ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1987, p. 1301–1306
0066-4804/87/091301-06$02.00/0

Vol. 31, No. 9

Cloning and Expression of a Tetracycline Resistance Determinant from *Campylobacter jejuni* in *Escherichia coli*

FRED C. TENOVER,[1,2]* DONALD J. LEBLANC,[3] AND PATRICIA ELVRUM[1]

Medical Research Service, Veterans Administration Medical Center, Seattle, Washington 98108[1]; Departments of Laboratory Medicine and Microbiology, University of Washington, Seattle, Washington 98195[2]; and Bacterial Virulence Section, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Fort Detrick, Frederick, Maryland 21701-1013[3]

Received 4 March 1987/Accepted 16 June 1987

The tetracycline resistance gene (*tet*) from the *Campylobacter jejuni* plasmid pFKT1025 was cloned into both pUC18 and pBR322 and was expressed when the chimeric plasmids were introduced into *Escherichia coli*. The location of the *tet* determinant on the chimeric plasmids was determined by BAL 31 deletion mapping within a 2.25-kilobase (kb) *Rsa*I-*Hin*cII fragment. A protein of approximately 70 kilodaltons was consistently produced by *E. coli* maxicells harboring the cloned *tet* determinant. A 500-base-pair restriction fragment from within the 2.25-kb *tet* region was shown to hybridize only to DNA from tetracycline-resistant strains of *C. jejuni* and *C. coli*, but not to the DNA of organisms known to carry the streptococcal *tetM* determinant. No homology was noted between the DNA of 10 tetracycline-resistant isolates of campylobacter and the streptococcal *tetL*, *tetM*, or *tetN* determinants when tested under conditions of high stringency. However, homology was noted between a 5.0-kb *Hin*cII restriction fragment containing the *tetM* determinant and two *C. jejuni tet* R factors under conditions of reduced stringency.

Tetracycline resistance (Tc^r) is relatively common among microorganisms in nature (5, 14, 18–20). Multiple genes can encode resistance to this antimicrobial agent in both gram-positive (5) and gram-negative (14) bacteria. Isolates of the enteric pathogens *Campylobacter jejuni* and *Campylobacter coli* (2) are likewise often Tc^r. In the Seattle, Wash., area for instance, 24% of all campylobacter isolates were resistant to this antimicrobial agent (28). Other researchers have reported the prevalence of Tc^r strains to vary from 2.0 to 37.9% (3, 12, 17, 24, 30).

The *tet* gene in campylobacters is usually located on a plasmid (26, 28). Hybridization studies by both Tenover et al. (28) and Taylor et al. (26) have shown this gene to be unrelated to *tet* determinants from gram-negative enteric organisms. Recently, however, Taylor reported homology between a campylobacter *tet* determinant and the *tetM* determinant from streptococci (25). Martin et al. (16), after sequencing the *tetM* gene, suggested that the homology noted by Taylor between the campylobacter *tet* gene and the *tetM* determinant was due to sequences on the *tetM* probe outside the coding region of the *tetM* gene and not to sequences within the open reading frame. This was confirmed when an intragenic probe from the *tetM* determinant failed to hybridize to a campylobacter R factor carrying a *tet* gene (16).

