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Nucleotide Sequence of the 18-kb Conjugative Transposon Tn*916* from *Enterococcus faecalis*

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Conjugative transposon Tn*916* from *Enterococcus faecalis* DS16 encodes tetracycline resistance (Tet M) as well as determinants necessary for its own movement. Determination of the nucleotide sequence of Tn*916* has been completed. The element is 18,032 bp in length and has an overall G+C content of 38.8%. Twenty-four potential open reading frames (ORFs) were identified based on sequence analysis. Similarities of the ORFs to other known determinants, which were revealed by database searches, are discussed.

Transposon Tn*916* (Franke and Clewell, 1981; Gawron-Burke and Clewell, 1982, 1984) represents a unique class of mobile bacterial DNA elements whose members have the ability not only to move from place to place within a cell but also to conjugatively transpose from one cell to another. Movement proceeds via a circular intermediate (Scott *et al.*, 1988) after excision of the element from its donor site. These elements, designated "conjugative transposons," display a remarkably broad host range, especially among gram-positive strains. Their known host range encompasses at least 47 species in 23 genera (both gram-positive and gram-negative) and is expanding as more species are examined (Clewell and Flannagan, 1993). Originally from *Enterococcus faecalis* DS16 (Franke and Clewell, 1981), Tn*916* is the prototype; members of the related family include Tn*1545* (Courvalin and Carlier, 1987; Trieu-Cuot *et al.*, 1991) and Tn*5251* (Ayoubi *et al.*, 1991) from *Streptococcus pneumoniae*, Tn*918* (Clewell *et al.*, 1985), Tn*920* (Murray *et al.*, 1988), Tn*925* (Christie *et al.*, 1987), Tn*3702* (Horaud *et al.*, 1990), Tn*5381* and Tn*5383* (Rice *et al.*, 1992) from *E. faecalis*, Tn*5031*, Tn*5032*, and Tn*5033* from *Enterococcus faecium* (Fletcher *et al.*, 1989), Tn*919* (Fitzgerald and Clewell, 1985) from *Streptococcus sanguis*, and Tn*3704* (Clermont and Horaud, 1994) from *Streptococcus anginosus*. These transposons typically confer tetracycline resistance of the Tet M variety (Burdett *et al.*, 1982), which is mediated at the level of protein synthesis and alleviates the effect of tetracycline on the ribosome (Burdett, 1986, 1991). Multiple antibiotic resistance genes are carried by some, such as Tn*1545* (tetracycline, erythromycin, and kanamycin resistances) and Tn*3704* (tetracycline and erythromycin resistances). Several larger, more complex and unrelated conjugative transposons that encode multiple antibiotic resistances or that carry other determinants, such as for nisin production and sucrose fermentation, have been identified. For reviews of conjugative transposons, see Clewell and Flannagan (1993), Clewell and Gawron-Burke (1986), and Scott (1992, 1993).

Several regions of Tn*916* have been analyzed in some detail. The *tet*(M) gene has been sequenced (Burdett, 1990; Su *et al.*,

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1992) and a model has been proposed (Su *et al.*, 1992) for its regulation based on transcriptional attenuation. Determinants *xis-Tn* and *int-Tn*, involved in excision and integration, are present near the left end of the element (Clewell *et al.*, 1991; Su and Clewell, 1993) and are nearly identical to determinants described for Tn*1545* (Poyart-Salmeron *et al.*, 1989, 1990). The area between the left region and *tet*(M) contains several open reading frames (ORFs),[3] one of which, ORF5, is necessary for conjugative transfer (Su and Clewell, 1993). In addition, the sequences of the extreme left and right terminal regions have been reported (Clewell *et al.*, 1988). The region in the central part of the transposon and toward the right end has been shown, by insertional mutagenesis (Senghas *et al.*, 1988), to be necessary for conjugation. Conjugative transfer potential of Tn*916* varies from less than 10^{-8} to greater than 10^{-4} per donor depending on the particular insert (Clewell and Flannagan, 1993; Gawron-Burke and Clewell, 1982; Jaworski and Clewell, 1994). Short junction sequences created upon insertion influence the frequency with which a particular insert will subsequently transpose (Jaworski and Clewell, 1994), and individual inserts with high donor potential appear able to stimulate the transfer of other copies of the transposon located within the same cell by a *trans*-activation process (Flannagan and Clewell, 1991).

To further characterize the overall structure and genetic components of Tn*916*, determination of the nucleotide sequence has been completed. After cloning in *Escherichia coli*, sequencing was accomplished using Sequenase enzyme (United States Biochemical Corp.) and standard dideoxy chain termination methods essentially as previously described (Su and Clewell, 1993; Su *et al.*, 1992) for the left part of the transposon. For the central region, Tn*5* *lac* inserts were generated in pAM620 (vector pVA891 carrying the entire Tn*916* inserted into a small enterococcal fragment; Yamamoto *et al.*, 1987) using P1::Tn*5* *lac* (Kroos and Kaiser, 1984). Sequencing was performed using specific left and right primers that read from the inside of Tn*5* *lac* out into the bordering (Tn*916*) DNA. Sequence information for the rightward region was generated using Tn*5* inserts (Senghas *et al.*, 1988) in Tn*916* which were digested at a site internal to Tn*5* and subcloned, allowing sequencing of the bordering (Tn*916*) DNA by use of an outward-reading primer specific for the end of Tn*5*. Some areas were sequenced using Tn*916*-specific primers and template pAM120 (vector pGL101 carrying the entire Tn*916* inserted into a small enterococcal fragment; Gawron-Burke and Clewell, 1984). All specific (custom-designed) primers were synthesized at the Biomedical Research DNA Core Facility of the University of Michigan. Sequences were entered into a Macintosh IIci computer and aligned using MacVector and Assembly-LIGN software (International Biotechnologies, Inc.). The GenBank database accession number is U09422.

