Conjugal Transfer of Transposon Tn916 from *Streptococcus faecalis* to *Mycoplasma hominis*

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Transposon Tn916 was transferred from *Streptococcus faecalis* to *Mycoplasma hominis* by a mating process resembling conjugation with a frequency of 10^{-6} to 10^{-7} . Tn916 was inserted into the mycoplasmal chromosome in single and multiple copies.

Mycoplasmas are the smallest free-living procaryotes. Their small physical size (0.3 μ m in diameter), small genome size (5×10^8 daltons), and low guanine-plus-cytosine content (28% for *Mycoplasma hominis*) have led to the hypothesis that they represent the minimal free-living cell, with a coding capacity of around 700 proteins (20, 30). Mycoplasmas grow poorly in culture and require serum for growth in a complex, undefined medium (13). Mycoplasmas apparently descended from gram-positive bacteria, presumably by degenerative evolution rather than representing modern versions of a primordial procaryote (20, 30). A major deletion in these organisms has been the loss of a cell wall, leaving the organism surrounded only by a unit membrane which contains cholesterol, a component not found in other bacteria.

Recently, we have shown that high-level tetracycline-resistant (Tc^r) clinical isolates of both *M. hominis* (16) and *Ureaplasma urealyticum* (another member of the order *Mycoplasmatales*) contain DNA sequences which hybridized with the tetracycline resistance element *tetM* (24, 25), a representative of a family of related determinants in streptococci (1, 2, 4, 6, 9, 10, 12). The best-studied *tetM* transposon is Tn916 isolated from *Streptococcus faecalis* (4). Tn916 can be transferred between streptococcal strains by a process resembling conjugation and can be found in the chromosomes of the recipients (4, 9, 10, 12) or on occasion transferred to conjugative streptococcal plasmids such as pPD1 and pPD5 (17). This family of transposons appears to have a wide host range and has been transferred in the laboratory to such diverse gram-positive species as *Bacillus anthracis* (B. Ivins, G. Knudson, and D. Le Blanc, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, D-81, p. 79) and *Listeria monocytogenes* (8). We have also found *tetM* DNA sequences in clinical urogenital organisms such as *Neisseria gonorrhoeae* (a gram-negative organism) (19) and *Gardnerella vaginalis* (an organism of uncertain taxonomic status) (21). The *tetM* determinant appears to be located in the chromosome of *G. vaginalis*, *M. hominis*, and *U. urealyticum* (21, 24, 25), whereas it is located on a conjugative 25.2-megadalton plasmid in *N. gonorrhoeae* (19). More recently, Tn916, cloned in an *Escherichia coli* vector, has been transformed into two strains of *Acholeplasma laidlawii* and one strain of *Mycoplasma pulmonis*, two other members of the class *Mollicutes* (5). We have recently cloned the Tc^r determinant from a Tc^r *U. urealyticum* strain. This gene is expressed in *E. coli*, and the restriction pattern of the cloned fragment is very similar to the two cloned *tetM* genes

isolated from *Streptococcus pneumoniae* and *S. agalactiae*. This work provides proof that clinical isolates of the order *Mycoplasmatales* carry a highly conserved *tetM* gene and suggests that the *tetM* gene cannot be used to examine whether the UGA codon is read as a stop (28) or as an amino acid (J. T. Martin and M. C. Roberts, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, A-123, p. 21).

In our previous study (25), we found that Southern blots of Tc^r *M. hominis* isolates showed significant differences, leading us to believe that the Tc^r determinant had been introduced on multiple occasions into *M. hominis* strains rather than having been introduced into a single clone which then disseminated. Therefore, we sought to transfer the conjugative transposon Tn916 from streptococci into *M. hominis*. We used three donor *S. faecalis* strains: DS160 (Tn916 located in the chromosome), OG1-10(pPD1::Tn916) (Tn916 located on the pPD1 plasmid), and OG1-10(pPD5::Tn916) (Tn916 located on a different plasmid, pPD5). Strain OG1-10(pPD1) and the plasmid-free strain JH203 were used as controls (6, 17). We used three tetracycline-susceptible (Tc^s) *M. hominis* strains: two prototypes from the American Type Culture Collection (ATCC) and GX55, a wild strain in its fifth passage from the patient (Table 1).

