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Characterization of Tn*916*S, a Tn*916*-Like Element Containing the Tetracycline Resistance Determinant *tet*(S)

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We have characterized a transferable tetracycline resistance (Tc^r) element from a *Streptococcus intermedius* isolate. The gene responsible for this resistance was identified by PCR and Southern hybridization as *tet*(S). Furthermore, the genetic support for this determinant was shown to be a conjugative transposon closely related to Tn*916*. This element has been designated Tn*916*S.

Tetracycline-resistant streptococci are frequently isolated from the oral cavity of humans (13), and resistance is most commonly conferred by Tet(M), a ribosomal protection protein often associated with the conjugative transposon (cTn) Tn*916* (4). Tn*916* belongs to a family of cTns that are composed of functional modules (10, 12) involved in conjugation, antibiotic resistance, regulation, and integration and excision. Different members of this family of cTns are comprised of different modules.

The Tc^r determinant *tet*(S) encodes a ribosomal protection protein showing 79% amino acid identity with Tet(M). It was initially identified in a multiresistant *Listeria monocytogenes* strain on a 37-kb conjugative plasmid, pIP811 (2). Subsequently *tet*(S) has been found on plasmid pK214 from *Lactococcus lactis* (8) and in the chromosome of *Enterococcus faecalis* (3). The *tet*(S) gene in *L. lactis* and *L. monocytogenes* is linked to homologues of the Tn*916 orf6*, *orf9*, and *orf7*. In this work, we show that the *tet*(S) gene from a *Streptococcus intermedius* isolate, originally isolated from a 5-year-old human child, is contained within a functional Tn*916*-like element.

All chemicals were purchased from BDH (Poole, United Kingdom). Antibiotics were purchased from Sigma-Aldrich (Poole, United Kingdom) and used at concentrations of 8 μg/ml for tetracycline and 25 μg/ml for rifampin. All enzymes were purchased from Promega (Southampton, United Kingdom), and all growth media were purchased from Oxoid (Basingstoke, United Kingdom). All bacterial strains and plasmids used are shown in Table 1. The primers used are shown in Table 2. The *S. intermedius* strain 15.3T.2 was grown on Iso-Sensitest agar containing 5% defibrinated horse blood (E&O Laboratories, Bonneybridge, United Kingdom) and tetracycline at 37°C in an anaerobic cabinet (Don Whitley Scientific Ltd., Shipley, United Kingdom) containing a mixture of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide. All other strains were grown aerobically at 37°C. The filter-mating recipients *E. faecalis* and *Streptococcus* spp. were grown in brain heart infusion broth.

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Filter-mating experiments were carried out as previously described (14). Transconjugants were selected on brain heart infusion agar, containing 5% horse blood, rifampin, and tetracycline at 4 μg/ml. Spontaneous mutations to rifampin resistance in the donor were not detected. The streptococcal transconjugants were also subcultured onto esculin agar to confirm that they were negative for hydrolysis, in order to distinguish them from the donor, which was positive.

S. intermedius 15.3T.2 was grown overnight, and genomic DNA was extracted with the Yeast and Gram Positive Bacteria Genomic DNA kit (Genetra, Minneapolis, Minn., supplied through Flowgen) according to the manufacturer's instructions. PCR for the detection of a variety of Tc^r genes was carried out as described by Ng et al. (7) and Villedieu et al. (13). Positive PCR products were sequenced with the Big Dye Terminator ready reaction mixture (PE Biosystems, Warrington, United Kingdom) and an ABI310 genetic analyzer (PE Biosystems) or sent to Oswel Sequencing (Romsey, United Kingdom).

Southern blotting and hybridization were carried out with an ECL Direct Nucleic Acid Labeling and Detection system (Amersham Biosciences, Little Chalfont, United Kingdom). Southern blots were probed with pAM120 (Table 1) and PCR products derived from *tet*(S), *tet*(M), and the *int* and *xis* genes of Tn*916* (Table 1). PCR assays were also carried out as previously described by Wang et al. (15) to detect all regions of Tn*916*. The region between *orf13* and *orf6* (RT1 to RT4 on Fig. 1) was sequenced in triplicate.