To learn more about the molecular biology of the campylobacter *tet* gene, the determinant from the campylobacter R factor pFKT1025 was cloned into both pUC18 and pBR322 and the chimeric plasmids were introduced into several strains of *Escherichia coli*. The protein products of this determinant and its genetic relatedness to the *tet* determinants of gram-positive cocci are the subject of this study.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains used are described in Table 1. *C. jejuni* PS1025 (28) was used as the source of pFKT1025 plasmid DNA. *C. jejuni* PS946, PS968, PS973, PS975, PS1156, PS1318, PS1455, PS1460, PS1709, and PS2051 and *C. coli* PS1148, all of which are Tc^r strains carrying R factors ranging in size from 45 to 109 kilobases (kb), were used in hybridization studies (see below). *C. jejuni* PS1234 was included as a tetracycline-susceptible (Tc^s) control. All of these strains were obtained from humans or chickens during a recent Food and Drug Administration project to study the flow of campylobacters and salmonellae from food sources of animal origin to humans (10, 11, 28). Two Tc^r isolates of *Neisseria gonorrhoeae*, two Tc^r isolates of *Gardnerella vaginalis*, two Tc^r isolates of *Mycoplasma hominis*, and one Tc^r isolate each of *Ureaplasma* sp. and *Neisseria meningitidis*, previously shown to contain the *tetM* determinant (18–20), were obtained from M. Roberts, Seattle, Wash. *Streptococcus faecalis* strains containing either Tn*916*, pAMα1, or pMV120, each of which carries the *tetM* resistance determinant, were used as *tetM*-positive controls. *E. coli* C600 (15) was obtained from L. Tompkins, Stanford, Calif.; *E. coli* JM83 (31) from V. Clark, Rochester, N.Y.; and strain JC2926, a *recA13* derivative of AB1157 (15), from M. Yanofsky, Seattle, Wash.

Plasmid methods. Plasmid DNA was prepared by CsCl-ethidium bromide density gradient centrifugation as previously described (28). Restriction endonucleases and T4 DNA ligase were purchased from New England BioLabs, Inc., Beverly, Mass., and used according to the manufacturer's directions. Restriction endonuclease maps of plasmids used in this study were prepared by standard methods (15).

TABLE 1. Bacterial strains

Organism and strain	Plasmid (size, kb)	Parent[a]	Phenotype
C. jejuni PS1025	pFKT1025 (48)	NA	Tc^r
E. coli			
PS3601	pBR322 (4.4)	NA	Tc^r
PS3312	pFKT686 (15.0)	NA	Tc^r
PS3748	pFKT3748 (3.6)	pFKT686	Tc^s
PS3750	pFKT3750 (6.7)	pFKT686	Tc^s
PS3744	pFKT3744 (6.8)	pFKT686	Tc^s
PS3745	pFKT3745 (7.8)	pFKT686	Tc^s
PS3737	pFKT3737 (9.4)	pFKT686	Tc^s
PS3738	pFKT3738 (11.4)	pFKT686	Tc^s
PS3406	pFKT3406 (11.8)	pFKT686	Tc^s
PS3602	pFKT3602 (12.6)	pFKT686	Tc^r
PS3741	pFKT3741 (13.0)	pFKT686	Tc^r
PS3749	pFKT3749 (13.2)	pFKT686	Tc^r
PS3783	pFKT3783 (14.7)	pFKT686	Tc^r
PS3724	pFKT212 (11.0)	NA	Tc^r
PS3751	pFKT3751 (3.0)	pFKT212	Tc^s
PS3752	pFKT3752 (4.8)	pFKT212	Tc^s
PS3739	pFKT3739 (5.0)	pFKT212	Tc^s
PS3740	pFKT3740 (6.0)	pFKT212	Tc^s
PS3409	pFKT3409 (7.5)	pFKT212	Tc^s
PS3603	pFKT3603 (9.1)	pFKT212	Tc^s
PS3408	pFKT3408 (10.0)	pFKT212	Tc^r

[a] NA, Not applicable.

Cloning procedure. The chimeric plasmid pFKT212 was constructed by digesting the CsCl-purified pFKT1025 DNA with *Bgl*II and *Xho*I and the pUC18 DNA with *Sal*I and *Bam*HI for 2 h at 37°C and then ligating the digests with T4 ligase overnight at 16°C as recommended by the manufacturer. The ligation mixture was transformed into *E. coli* JM83 by the method of Cohen et al. (7). Transformants were selected on X-Gal plates (31) containing 30 μg of ampicillin per ml. After 48 h, colorless colonies were replica plated onto brucella agar containing 15 μg of tetracycline per ml. Tc^r clones were screened for chimeric plasmids by an alkaline-sodium dodecyl sulfate (SDS) lysis method as previously described (28).

