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# Plasmid-Mediated Tet M in *Haemophilus ducreyi*

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**A tetracycline-resistant *Haemophilus ducreyi* strain isolated in the United States was shown to carry a 34-megadalton plasmid which hybridized with the 1.8-kilobase *Kpn*I-*Hin*dIII Tet M probe. The complete Tn*916* transposon hybridized with five different bands from this plasmid, suggesting homology throughout the length of the transposon.**

The Tet M determinant has been found in the chromosome of commensal *Neisseria* species (14, 28) and gram-negative anaerobic species (28), as well as in a variety of gram-positive species (5, 12, 28). This gene codes for a protein that binds to the ribosomes, protecting them from tetracycline (3), and shares amino acid sequence homology with various elongation factors (30).

Five tetracycline-resistant *Haemophilus ducreyi* strains isolated in Jacksonville, Fla., were obtained from the Centers for Disease Control. The *H. ducreyi* strains were grown and cleared lysates were prepared (19, 22). Duplicate dot blots were hybridized with a 1.8-kilobase (kb) *Kpn*I-*Hin*dIII internal Tet M fragment from plasmid pUW-JKB1 (2, 31) or an internal 1.27-kb *Hin*cII Tet B fragment from plasmid pRT11 (17, 32). Four of the strains hybridized with the Tet B probe as described previously (17) and were not examined further, while the fifth strain (86.039418) hybridized only with the Tet M probe and was chosen for further study.

The DNA from this strain was prepared and electrophoresed through an agarose gel (19). A small plasmid which hybridized with the TEM β-lactamase gene from plasmid pJI3 was observed (4, 27) and thus assumed to be a β-lactamase plasmid (1, 7). A large plasmid of 34 megadaltons (MDa) was also seen as a faint band in some DNA preparations and was named pMR9418. The pMR9418 DNA was found to hybridize with the Tet M probe, while no hybridization was observed between the Tet M probe and the chromosome or the small plasmid.

DNAs from strain 86.039418, *Enterococcus faecalis* strain DS160 (5), and Tc$^r$ *Neisseria gonorrhoeae* strain 83.022650 (15, 18) (Table 1) were treated with the restriction enzyme *Hin*cII or *Hin*cII and *Eco*RI. Southern blots were prepared and probed with the 1.8-kb Tet M determinant or the Tn*916* probe. The Tn*916* probe used was a 17.7-kb fragment from plasmid pAM120, which carries the complete Tn*916* DNA sequence, originally from DS160 (5). When the 1.8-kb Tet M probe was used, all three DNAs had one hybridizing band. This is similar to what has been observed for other Tet M-containing species (2, 23, 27, 28). With the Tn*916* probe, at least five hybridizing bands were seen with the *H. ducreyi* DNA, and multiple bands were seen with the *E. faecalis* DNA (5, 12) (Fig. 1). In contrast, the *N. gonorrhoeae* DNA hybridized only with a single band. A 4.9-kb *Hin*cII Tet M fragment from this *N. gonorrhoeae* strain has been cloned and shown to contain the structural Tet M determinant, which hybridizes with one band with either probe (my unpublished data). Therefore, I conclude that the *N. gonorrhoeae* does not contain the entire transposon, while the *H. ducreyi* strain appears to have homology along the whole length of the Tn*916* transposon. This is similar to the Tc$^r$ *Clostridium difficile* strains, except in this species the Tet M determinant is located in the chromosome (12).

In most of the species with the Tet M determinant, including streptococci, the determinant is located in the chromosome (5, 12, 23, 24, 27, 28). The one exception is the 25.2-MDa plasmid (14, 15, 18). This plasmid is highly related to the indigenous gonococcal 24.5-MDa conjugative plasmid, and we have hypothesized that it was created by the transposition of the Tet M determinant into the 24.5-MDa plasmid (14, 18). We examined whether plasmid pMR9418 was related to the 25.2-MDa plasmid family by using a 24.5-MDa plasmid, pMR388, which had >60% of its DNA sequences in common with pMR650 but does not carry the Tet M determinant, as a probe (14). The large R plasmids described previously in *H. ducreyi* share 70 to 80% of their DNA sequences in common with R plasmids isolated from *H. influenzae* (1, 9, 22, 29). We used a well-characterized *H. influenzae* plasmid, RSF007 (8), to determine whether the *H. ducreyi* plasmid was related to this plasmid class as well. Southern blots were prepared and hybridized under stringent conditions (50% formamide). No homology was found between plasmids pMR9418 and pMR388, or with the RSF007 plasmid, suggesting that the pMR9418 plasmid is not highly related to either plasmid family.