Tc^r from the *S. intermedius* 15.3T.2 donor was transferable to *E. faecalis* JH2-2, *S. sobrinus*, and *S. sanguinis* at frequencies of 4.5×10^{-7}, 2.5×10^{-7}, and 1.0×10^{-5} per donor, respectively. PCR amplifications specific for *tet*(S) on *S. intermedius* 15.3T.2 and transconjugant genomic DNA demonstrated that *tet*(S) was present. No other Tc^r genes could be amplified. No *tet* genes could be detected in the recipients. PCRs for the entire length of Tn*916* were carried out on *S. intermedius* 15.3T.2, the *E. faecalis* transconjugant, and the *E. faecalis* JH2-2 recipient. *S. intermedius* 15.3T.2 and the transconjugant had PCR amplicons [with the exception of the *tet*(M)-containing amplicon] the same size as the positive control of *Bacillus subtilis* BS34A (which contains a single copy of Tn*916*), indicating that Tn*916*S has the same genetic organization as

TABLE 1. Bacterial strains and plasmids used throughout this study

Strain or plasmid	Comments[a]	Source or reference
Strains		
S. intermedius 15.3T.2	Tc^r donor strain	This study
B. subtilis BS34A	Tc^r *B subtilis*::Tn*916*	11
E. faecalis JH2-2	Rif^r recipient strain	14
E. coli pAT451	Tc^r *E. coli*::pAT451[b]	1
S. sobrinus NCTC 12279 (type strain)	Rif^r recipient strain	Health Protection Agency[c]
S. sanguinis NCTC 7863 (type strain)	Rif^r recipient strain	Health Protection Agency
Plasmids		
pAM120	pUC18::Tn*916*	5
pAT451	pUC18::*tet*(S)	1

[a] Tc^r; tetracycline resistant, Rif^r; rifampin resistant.
[b] pUC18 carrying a 4.5-kb ClaI fragment of pIP811 with the *tet*(S) gene.
[c] www.hpa.org.uk.

Tn*916*. The PCR assay on the recipient (*E. faecalis* JH2-2) yielded no amplicons. The sequence data of the region between RT1 and RT4 (Fig. 1A) showed that *tet*(S) has effectively replaced *tet*(M) with the upstream region lacking repeat regions that are involved in transcriptional control in Tn*916*.

DNA from the parents and one of the transconjugants was subjected to Southern blot analysis (Fig. 2). HindIII digests were probed with *tet*(S), and two hybridizing fragments were observed. As there is one HindIII site in the *tet*(S) gene, the two hybridizing fragments are likely to be Tn*916*S-genome junction regions. A single HincII fragment is seen in both the donor and transconjugant, which corresponds to an internal fragment (9).

When probed with *int*/*xis*, two HindIII fragments are observed in the donor and three are observed in the transconjugant. As this probe should hybridize to one junction fragment within Tn*916*S, this means either that there are two copies of the element in the donor and three in the transconjugant or that there is another *int*/*xis*-containing genetic element in the donor that may have transferred to the recipient. We prefer the latter explanation, as the 4.8-kb hybridizing HindIII fragment with the *int*/*xis* probe is the same size as one of the fragments when *tet*(S) is used as the probe, indicating that the *tet*(S) gene and one of the *int*/*xis* regions are linked. When pAM120 is used to probe the blots, HincII digestion shows similar hybridizing fragments, as would be expected from DNA containing an integrated copy of Tn*916* but with the extra HincII fragment (see above).