The size of the entire element was found to be 18,032 bp [for the form of the element having four T residues on the right end (Clewell and Flannagan, 1993)] with a G+C content of 38.8%. Digestion of Tn*916* by *Hin*cII restriction endonuclease is known to yield five internal fragments (Yamamoto *et al.*, 1987), which have been widely utilized for comparison of Tn*916*-like and Tn*916*-modified elements (Bentorcha *et al.*, 1992; Hachler *et al.*, 1987; Roberts, 1990). The designations and precise sizes of the *Hin*cII internal fragments are as follows: B [containing *tet*(M)], 4911 bp; A, 5624 bp; F, 1775 bp; H, 462 bp; G, 1211 bp.

Twenty-four potential ORFs have been identified. These are shown in Fig. 1. The sizes and masses of the corresponding putative protein products are listed in Table 1. For the areas not previously reported (i.e., to the right of *orf12*), the open reading frames were proposed based on size (greater than 25 codons) and the presence of suitably located possible ribosome binding sites. It should be noted that there are a few other small poten-

[3] Abbreviation used: ORF, open reading frames.

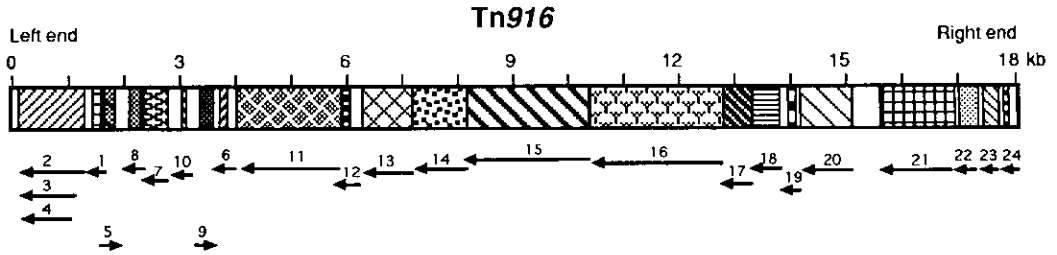

FIG. 1. Linear map of Tn*916* (18.0 kb) showing open reading frames (ORFs) represented by patterned segments. The assigned number for each ORF is shown above an arrow that indicates the direction of transcription and the approximate extent of the ORF (the arrows for the smaller ORFs are shown larger than scale for diagrammatic clarity). The sequence entered into the GenBank database reads from right to left, in the same direction as that of the majority of the ORFs.

tial open reading frames not included that fit the above criteria for size and ribosome binding site (although weak); however, they would be transcribed within, and in a direction counter to, one of the larger open reading frames.

Similarity of some of the ORFs to other known determinants has previously been discussed (Su and Clewell, 1993; Su *et al.*, 1992). Concerning those to the right of the *tet*(M) leader peptide, computer-assisted homology analyses, in some cases using the *Entrez* retrieval system (National Library of Medicine) and the BLAST algorithm (Altschul *et al.*, 1990), revealed similarities with known determinants in GenBank (Bilofsky and Burks, 1988) and other databases as follows. The nucleotide sequence of *orf13* showed significant homology with regions upstream of the *tet*(M) genes of *Ureaplasma urealyticum* (Sanchez-Pescador *et al.*, 1988) and *Streptococcus agalactiae* (Wang and Taylor, 1991). In the carboxy-terminal region, ORF14 displayed similarity with p60, a major extracellular invasion-associated protein of *Listeria monocytogenes* (Kohler *et al.*, 1990). ORF18 exhibited similarity with the antirestriction proteins Ard of plasmid Co1Ib-P9 (Delver *et al.*, 1991) and ArdA of pKM101 (Belogurov *et al.*, 1992). ORF23 showed local homology with the MbeA mobilization protein (relaxase) of plasmid ColE1 (Boyd *et al.*, 1989).

TABLE 1

DEDUCED PROTEIN PRODUCTS FOR EACH OPEN READING FRAME (ORF)

ORF[a]	Deduced protein[b] Size	Mass	ORF	Deduced protein Size	Mass
1 *xis-Tn*	67	8.1	13	310	35.7
2 *int-Tn*	405	47.1	14	333	36.8
3	361	41.7	15	754	84.9
4	324	37.6	16	815	93.7
5	83	9.1	17	168	19.1
6	62	7.4	18	165	19.1
7	157	18.4	19	73	8.1
8	76	8.9	20	329	39.1
9	117	13.5	21	461	53.4
10	23	2.9	22	128	14.4
11 *tet*(M)	639	72.5	23	104	11.9
12 *tet*(M) leader	28	3.4	24	39	4.6

[a] Designations for those determinants that have been named are shown next to the ORF number.

[b] Size is given in number of amino acids and mass in kilodaltons.

Tn*916* is the first conjugative transposon for which the nucleotide sequence has been completely determined. The data should greatly facilitate further studies of this prototype element.

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