Mycoplasmas were grown in dialysate broth (13) supplemented with 20% "agamma" horse serum and 200 U of penicillin per ml. Overnight cultures were prepared by inoculating broth cultures with 1:100 and 1:1,000 dilutions of *M. hominis* strains. These tubes were mixed together in equal volumes to assure that peak populations of mycoplasmas were present (mycoplasmas show almost no turbidity during the growth cycle and die rapidly after peak growth is achieved). The mycoplasmal mixture (100 ml) was mixed with 10 ml of a log-phase culture of *S. faecalis*. The mixture was centrifuged at $8,000 \times g$ for 30 min at room temperature and suspended in 1/10 volume of mycoplasmal medium, and 0.3 ml was plated on H agar (14) containing 2 μ g of tetracycline per ml and 200 U of penicillin per ml at 0, 1, and 2 h. Longer matings of 12 to 24 h gave fewer transconjugants, probably because mycoplasmal counts had decreased. Plates were observed daily for the presence of colonies, and tetracycline-resistant colonies were transferred to broth medium which was incubated overnight and then stored at -70°C . Controls with streptococci alone or mycoplasmas alone were similarly processed.

All three recipients accepted and maintained the Tn916 determinant (Table 1). Transconjugant colonies could be clearly distinguished from the background debris on plates by day 3 or 4 and were subcultured at that time. In all of the

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TABLE 1. Transfer of *tetM* (Tn916) from *S. faecalis* to *M. hominis*

Donor streptococcal strain or plasmid	Recipient <i>M. hominis</i> strain	Frequency of Tc ^r mycoplasmas
DS160 ^a	GX55	0.3×10^{-6} – 6.0×10^{-6}
OG1-10(pPD1::Tn916) ^a	GX55	0.2×10^{-6} – 4.4×10^{-6}
OG1-10(pPD5::Tn916)	GX55	7.4×10^{-7}
OG1-10(pPD1::Tn916)	ATCC 23114	7.0×10^{-7}
OG1-10(pPD5::Tn916)	ATCC 23114	1.4×10^{-6}
OG1-10(pPD1::Tn916)	ATCC 14027	1.0×10^{-6}
OG1-10(pPD1) ^b	GX55	$<10^{-8}$
JH203 ^b	GX55	$<10^{-8}$
JH203	ATCC 23114	$<10^{-8}$
OG-10(pPD1)	ATCC 23114	$<10^{-8}$
JH203	ATCC 14027	$<10^{-8}$

^a In the mating of GX55 with DS160 or OG1-10(pPD1::Tn916), the range of frequencies shown was obtained in five separate experiments. The other frequencies are averages of two experiments. Tn916 was present in the chromosome of DS160 and on a plasmid in OG1-10(pPD1::Tn916).

^b Tetracycline-susceptible controls.

experiments combined (over 20), only one spontaneous Tc^r *M. hominis* mutant was observed among the controls. The medium used for conjugation contained 200 U of penicillin per ml, which would lead to production of protoplasts from the streptococci, a condition which may have facilitated conjugation. The streptococci retained viability throughout the experiment, as determined by plate counts. The viability of the streptococci was probably due to the serum in the mating mixture, which is known to stabilize wall-less forms of bacteria (11). Polyethylene glycol-treated cells were also used (26). Transconjugants were recovered, but the frequency of Tn916 transfer was no higher. Neither the location of the Tn916 in the donor streptococci (plasmid versus chromosomal) nor the presence of DNase in the mating mixture appeared to influence the frequency of transfer into *M. hominis* (approximately 10^{-6} to 10^{-7} per recipient). This is similar to the transfer frequency of 10^{-6} from *S. faecalis* DS160 to other *S. faecalis* strains (4, 6).

Tc^r transconjugants were tested by dot blot for homology with nick-translated, radiolabeled probes made from pJ13, a chimeric plasmid containing a 5-kilobase-pair *Hinc*II fragment carrying the structural *tetM* gene (3, 25); pJ12.14, which does not confer Tc^r but carries transposon-specific sequences to the right of the structural Tc^r gene (21); and whole chromosomal DNA from the streptococcal strain JH203, as previously described (22, 24). Regardless of the donor, no *M. hominis* transconjugant hybridized with the whole-chromosomal DNA probe, whereas all strains isolated from matings with Tn916 carrying donors hybridized with the *tetM* probes pJ13 and pJ12.14 (data not shown). This indicates that the transconjugants acquired not only the structural *tetM* gene but also most, if not all, of the Tn916 transposon as well. When transconjugants from the OG1-10(pPD1::Tn916) matings were probed with the plasmid pPD1, no strains hybridized with the probe, suggesting that plasmid DNA sequences were not present in the transconjugants. Perhaps the conjugative plasmid pPD1::Tn916 was transferred into *M. hominis* intact; thereafter, Tn916 was excised from the plasmid and inserted into the recipient mycoplasmal chromosome, an event relatively common in mating of streptococci (4, 6). Alternatively, Tn916 may have been transferred as an independent determinant in a manner similar to that proposed for the transfer from the chromosome of strain DS160 (4, 6). One spontaneous Tc^r mutant appeared on one of the plates which had no donor. This