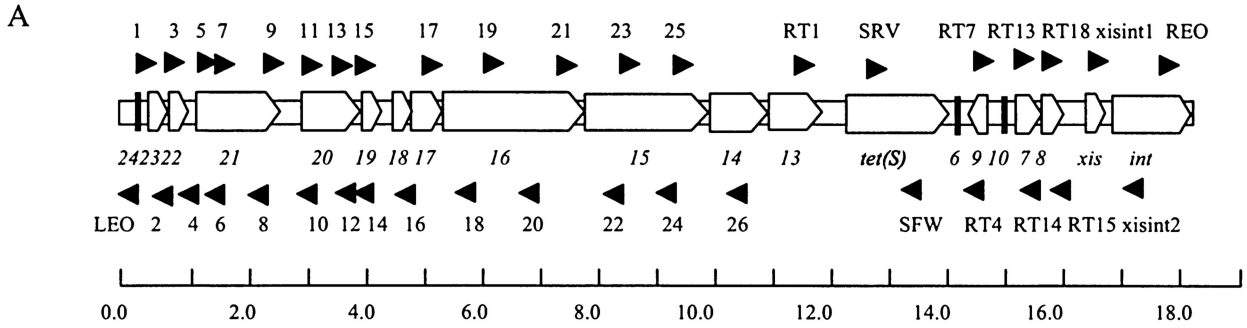
When the blots were probed with *tet*(M), there was no hybridization (data not shown). Taken together, these data indicate the donor strain contains at least two mobile elements containing *xis* and *int*, one of which contains *tet*(S). Both are capable of transfer to the recipient.

The finding of *tet*(S) in the same relative position as *tet*(M) in a broad-host-range Tn*916*-related element supports the view that conjugative transposons are composed of modules that are able to exchange with modules from other elements (10, 12), possibly by homologous recombination. It now seems apparent that not only is Tn*916* involved in the dissemination of *tet*(M), it is also involved in the dissemination of *tet*(S).

TABLE 2. Oligonucleotide primers used in this study

Primer name	Primer sequence or reference	Expected PCR product
1–26	15[a]	*orf24* to *orf14* of Tn*916*
RT1	5′-CTCTATCCTACAGCGACAGC-3′	PCR product containing *orf13*, *orf12*, *tet*(M), and *orf6*
RT4	5′-TCTTTGCGTCTGGCTCTGTA-3′	
RT6	5′-TATGATTTTAGAGCCCTTTGG-3′	PCR product containing *orf6* and *orf9*
RT11	5′-ACAGAGCCAGACGCAAAGAC-3′	
RT7	5′-CTTGTATGCTGGGGTGTTGC-3′	PCR product containing *orf9* and *orf7*
RT14	5′-TAATTCTTCCGCTCGTCGTC-3′	
RT13	5′-AAAGAAAGGGGGTGAAC-3′	PCR product containing *orf7* and *orf8*
RT15	5′-GGTTAATCGCTTCTGTATCG-3′	
RT12	5′-ATTTCACGTTTCTTGTCTGG-3′	Primer reading upstream from within *orf7*
RT18	5′-AAGTATGGTCGTTGATGAAG-3′	Primer reading downstream from within *orf8*
SRV	7	667-bp product of the *tet*(S) gene
SFW	7	
intxis1	6	*xis* and *int* fragment of Tn*916*
intxis2	6	
REO	14	Joint of the circular form of Tn*916*
LEO	14	
SFW(2)	5′-GTGTCCAGGAGTATCTAC-3′	Sequencing between RT1 and RT4
RT1(2)	5′-CTGTCAATTAGATAGCGGG-3′	Sequencing between RT1 and RT4
RT4rev	5′-GATTTGAATTAAAGTGTAAAGGAGG-3′	Primer reading downstream from within *orf6*
RT7rev	5′-ATGTATAGAGTGGTCTACTATGCG-3′	Primer reading upstream from within *orf9*

[a] Thirteen pairs of sequences.



