise to composite elements, like those of category II. Finally, we also report here that several strains can carry at least two independent elements. Since Tn*3701* is the first composite element to be described and is widely spread among streptococci (47% of the strains studied here), we propose that it be considered the prototype of this class of genetic elements.

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