strain did not hybridize with the *tetM* probe. No colonies appeared when the streptococcal donor was JH203 or OG1-10(pPD1). The transfer of Tc^r from *M. hominis* back into *S. faecalis* was examined. Ten different *tetM*-containing wild isolates of *M. hominis* were used as donors, and *S. faecalis* JH203 was the recipient. We were unable to detect transfer of the Tc^r phenotype. However, since only a small portion of streptococcal transconjugants created in the laboratory (9) or existing as natural isolates (10, 21) are able to act as donors, a large number (200 to 300) of independent Tc^r *M. hominis* strains will need to be examined before we can rule out the possibility of transfer from *M. hominis* to *S. faecalis*.

The tetracycline sensitivities of the transconjugants and their isogenic parental strains were determined by the agar dilution method (15). The MICs for the parental strains were 0.25 µg/ml for GX55 and 0.5 µg/ml for ATCC 23114 and ATCC 14027. The MICs for all 24 transconjugants tested were ≥ 32 µg/ml, values similar to those of naturally occurring strains of *M. hominis* carrying *tetM* sequences (25) and significantly higher than those of the original parents. The spontaneous mutant isolated from the control plate had an MIC of 1 µg/ml, which was significantly lower than those of the strains carrying the *tetM* determinant but four times higher than that of the GX55 parent.

Transfer of Tn916 from one strain of *S. faecalis* to another leads to insertion of Tn916 into different sites within the chromosome and can lead to multiple insertions of the determinant (6, 9). To determine whether a similar mechanism was evident with the *M. hominis* transconjugants, we prepared DNA from some of the Tc^r transconjugants, digested them with restriction enzymes, and prepared Southern blots (18, 27) (Fig. 1). *Hind*III cuts Tn916 once (4, 6) and is a convenient enzyme with which to study this

FIG. 1. Southern hybridization analysis of transconjugants. Lanes 1 to 6, chromosomal DNAs (3 µg) from different Tc^r GX55 transconjugants isolated from different experiments were digested with the restriction endonucleases *Pvu*II and *Hind*III and then electrophoresed on a 1.0% agarose gel. DNA fragments were transferred to nitrocellulose sheets and then hybridized with ³²P-labeled pJ13 DNA. Sizes (in kilobase pairs) of DNA *Hind*III-generated restriction fragment markers are noted on the right. In lane 3, arrows point out multiple hybridizing bands, indicating that at least two copies of Tn916 are present in the chromosome.

question. Since this enzyme does not readily or consistently cut *M. hominis* DNA (unpublished data), we cut the DNA with both *Hind*III and *Pvu*II to generate suitable fragments for probing with the *tetM* probe. *Pvu*II does not cut Tn916 but does cut the *M. hominis* chromosome (unpublished data); therefore, a single Tn916 determinant present in the strain would be identified by the presence of two fragments hybridizing with the *tetM* probe. In each transconjugant tested, at least two fragments hybridized with the *tetM* probe; however, as with the *S. faecalis* transconjugants, multiple copies of the transposon were evident in some transconjugants because multiple hybridizing bands were seen in some transconjugants (Fig. 1, lane 3). The sizes of the hybridizing bands were not identical in all of the transconjugants, suggesting that Tn916 was inserted in different sites in some of the transconjugants. The number of copies or the apparent location of Tn916 in the transconjugant did not seem to influence the MIC of the strain.

Furness and Creone (7) attempted to transfer tetracycline resistance from *M. hominis* to *Mycoplasma salivarium* by transformation with DNA from the donor strain, but they found that the transformed strain had gained relatively little resistance. We tested their donor strain (ATCC 33131) and found that it contained *tetM* but the transformed *M. salivarium* strain (ATCC 33130) did not contain *tetM*; these results indicate that the *tetM* resistance determinant had not been transferred to the recipient. In limited experiments, we failed to transform *M. hominis* by using either the *E. coli* CaCl₂ or the streptococcal polyethylene glycol transformation method (26). Moreover, we failed to transform *tetM* into *M. hominis* with purified plasmid DNA from OG1-10(pPD1::Tn916) or chromosomal DNA from strain DS160 when the standard mating method was used with recipient and pure DNAs.