We examined whether pMR9418 could be transferred to other *Haemophilus* spp. We chose, as recipients, the well-characterized *H. influenzae* strains Rd (29) and G32, carrying the 4.1-MDa β-lactamase plasmid (RSF0885) isolated from *H. parainfluenzae* HR-8 (8). The *H. influenzae* strains were grown as described previously (22), while the *H. ducreyi* strain was grown overnight on four agar plates supplemented with tetracycline. The *H. ducreyi* cells were removed and suspended in 1 ml of brain heart infusion to which broth-grown *H. influenzae* was added. The suspension was mixed, plated directly on four agar plates, incubated for 16 h, and then removed. A 1:10 dilution series was cultured on agar plates supplemented with rifampin, rifampin and tetracycline, rifampin and ampicillin, tetracycline and ampicillin, ampicillin, or tetracycline at the following concentrations (micrograms per milliliter): tetracycline, 10; streptomycin, 250; rifampin, 10; ampicillin, 10. Plates were incubated at 36.5°C in 5% $CO_2$ and were examined for growth at 24 and 48 h. Colonies that grew on the antibiotic-containing media were scored for streptomycin resistance if the recipient strain was Rd, and all transconjugants were screened for plasmids.

Both recipients, Rd and G32, acquired tetracycline resistance from the donor *H. ducreyi* strain at a frequency that

TABLE 1. Bacterial strains and their plasmids

| Strain | Plasmid (MDa) | Resistance[a] | Reference |
|---|---|---|---|
| *H. ducreyi* 86.039418 | 7.0, 34 (pMR9418) | $Ap^r$ $Tc^r$ | This study |
| *H. influenzae* Rd | None | $Ery^r$ $Rif^r$ $Str^r$ | 29 |
| G32(RSF0885)[b] | 4.1 | $Ap^r$ | 8 |
| *N. gonorrhoeae* 83.022650 | 25.2 (pMR650) | $Tc^r$ | 18 |
| *E. faecalis* DS160 | None | $Tet^r$ | 5 |

[a] Plasmid-mediated resistance: $Ap^r$, ampicillin; $Tc^r$, tetracycline. Chromosomal resistance: $Ery^r$, erythromycin; $Rif^r$, rifampin; $Str^r$, streptomycin; $Tet^r$, tetracycline (original Tn*916*).

[b] RSF0885 is an *H. parainfluenzae* plasmid which has been transformed into *H. influenzae* G32 (8).

ranged from $10^{-6}$ to $10^{-7}$ per recipient (Table 2). When the transconjugants were lysed, a plasmid of 34 MDa was found in all strains and was shown to hybridize with the 1.8-kb Tet M probe.

Cross-mating experiments were done by using the filter-mating technique (29) (Table 2). Both strains Rd and G32 could transfer the 34-MDa plasmid at higher frequencies ($10^{-1}$ to $10^{-4}$) compared with the original *H. ducreyi* donor strain (Table 2). This is not unusual, since transfer frequencies are often higher when both donor and recipient are from the same species than when the two represent different species (11, 25).

*H. ducreyi* R plasmids, in general, do not mobilize the small β-lactamase plasmids to recipients by conjugation. Only one cryptic *H. ducreyi* plasmid has been described which does (7). There was apparent transfer of the ampicillin determinant into strain Rd from the *H. ducreyi* strain. However, we could not maintain the transconjugants for more than one passage on ampicillin media and were never