A second chimeric plasmid, pFKT686, was constructed by inserting a 10.7-kb *Bgl*II restriction fragment from pFKT1025 (Fig. 1) into the *Bam*HI site within the *tet* gene of pBR322 and transforming the ligated plasmid mixture into *E. coli* C600, selecting for Tc^r (15 μg/ml) on brucella agar. Clones were transferred to nitrocellulose filters (BA45; Schleicher & Schuell, Inc., Keene, N.H.) and hybridized with gradient-purified, ^{32}P-labeled pFKT1025 DNA to verify the presence of campylobacter DNA in the clones. We had previously determined that pFKT1025 and pBR322 share no homology (28), and we hypothesized that the likelihood of producing an active *tet* fusion protein that would result in a Tc^r phenotype was low. A chimeric plasmid showing a 10.7-kb insert that hybridized to pFKT1025 DNA was selected for further study.

Localization of the *tet* gene. The chimeric plasmids pFKT212 and pFKT686 were cleaved into a single linear fragment with *EcoR*V and *Xho*I, respectively, and digested with BAL 31 (New England BioLabs) at concentrations of 1 and 5 U of activity for 1, 2, and 5 min according to the manufacturer's directions. The digestion mixtures were ligated with T4 DNA ligase and transformed into *E. coli* C600 with selection for ampicillin resistance (100 μg/ml). Clones were replica plated onto brucella agar containing 100 μg of ampicillin per ml with or without the addition of 15 μg of tetracycline per ml. The *Hin*cII, *Hin*dIII, and *Rsa*I restriction maps of 14 chimeric plasmids representing nine Tc^s and five Tc^r clones were determined to localize the extent of the BAL 31 deletions.

Maxicells. Maxicells were prepared by the method of Sancar et al. (21) with *E. coli* JC2926, and the products were analyzed on 12 or 15% SDS-polyacrylamide gels as previously described (13, 27). Proteins were labeled with [^{35}S]methionine (1,112 Ci/mmol; New England Nuclear Corp., Boston, Mass.).

Hybridization procedures. Spot blots of bacterial isolates were prepared on nitrocellulose filters by the method of Gootz et al. (9) and hybridized with the campylobacter *tet* probe as described by Wahl et al. (32). Briefly, filters were prehybridized for 1 h at 42°C in a buffer containing 50% formamide, 1% glycine, 50 mM phosphate buffer (pH 6.5), 100 μg of sonicated calf thymus DNA per ml, 5× Denhardt solution (1× Denhardt solution is 0.02% polyvinylpyrrolidone plus 0.02% bovine serum albumin plus 0.02% Ficoll), and 5× SSPE buffer (20× SSPE buffer contains 18 mM NaCl, 200 mM $NaH_2PO_4 \cdot H_2O$, 22 mM disodium EDTA, and 110 mM NaOH [pH 7.7]). This solution was discarded and replaced with a hybridization buffer containing 50% formamide, 1% glycine, 20 mM sodium phosphate buffer (pH 6.5), 1× Denhardt solution, 100 μg of calf thymus DNA per ml, 5× SSPE, and 10% dextran sulfate. Hybridization was carried out at 42°C overnight. Filters were washed at room temperature twice in 2× SSPE and 0.1% SDS and twice at 50°C in 0.1× SSPE–0.1% SDS.

Spot blot hybridization reactions with the *tetL*, *tetM*, and *tetN* determinants were prehybridized in a solution containing 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 4× Denhardt solution, and 10 μg of whole yeast RNA per ml for 4 h at 68°C and hybridized overnight under the same conditions with fresh buffer containing denatured probe plus 120 μg of sheared salmon sperm DNA per ml (29). After hybridization, the blots were washed four times in

