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# Cloning and Characterization of *tetM* Gene from a *Ureaplasma urealyticum* Strain

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**We have cloned a 4.9-kilobase (kb) *Hin*cII fragment which contains a tetracycline resistance determinant (*tetM*) from the chromosome of *Ureaplasma urealyticum*. The 4.9-kb *Hin*cII fragment contains DNA in addition to the structural gene, is closely related to the previously characterized 5.0-kb fragment from pJI3, and contains no normal ureaplasmal DNA sequences.**

Tetracycline disrupts protein synthesis by interfering with the binding of the aminoacyl-tRNA-GTP-elongation factor Tu (EF-Tu) complex to the acceptor site of the ribosome (12). At least five genetically different tetracycline resistance ($Tc^r$) genes have been defined in gram-negative bacteria (18, 21). These genes are generally borne by plasmids or transposons and code for an energy-dependent efflux mechanism which prevents accumulation of the drug (20). In gram-positive streptococci, three $Tc^r$ determinants have been well characterized (3–5). *tetL* is generally plasmid encoded and has been shown to decrease accumulation of tetracycline, presumably because of an active efflux of tetracycline similar to that of the gram-negative determinants. In contrast, the *tetM* and *tetN* determinants mediate $Tc^r$ with a cytoplasmic protein which interferes with the interaction of tetracycline with the ribosomes (4). *tetN* is plasmid mediated and seems to be rare, whereas *tetM* is not only found in gram-positive streptococci, as originally described (3, 8), but also dispersed in such diverse organisms as cell wall-free bacteria (*Ureaplasma urealyticum* [24, 25] and *Mycoplasma hominis* [27]), bacteria of uncertain taxonomic status (*Gardnerella vaginalis* [23]), gram-negative bacteria (*Neisseria gonorrhoeae* [22]), and a strict anaerobic gram-positive bacterium (*Clostridium difficile* [13]). The frequent location of *tetM* determinants on conjugative transposable elements may account for this broad distribution (6, 8, 11).

The first $Tc^r$ *U. urealyticum* strain was isolated in 1974 (10) in British Columbia. Currently, more than 15% of *U. urealyticum* strains isolated from patients of the Seattle Sexually Transmitted Disease Clinic are resistant to tetracycline (25). A total of 19 high-level $Tc^r$ *U. urealyticum* strains isolated in British Columbia, Washington, Massachusetts, North Carolina, and Alabama (24, 25) were tested, and all strains had DNA sequences which hybridized with the *tetM* determinant. The determinant appeared to be located in the chromosome (24, 25). We chose one $Tc^r$ *U. urealyticum* strain isolated in Seattle, Wash., in 1984 (25) from which to clone the tetracycline resistance gene.

*U. urealyticum* K was grown in liquid medium and whole-cell DNA prepared as previously described (1, 24). The DNA was digested with *Hin*cII restriction enzyme and run on a 0.8% agarose gel, and a Southern blot was prepared (29, 32). The blot was hybridized with a nick-translated, radiolabeled 5-kilobase (kb) *Hin*cII fragment from pJI3 as previously described (23, 24). A single hybridizing fragment which migrated at between 4.0 and 8.0 kb was observed. Fragments in the size range of 4.0 to 8.0 kb were isolated by electroelution of *Hin*cII-digested chromosomal DNA (17). The fragments were ligated into the vector pACYC177 (7) and cloned into *Escherichia coli* HB101 (2, 14, 17). Transformants were selected directly on L agar medium containing 10 μg of tetracycline per ml. Transformants were screened by dot blot with the 5-kb *Hin*cII radiolabeled fragment from pJI3. All transformants hybridized with the *tetM* probe, whereas the $Tc^r$ *E. coli* spontaneous mutants and tetracycline-susceptible ($Tc^s$) *Ureaplasma* strain did not hybridize. Restriction analysis of the plasmids carried by the $Tc^r$ transformants indicated that each contained an insert of approximately 4.9 kb.