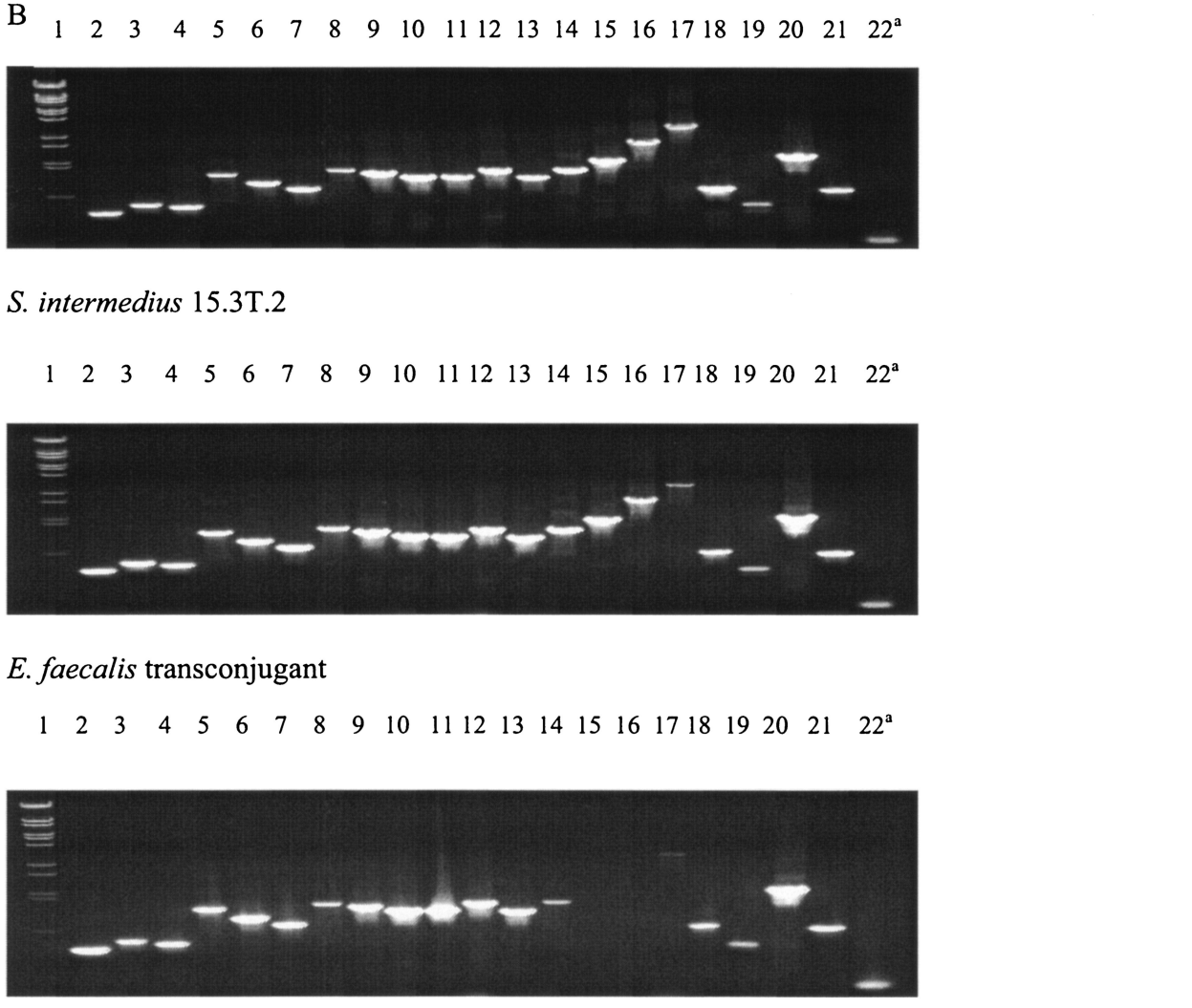


FIG. 1. (A) Predicted schematic of Tn*916*S based on the structure of Tn*916*. Shown are the positions and names of primers (solid triangles) used to amplify the amplicons shown in panel B below. The origins and sequences of the primers are given in Table 1. The last line represents the scale in kilobases. (B) Amplicons from *S. intermedius* 15.3T.2, the *E. faecalis* transconjugant, and BS34A (contains Tn*916*). Lanes: 1, lambda BstEII molecular marker; 2, primers 1 and 2; 3, primers 3 and 4; 4, primers 5 and 6; 5, primers 7 and 8; 6, primers 9 and 10; 7, primers 11 and 12; 8, primers 13 and 14; 9, primers 15 and 16; 10, primers 17 and 18; 11, primers 19 and 20; 12, primers 21 and 22; 13, primers 23 and 24; 14, primers 25 and 26; 15, primers RT1 and SFW; 16, primers RT4 and SRV [reactions 15 and 16 should be negative for BS34A, as this strain does not contain *tet*(S)]; 17, primers RT1 and RT4; 18, primers RT7 and RT14; 