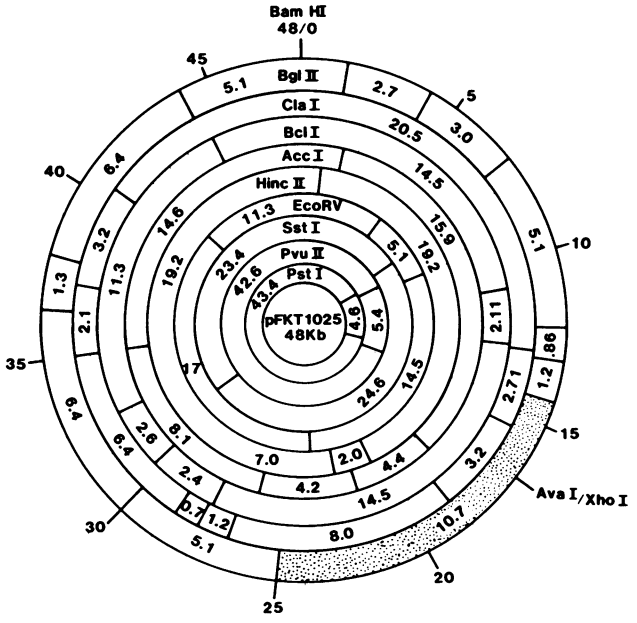

FIG. 1. Physical map of pFKT1025. Units are expressed in kilobases. The stippled area indicates the cloned fragments.

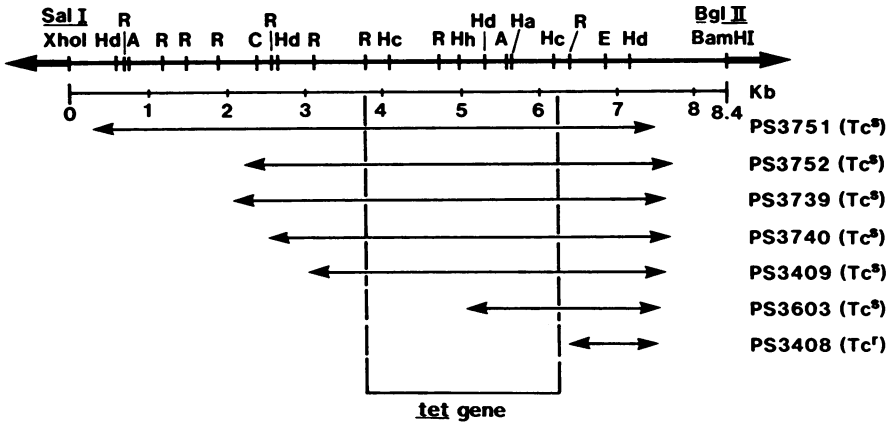

FIG. 2. Physical map of pFKT212 and BAL 31 deletion variants. *tet* gene, Tc^r gene. Distances are expressed in kilobases. Abbreviations: Hd, *Hin*dIII; R, *Rsa*I; C, *Cla*I; Hc, *Hin*cII; Hh, *Hha*I; A, *Acc*I; Ha, *Hae*III; E, *Eco*RV. Arrows represent deletions produced by BAL 31 digestion.

0.1% SDS–0.1× SSC and four times in 0.1× SSC, for 15 to 30 min each, at 52°C.

To determine the level of stringency at which the *tetM* determinant hybridized to the campylobacter *tet* determinant, the restriction fragments generated by *Bgl*II digestion of the campylobacter *tet* R factors pFKT946 and pFKT1156 (28) and the fragments generated by *Hin*cII digestion of the *tetM* containing plasmid pJI3 were transferred to nitrocellulose filters by the method of Southern (22) and hybridized under three conditions of stringency with the *Hin*cII fragment, radiolabeled with ^{32}P by nick translation, as a probe. The three conditions were (i) hybridization in 50% formamide at 42°C with washing at 50°C, (ii) hybridization in 40% formamide at 37°C with washing at 43°C, and (iii) hybridization in 30% formamide at 37°C with washing at 36°C. After being dried, all blots (Southern and spot blots) were exposed to Kodak X-Omat AR X-ray film with intensifying screens at −70°C for 1 to 4 days.