Restriction analysis of the chimeric plasmid pUW-JKB1 indicated that the inserted fragment was 4.9 kb long and contained single *Hin*dIII, *Kpn*I, and *Sac*I (*Sst*I) sites along with several *Hpa*II sites; however, only one *Hpa*II site was mapped. The fragment did not appear to have restriction sites for *Bam*HI, *Cla*I, *Pst*I, or *Sma*I (Fig. 1). The restriction map of pUW-JKB1 is very similar to the published maps for pJI3 (5, 16) and pAT183 (19), cloned from the chromosomes of *Streptococcus agalactiae* and *Streptococcus pneumoniae*, respectively (Fig. 1). Both pUW-JKB1 and pJI3 lack *Cla*I restriction sites. However, since the host *E. coli* was a $Dam^+$ strain, which methylates its DNA, the *Cla*I restriction site may be present but not cleaved because of methylation.

Various fragments from pUW-JKB1 and pJI3 were subcloned into pUC19, transformed into *E. coli* TB1 (31), and selected on L agar supplemented with 50 μg of ampicillin and 40 μg of 5-bromo-4-chloro-3-indoyl-β-D galactopyranoside per ml (Fig. 2) (17, 31). All transformants were $Tc^s$, suggesting that the structural gene for $Tc^r$ is larger than the *Kpn*I-*Hin*dIII subcloned fragment from pUW-JKB1 (pUW-JKB3) and larger than the *Sac*I-*Hin*dIII subcloned fragment from pJI3 (pMR31). Our data are compatible with the DNA sequence published for pAT183 (19).

To determine if the 4.9-kb fragment from $Tc^r$ *U. urealyticum* K is specific to the $Tc^r$ gene, chromosomal DNA (average length, 500 base pairs [bp]) from $Tc^s$ *U. urealyticum* serovar 8 (28) was radiolabeled and hybridized to a Southern blot containing enzyme-restricted plasmids pUW-JKB1, pUW-JKB2, pUW-JKB3, pUW-JKB4, pMR31, and pMR32 and enzyme-restricted whole-cell DNA from *U. urealyticum* K and serovar 8. No hybridization was observed with any of the plasmid DNAs tested, suggesting that the cloned fragments do not contain any normal ureaplasmal DNA and, like the 5-kb *Hin*cII insert of pJI3, are specific to the $Tc^r$ gene. In

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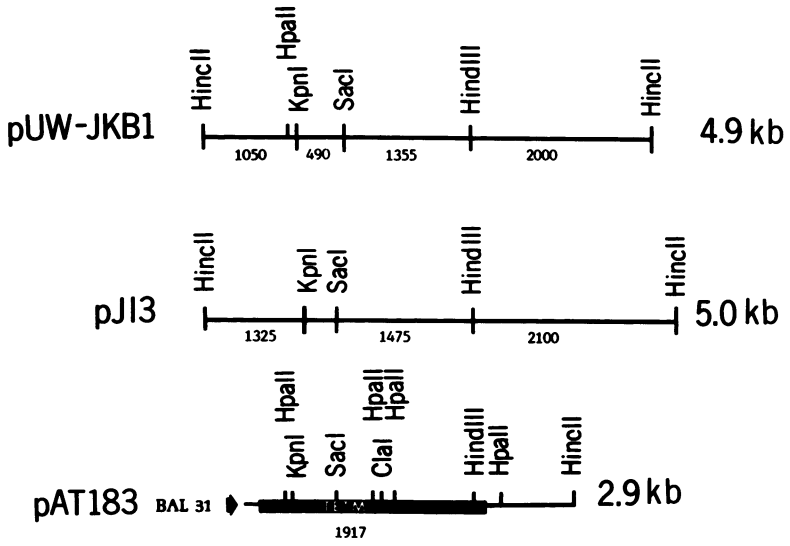


FIG. 1. Restriction maps of the 4.9-kb *Hin*cII fragment from pUW-JKB1 (*U. urealyticum*); a 5.0-kb *Hin*cII fragment from pJI3 (*S. agalactiae*; data primarily from reference 5); and pAT183, a 2.9-kb deletion derivative of pAT182 (Tn*1545*) (*S. pneumoniae*; 19). The heavy line indicates the structural *tetM* gene sequenced in pAT183.

contrast, plasmids containing cloned 1- to 5-kb chromosomal DNA from strain K hybridized with the radiolabeled probe prepared from *U. urealyticum* serovar 8 (unpublished observations). Extensive hybridization was seen with *Hin*cII-digested DNA from *U. urealyticum* K and serotype 8 (data not shown). When the 1.8-kb fragment from pUW-JKB3 was used as the source of the probe, it did not hybridize with the $Tc^s$ *U. urealyticum* strain or with pMR32, but hybridized with pMR32, pJI3, and pUW-JKB4. We have also used the 1.8-kb fragment from the pUW-JKB3 fragment to test other $Tc^r$ *U. urealyticum* strains and determined that this fragment hybridized with the same strains as did the larger *Hin*cII fragment from pJI3 and had the advantage of carrying only a part of the *tetM* determinant and none of the flanking sequences (data not shown).