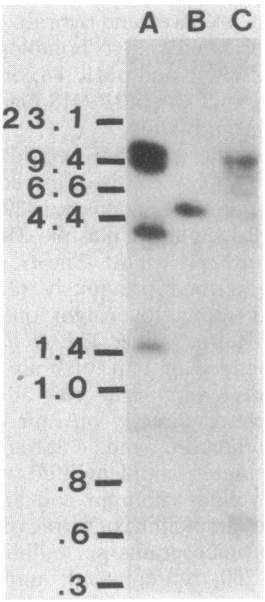


FIG. 1. Southern blot of 1.0% agarose gel probed with a $^{32}P$-radiolabeled 17.7-kb fragment carrying the complete Tn*916* transposon. Lane A, *Hin*cII-restricted DNA from *E. faecalis* DS160; lane B, *Hin*cII- and *Eco*RI-restricted DNA from *N. gonorrhoeae* 83.022650; lane C, *Hin*cII- and *Eco*RI-restricted DNA from *H. ducreyi* 86.039418. Numbers on the side represent sizes, in kilobases, of the *Hin*dIII lambda and φX174 *Hae*II fragments.

TABLE 2. Transfer of tetracycline and ampicillin plasmids by conjugation[a]

| Donor | Frequency with given recipient | | |
|---|---|---|---|
| | Rd | | G32(RSF0885) ($Tc^r$) |
| | $Tc^r$ | $Ap^r$ | |
| 86.039418 | $3.2 \times 10^{-7}$ | $4.5 \times 10^{-6}$[b] | $2.6 \times 10^{-6}$ |
| 83.022650 | $2.6 \times 10^{-6}$ | | $1.4 \times 10^{-4}$ |
| G32(RSF0885, pMR9418) | $1.4 \times 10^{-4}$ | $2.3 \times 10^{-6}$[b] | ND[d] |
| G32(RSF0885, pMR650) | $7.8 \times 10^{-6}$ | $6.5 \times 10^{-6}$[c] | ND |
| Rd(pMR9418) | ND | | $3.0 \times 10^{-1}$ |
| Rd(pMR650) | ND | | $3.0 \times 10^{-5}$ |

[a] Average of three or more separate experiments.

[b] Apparent transfer of β-lactamase plasmids, but not confirmed by agarose gel.

[c] Confirmed transfer of β-lactamase plasmids, validated by agarose gel visualization.

[d] ND, Not done.

able to demonstrate the β-lactamase plasmid in the transconjugants. This suggests that pMR9418 could not mobilize the small β-lactamase plasmids. Thus, plasmid pMR9418 is similar to other conjugative *Haemophilus* R plasmids in this characteristic and differs from the cryptic *H. ducreyi* conjugative plasmid described previously (7). This, along with the fact the 25.2-MDa plasmid retained its ability to mobilize β-lactamase plasmids (25, 26), suggests that the ancestor of pMR9418 may not be the cryptic *H. ducreyi* plasmid described previously, but more work is needed to confirm this hypothesis.

We transferred plasmid pMR650 from *N. gonorrhoeae* into the two *H. influenzae* recipients by conjugation (Table 2). These transconjugants could then act as donors in further matings (Table 2). When an *H. influenzae* strain carried both the 25.2-MDa plasmid and the 4.1-MDa β-lactamase plasmid, it could transfer both plasmids into a recipient and the β-lactamase plasmid was stable and could be visualized by agarose gels. The resulting transconjugants, which carried both the 25.2- and 4.1-MDa plasmids, could then act as donors and transfer both plasmids to other strains (data not shown).

We have shown that plasmid pMR650 from *N. gonorrhoeae* can transfer into the genus *Haemophilus*, suggesting that at some time we will see clinical isolates with the 25.2-MDa plasmid. This, together with the discovery of the *H. ducreyi* plasmid pMR9418, indicates that it is possible for strains of *Haemophilus* spp. to acquire the Tet M determinant. It also demonstrates that the antibiotic resistance gene pool in *H. ducreyi*, and probably in all bacteria, is not stagnant and indicates that, even in a bacterial population which has a high carriage of one tetracycline determinant (Tet B) (1), a second unrelated determinant can be introduced. How common the Tet M determinant is in *H. ducreyi* is not known and will require the screening of new $Tc^r$ strains. However, as carriage of the Tet M determinant increases in the bacterial populations of humans, the probability that the Tet M determinant will be introduced into other strains within the genus *Haemophilus* would be expected to increase.

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