The *tetL* probe was whole pAMα1 plasmid DNA (6), the *tetM* probe was pJI3 plasmid DNA or the 5-kb *Hin*cII fragment containing the *tetM* determinant, and the *tetN* probe was whole pMV120 DNA (6).

RESULTS

Construction of the physical map of pFKT1025 and cloning of the Tc^r gene. The physical map of the campylobacter *tet* R factor pFKT1025 was determined by restriction endonuclease analysis and is presented in Fig. 1.

The 8.3-kb *Xho*I-*Bgl*II restriction fragment from pFKT1025 (Fig. 1) was inserted into pUC18 after cleavage of the vector with *Sal*I-*Bam*HI. This chimeric plasmid conferred Tc^r to the recipient *E. coli* JM83 strain. This plasmid was designated pFKT212. Restriction endonuclease mapping of this plasmid revealed that a small insertion of DNA (~0.2 kb) occurred at a site located at 2.0 kb on the pFKT212 map (Fig. 2). We were unable to determine the source of this inserted DNA.

The 10.7-kb *Bgl*II fragment of pFKT1025 (Fig. 1) was isolated and inserted into the *Bam*HI site in pBR322, and the presence of campylobacter DNA in the clone was confirmed by hybridization with pFKT1025 DNA. This plasmid, pFKT686, also conferred Tc^r to a recipient strain of *E. coli*. The physical map of this plasmid is presented in Fig. 3.

Localization of the *tet* resistance determinant on the cloned

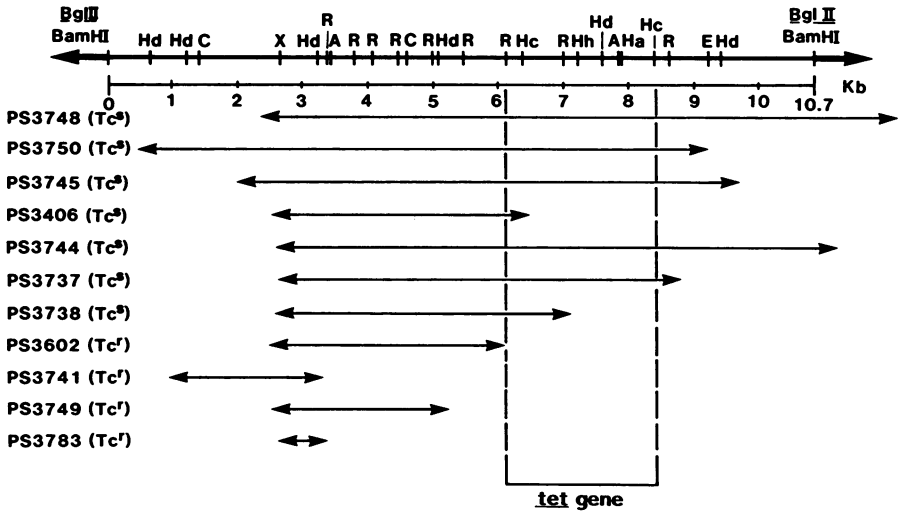

FIG. 3. Physical map of pFKT686 and BAL 31 deletion variants. *tet* gene, Tc^r gene. Distances are expressed in kilobases. Arrows represent deletions produced by BAL 31 digestion. For abbreviations, see the legend to Fig. 2.

