Cloning and Characterization of \textit{tetM} Gene from a \textit{Ureaplasma urealyticum} Strain

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We have cloned a 4.9-kilobase (kb) \textit{HincII} fragment which contains a tetracycline resistance determinant (\textit{tetM}) from the chromosome of \textit{Ureaplasma urealyticum}. The 4.9-kb \textit{HincII} fragment contains DNA in addition to the structural gene, is closely related to the previously characterized 5.0-kb fragment from pJ13, 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 (Tcr) 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\textsuperscript{r} determinants have been well characterized (3-5). \textit{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 \textit{tetM} and \textit{tetN} determinants mediate Tc\textsuperscript{r} with a cytoplasmic protein which interferes with the interaction of tetracycline with the ribosomes (4). \textit{tetN} is plasmid mediated and seems to be rare, whereas \textit{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 (\textit{Ureaplasma urealyticum} [24, 25] and \textit{Mycoplasma hominis} [27]), bacteria of uncertain taxonomic status (\textit{Gardnerella vaginalis} [23]), gram-negative bacteria (\textit{Neisseria gonorrhoeae} [22]), and a strict anaerobic gram-positive bacterium (\textit{Clostridium difficile} [13]). The frequent location of \textit{tetM} determinants on conjugative transposable elements may account for this broad distribution (6, 8, 11).

The first Tc\textsuperscript{r} \textit{U. urealyticum} strain was isolated in 1974 (10) in British Columbia. Currently, more than 15% of \textit{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\textsuperscript{r} \textit{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 \textit{tetM} determinant. The determinant appeared to be located in the chromosome (24, 25). We chose one Tc\textsuperscript{r} \textit{U. urealyticum} strain isolated in Seattle, Wash., in 1984 (25) from which to clone the tetracycline resistance gene.

\textit{U. urealyticum} K was grown in liquid medium and whole-cell DNA prepared as previously described (1, 24). The DNA was digested with \textit{HincII} 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) \textit{HincII} fragment from pJ13 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 \textit{HincII}-digested chromosomal DNA (17). The fragments were ligated into the vector pACYC177 (7) and cloned into \textit{Escherichia coli} HB101 (2, 14, 17). Transformants were selected directly on L agar medium containing 10 \textmu g of tetracycline per ml. Transformants were screened by dot blot with the 5-kb \textit{HincII} radiolabeled fragment from pJ13. All transformants hybridized with the \textit{tetM} probe, whereas the Tc\textsuperscript{r} \textit{E. coli} spontaneous mutants and tetracycline-susceptible (Tcs) \textit{Ureaplasma} strain did not hybridize. Restriction analysis of the plasmids carried by the Tc\textsuperscript{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 \textit{HindIII}, \textit{KpnI}, and \textit{SacI} (\textit{SstI}) sites along with several \textit{HpaI} sites; however, only one \textit{HpaI} site was mapped. The fragment did not appear to have restriction sites for \textit{BamHI}, \textit{ClaI}, \textit{PstI}, or \textit{Smal} (Fig. 1). The restriction map of pUW-JKB1 is very similar to the published maps for pJ13 (5, 16) and pAT183 (19), cloned from the chromosomes of \textit{Streptococcus galactiae} and \textit{Streptococcus pneumoniae}, respectively (Fig. 1). Both pUW-JKB1 and pJ13 lack \textit{ClaI} restriction sites. However, since the host \textit{E. coli} was a Dam\textsuperscript{+} strain, which methylates its DNA, the \textit{ClaI} restriction site may be present but not cleaved because of methylation.

Various fragments from pUW-JKB1 and pJ13 were subcloned into pUC19, transformed into \textit{E. coli} TB1 (31), and selected on L agar supplemented with 50 \textmu g of ampicillin and 40 \mug of 5-bromo-4-chloro-3-indolyl-\textbeta-D-galactopyranoside per ml (Fig. 2) (17, 31). All transformants were Tc\textsuperscript{r}, suggesting that the structural gene for Tc\textsuperscript{r} is larger than the \textit{KpnI-HindIII} subfragment from pUW-JKB1 (pUW-JKB3) and larger than the \textit{SacI-HindIII} subfragment from pJ13 (pMR31). Our data are compatible with the DNA sequence published for pAT183 (19).

To determine if the 4.9-kb fragment from Tc\textsuperscript{r} \textit{U. urealyticum} K is specific to the Tc\textsuperscript{r} gene, chromosomal DNA (average length, 500 base pairs [bp]) from Tc\textsuperscript{r} \textit{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 \textit{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 \textit{HincII} insert of pJ13, are specific to the Tc\textsuperscript{r} gene. In
VOL. 31,agalactiae; data line indicates contrast, U. Tcs from prepared from DNA from pMR32, pJI3, DNA from digested vations). Extensive as the used Tcr U. shown). When not hybridized fragment 1.8-kb (data sequences region.

FIG. 1. Restriction maps of the 4.9-kb HincII fragment from pUW-JKB1 (U. urealyticum); a 5.0-kb HincII fragment from pJ13 (S. agalactiae; data primarily from reference 5); and pAT183, a 2.9-kb deletion derivative of pAT182 (Tn1545) (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 HincII-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 TcR U. urealyticum strain or with pMR32, but hybridized with pMR32, pJ13, and pUW-JKB4. We have also used the 1.8-kb fragment from the pUW-JKB3 fragment to test other TcR U. urealyticum strains and determined that this fragment hybridized with the same strains as did the larger HincII fragment from pJ13 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 HincII 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 TcR, 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 pJ13. The heavy line represents vector pACYC177. All fragments were ligated into pUC19, and all of the subclones were TcR. pUW-JKB2 contains part of the vector region and includes the HincII-KpnI fragment of pUW-JKB1. pUW-JKB3 contains the 1.845-bp KpnI-HindIII fragment, and pUW-JKB4 contains the 1.355-bp Sacl-HindIII region. pMR31 contains the 1.355-bp Sacl-HindIII fragment, and pMR32 contains the 2.100-bp HindIII-HincII fragment from pJ13.
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 Tn916 from *Streptococcus faecalis* to three strains of *Mycoplasma hominis* by conjugation (26). Tn916 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 *Accholeplasma 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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**LITERATURE CITED**