We have recently sequenced the structural *tetM* gene from the 4.9-kb *Hin*cII fragment (R. Sanchez-Pescador, J. T. Brown, M. C. Roberts, and M. S. Urdea, submitted for publication). The DNA sequence of the *U. urealyticum tetM* determinant is 95% homologous with the DNA sequence from pAT183 isolated from *S. pneumoniae*, indicating that the *tetM* determinant from *U. urealyticum* K has been conserved over time, change in geographical location, and passage into a cell wall-free bacterium.

The spread of antibiotic resistance among populations of unrelated genera may have major consequences for treatment of disease. With the emergence of $Tc^r$, the usefulness of tetracycline in treatment of urogenital disease must be reevaluated. *tetM* is generally located on a conjugative transposon (6, 8, 11, 15, 16) and is capable of self-mobilization. It is one of the two antibiotic resistance genes known to occur in both gram-positive and gram-negative

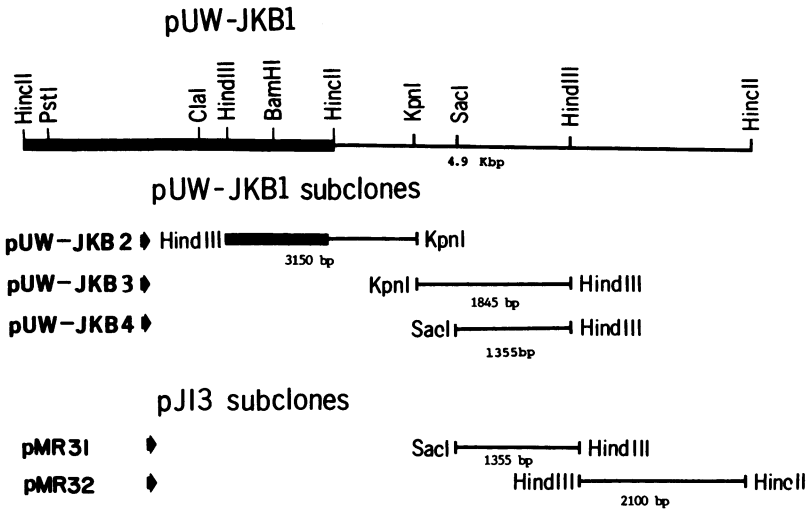


FIG. 2. Restriction sites of fragments subcloned from pUW-JKB1 and pJI3. The heavy line represents vector pACYC177. All fragments were ligated into pUC19, and all of the subclones were $Tc^s$. pUW-JKB2 contains part of the vector region and includes the *Hin*dIII-*Kpn*I fragment of pUW-JKB1. pUW-JKB3 contains the 1,845-bp *Kpn*I-*Hin*dII fragment, and pUW-JKB4 contains the 1,355-bp *Sac*I-*Hin*dIII region. pMR31 contains the 1,355-bp *Sac*I-*Hin*dII fragment, and pMR32 contains the 2,100-bp *Hin*dIII-*Hin*cII fragment from pJI3.

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bacteria in nature (30). Conjugal transfer between streptococci and other gram-positive bacteria has been demonstrated in the laboratory (8). We have been able to transfer Tn*916* from *Streptococcus faecalis* to three strains of *Mycoplasma hominis* by conjugation (26). Tn*916* often inserts at multiple sites on the chromosome of the recipient; this phenomenon appears to be similar to results of streptococcus-to-streptococcus conjugal transfer (26). *tetM* has also been introduced by transformation into strains of *Mycoplasma pulmonis* and *Acholeplasma laidlawii* (9), both members of the order *Mycoplasmatales*. The *tetM* class of determinants appears to have a wide host range, and since this determinant can be transferred by transformation or conjugation, we predict that in time, other species and genera will acquire the *tetM* determinant.

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