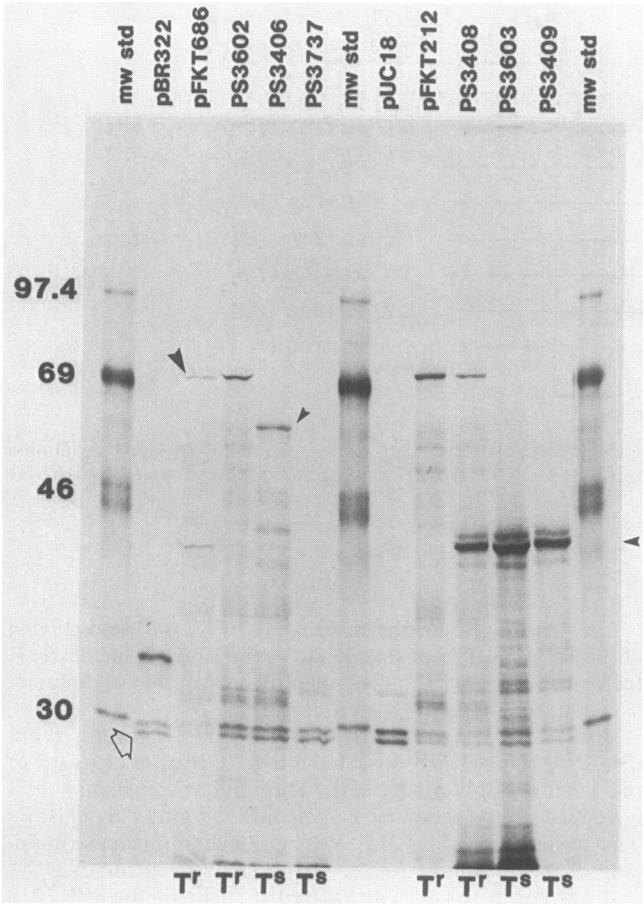

FIG. 4. Autoradiograph of 12% polyacrylamide gel containing plasmid-encoded products of chimeric plasmids expressed in *E. coli* JC2926 maxicells. Strains PS3602, PS3406, and PS3737 contain plasmids that are deletion derivatives of pFKT686 (see Table 1). Strains PS3408, PS3603, and PS3409 contain deletions of the chimeric plasmid pFKT212 (Table 1). T^r, Tc^r strain; T^s, Tc^s strain. Molecular weight standards are shown on the left. The proteins were labeled with [^{35}S]methionine. The large black arrow indicates the 70-kDa campylobacter *tet*-encoded protein; the smaller arrows indicate the 62-kDa truncated protein and the 41-kDa protein; the open arrow indicates β-lactamase proteins.

fragments. The enzyme BAL 31 was used to delete portions of pFKT212 starting from the *Eco*RV site at approximately 6.8 kb. Seven new chimeric plasmids are shown in Fig. 2. The Tc^r phenotype was only preserved in strain PS3408 in which the *Hin*cII site at 6.2 kb remained intact. Any deletion extending beyond this site toward the *Sal*I-*Xho*I border resulted in a Tc^s phenotype (Fig. 2). Similarly, all BAL 31 deletions of pFKT686 extending from the *Xho*I site at 2.7 kb, past the *Rsa*I site at 6.2 kb, resulted in a Tc^s phenotype (Fig. 3). Thus, the *tet* gene appears to lie on an *Rsa*I-*Hin*cII fragment of approximately 2.25 kb.

Maxicell analysis of chimeric clones. The chimeric plasmids pFKT212 and pFKT686 and several of the BAL 31 deletions of each were transformed into the *E. coli* maxicell strain JC2926, and the plasmid-encoded proteins were examined by polyacrylamide gel electrophoresis (Fig. 4). Transformants demonstrating the Tc^r phenotype produced a novel protein of approximately 70 kilodaltons (kDa). A truncated protein of 62 kDa was noted from PS3406 (a BAL 31 deletion of pFKT686), suggesting that the C terminus of the protein is proximal to the *Rsa*I site located at 6.2 kb on the map of pFKT686 (Fig. 3). No truncated proteins were seen among the peptides produced from the BAL 31 deletions of pFKT212 initiated at the *Eco*RV site. This further supports the hypothesis that transcription proceeds from the *Hin*cII boundary of the *tet* gene toward the *Rsa*I boundary. A novel 41-kDa protein was noted in maxicell preparations of pFKT686 and in preparations of Tc^r and Tc^s BAL 31 deletions of pFKT212 strains, but not in preparations containing the original pFKT212 (Fig. 4). The function and exact coding region of this protein are currently unknown. The 28-kDa β-lactamase protein and its 30-kDa precursor can be seen in all of the maxicell preparations (8).