Transformation of Tn916 has been reported (5) for *A. laidlawii* and *M. pulmonis* by a polyethylene glycol transformation system which differed from the one we used. In this system, Tn916, cloned in an *E. coli* vector (5), was shown to insert itself into different sites in the chromosome, but only one copy of the gene appeared to be present (only two bands hybridized with *Hind*III-restricted DNA). Neither organism is known to carry *tetM* in nature; therefore, their transformation may not reflect the origin of *tetM* in natural populations of *M. hominis* and *U. urealyticum*. We tested the ability of Tn916 to be transferred into *M. hominis*, a species which naturally contains the *tetM* determinant, by using conjugation. Conjugation is a likely system for the natural spread of this class of determinants (4). We found that the location (plasmid or chromosomal) of Tn916 on the donor streptococci did not influence the frequency of transfer. Only transposon-specific sequences were identified in the transconjugants, and all transconjugants tested carried more than just the structural *tetM* gene as measured by hybridization with the Tc^s pJ12.14 probe. Unlike the transformation system, some transconjugants had multiple hybridizing bands, indicating that more than a single copy of the transposon was present, findings similar to those found when *S. faecalis* DS160 transfers Tn916 into streptococcal recipients (9). Multiple hybridizing bands are also found in DNAs from natural *tetM*-carrying *M. hominis* and *U. urealyticum* digested with *Pvu*II and *Hind*III (24, 25).

The demonstration that Tn916 can be moved into members of the class *Mollicutes* by conjugation opens up the possibility of using this convenient method for delivering foreign DNA into the mycoplasmal genome in a manner similar to that described by Youngman et al. (29). Tn916 can

also be used to create insertional mutations or plasmid shuttle vectors which can be maintained and expressed in mycoplasmas, streptococci, and *E. coli*.

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LITERATURE CITED

- Burdett, V. 1980. Identification of tetracycline-resistant R-plasmids in *Streptococcus agalactiae* (group B). *Antimicrob. Agents Chemother.* 18:753-760.
- Burdett, V. 1986. Streptococcal tetracycline resistance mediated at the level of protein synthesis. *J. Bacteriol.* 165:564-569.
- Burdett, V., J. Inamine, and S. Rajagopalan. 1982. Heterogeneity of tetracycline resistance determinants in *Streptococcus*. *J. Bacteriol.* 149:995-1004.
- Clewell, D. B., and C. Gawron-Burke. 1986. Conjugative transposons and the dissemination of antibiotic resistance in streptococci. *Annu. Rev. Microbiol.* 40:635-659.
- Dybvig, K., and G. H. Cassell. 1987. Transposition of gram-positive transposon Tn916 in *Acholeplasma laidlawii* and *Mycoplasma pulmonis*. *Science* 235:1392-1394.
- Franke, A. E., and D. B. Clewell. 1981. Evidence for a chromosome-borne resistance transposon (Tn916) in *Streptococcus faecalis* that is capable of "conjugal" transfer in the absence of a conjugative plasmid. *J. Bacteriol.* 145:494-502.
- Furness, G., and A. M. Creone. 1979. The preparation of competent single-cell suspensions of *Mycoplasma hominis* tet^s and *Mycoplasma salivarium* tet^r for genetic transformation to tetracycline resistance by DNA extracted from *Mycoplasma hominis* tet^r. *J. Infect. Dis.* 139:444-451.
- Gaillard, J. L., P. L. Berche, and P. Sansonetti. 1986. Transposon mutagenesis as a tool to study the role of hemolysin in the virulence of *Listeria monocytogenes*. *Infect. Immun.* 52:50-55.
- Gawron-Burke, C., and D. B. Clewell. 1982. A transposon in *Streptococcus faecalis* with fertility properties. *Nature (London)* 300:281-284.
- Hartley, D. L., K. R. Jones, J. A. Tobian, D. J. LeBlanc, and F. L. Macrina. 1984. Disseminated tetracycline resistance in oral streptococci: implication of a conjugative transposon. *Infect. Immun.* 45:13-17.
- Horwitz, A. H., and L. E. Casida, Jr. 1976. Effects of magnesium, calcium, and serum on reversion of stable L-forms. *J. Bacteriol.* 136:565-569.
- Inamine, J., and V. Burdett. 1982. Genetic and physical characterization of two chromosomal resistance determinants of *Streptococcus agalactiae*, p. 170-173. In D. Schlessinger (ed.), *Microbiology—1982*. American Society for Microbiology, Washington, D.C.
- Kenny, G. E. 1967. Heat stability and organic solvent solubility of mycoplasma antigens. *Ann. N.Y. Acad. Sci.* 143:676-681.
- Kenny, G. E. 1985. Mycoplasmas, p. 407-411. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 4th ed. American Society for Microbiology, Washington, D.C.
- Kenny, G. E., F. D. Cartwright, and M. C. Roberts. 1986. Agar dilution method for determination of antibiotic susceptibility of *Ureaplasma urealyticum*. *Pediatr. Infect. Dis.* 5:S332-S334.
- Koutsky, L. A., W. E. Stamm, R. C. Brunham, C. E. Stevens, B. Cole, J. Hale, P. Davick, and K. K. Holmes. 1983. Persistence of *Mycoplasma hominis* after therapy: importance of tetracycline resistance and of coexisting vaginal flora. *Sex. Transm. Dis.* 11:374-381.
- LeBlanc, D. J., L. N. Lee, D. B. Clewell, and D. Behnke. 1983. Broad geographical distribution of a cytotoxin gene mediating beta-hemolysis and bacteriocin activity among *Streptococcus faecalis* strains. *Infect. Immun.* 40:1015-1022.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