19, primers RT13 and RT15; 20, primers RT18 and intxis2; 21, primers intxis1 and intxis2; and 22, primers REO and LEO ([a], this primer pair amplifies the circular form of the element).

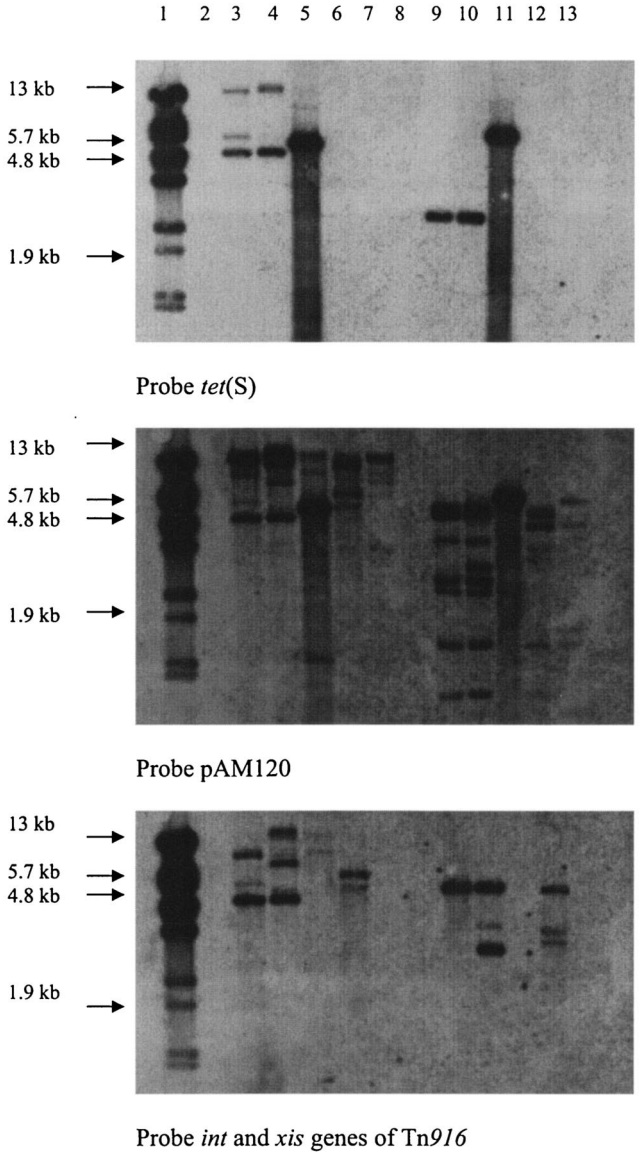


FIG. 2. Southern blot analysis of DNA from parents and transconjugants containing Tn*916*S. Lanes 3 to 7 contained HindIII-digested genomic DNA, and lanes 9 to 13 contained HincII-digested genomic DNA. Lanes: 1, lambda BstEII molecular marker; 2, blank; 3, *S. intermedius* 15.3T.2; 4, *E. faecalis* transconjugant; 5, *Escherichia coli* pAT451; 6, *B. subtilis* BS34A; 7, *E. faecalis* JH2-2; 8, blank; 9, *S. intermedius* 15.3T.2; 10, *E. faecalis* transconjugant; 11, *tet*(S)-positive strain; 12, *B. subtilis* BS34A; and 13, *E. faecalis* JH2-2.

Nucleotide sequence accession number. The sequence of the region between *orf13* and *orf6* has been deposited in GenBank under accession no. AY534326.

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