19. Morse, S. A., S. R. Johnson, J. W. Biddle, and M. C. Roberts. 1986. High-level tetracycline resistance in *Neisseria gonorrhoeae* is result of acquisition of streptococcal *tetM* determinant. *Antimicrob. Agents Chemother.* **30**:664-670.
20. Razin, S. 1985. Molecular biology and genetics of mycoplasmas (*Mollicutes*). *Microbiol. Rev.* **49**:419-455.
21. Roberts, M. C., S. L. Hillier, J. Hale, K. K. Holmes, and G. E. Kenny. 1986. Tetracycline resistance and *tetM* in pathogenic urogenital bacteria. *Antimicrob. Agents Chemother.* **30**:810-812.
22. Roberts, M. C., S. L. Hillier, F. D. Schoenknecht, and K. K. Holmes. 1984. Nitrocellulose filter blots for species identification of *Mobiluncus curtisii* and *Mobiluncus mulieris*. *J. Clin. Microbiol.* **20**:826-867.
23. Roberts, M. C., S. L. Hillier, F. D. Schoenknecht, and K. K. Holmes. 1985. Comparison of Gram stain, DNA probe, and culture for the identification of *Mobiluncus* spp. *J. Infect. Dis.* **152**:74-77.
24. Roberts, M. C., and G. E. Kenny. 1986. Dissemination of the *tetM* tetracycline resistance determinant to *Ureaplasma urealyticum*. *Antimicrob. Agents Chemother.* **29**:350-352.
25. Roberts, M. C., L. A. Koutsky, K. K. Holmes, D. J. LeBlanc, and G. E. Kenny. 1985. Tetracycline-resistant *Mycoplasma hominis* strains contain streptococcal *tetM* sequences. *Antimicrob. Agents Chemother.* **28**:141-143.
26. Smith, C. J. 1985. Polyethylene glycol-facilitated transformation of *Bacteroides fragilis* with plasmid DNA. *J. Bacteriol.* **164**:466-469.
27. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**:503-517.
28. Yamao, F., A. Muto, J. Kawauchi, M. Iwami, S. Iwayami, Y. Azumi, and S. Iwagami. 1985. UGA is read as tryptophan in *Mycoplasma capricolum*. *Proc. Natl. Acad. Sci. USA* **76**:2306-2309.
29. Youngman, P., J. B. Perkins, and R. Losick. 1984. Construction of a cloning site near one end of Tn917 into which foreign DNA may be inserted without affecting transposition in *Bacillus subtilis* or expression of the transposon-borne *erm* gene. *Plasmid* **12**:1-9.
30. Woese, C. R., J. Maniloff, and L. B. Zablen. 1980. Phylogenetic analysis of *Mycoplasmas*. *Proc. Natl. Acad. Sci. USA* **77**:494-498.