DNA-DNA homology studies with streptococcal *tet* determinants. The DNAs of nine Tc^r isolates of *C. jejuni* were examined for homology to the *tetL*, *tetM*, and *tetN* determinants by spot blot hybridization. No hybridization was noted between the DNAs of these strains and any of the three streptococcal *tet* probes under conditions of high stringency. The 5.0-kb *Hin*cII fragment of pJI3 was then radiolabeled and hybridized to *Bgl*II restriction digests of the campylobacter *tet* R factors pFKT946 and pFKT1156. Homology between the *tetM* probe and the campylobacter R factors was noted only when the concentration of formamide was reduced from 50 to 40% and the temperature of the posthybridization washes was reduced to at least 43°C (data not shown).

Similarly, eight isolates of Tc^r bacteria, each carrying the *tetM* determinant, were spotted onto nitrocellulose filters and probed with a 500-base-pair *Rsa*I-*Hin*cII fragment from the cloned campylobacter *tet* gene, presumed to be from within the open reading frame of the resistance gene (4.2 to 4.7 kb, Fig. 2). No homology was noted between this fragment and any of the *tetM*-carrying organisms, including *Mycoplasma hominis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Gardnerella vaginalis*, a *Ureaplasma* sp., and *E. coli*, which contain the cloned *tetM* sequence. Five Tc^s, plasmid-containing isolates of *C. jejuni* were also found to be negative by this method (data not shown). Only Tc^r *C. jejuni* strains gave positive signals.

DISCUSSION

The *tet* determinant from the *C. jejuni* plasmid pFKT1025 was cloned into two different vectors and was expressed when the chimeric plasmids were transformed into *E. coli*. The gene was localized to a fragment of approximately 2.25 kb by BAL 31 deletion mapping of the chimeric plasmids pFKT212 and pFKT686.

The cloned *tet* sequence appears to produce a protein of approximately 70 kDa when analyzed with *E. coli* maxicells. The fact that a truncated protein was noted only in the BAL 31-deleted plasmid of PS3406, a pFKT686 derivative, and not in any of the pFKT212 derivatives, suggests that transcription of the protein begins proximal to the *Hin*cII site at 8.4 kb on the pFKT686 map and proceeds toward the *Rsa*I site located at 6.2 kb (Fig. 3).

An additional protein of 41 kDa was found in maxicell preparations of the BAL 31-deleted pFKT212 and in the parent pFKT686 strain (Fig. 4). However, it was not consistently produced in either Tc^r or Tc^s organisms and could not be associated with any phenotypic trait.

Taylor has reported that the campylobacter *tet* gene and the *tetM* determinant from streptococci are homologous as shown by hybridization studies between a 5.0-kb *Hin*cII fragment from plasmid pJI3 and DNA from the 45-kb *C. jejuni* R factor pUA466 (25). We were able to demonstrate

homology between our campylobacter R factors and the same *Hin*cII fragment only under conditions of reduced stringency. Furthermore, a probe specific for the campylobacter *tet* gene failed to hybridize to a variety of *tetM*-containing organisms. This suggests that our campylobacter *tet* gene and the *tetM* determinant are unrelated. This notion is supported by the fact that restriction endonuclease maps of our cloned gene and restriction maps deduced from the DNA sequence of the *tetM* determinant (16) are quite dissimilar (data not shown). It is interesting, however, that the campylobacter *tet*-encoded protein and the *tetM*-encoded protein are similar in size (*tetM* is 68 kDa [16]). While the homology noted by Taylor may be due to flanking sequences outside the *tetM* gene, as suggested by Martin et al. (16), it is also possible that there are multiple *tet* genes within the genus *Campylobacter*.

Burdett has recently shown that the *tetM* determinant, unlike other *tet* determinants, appears to manifest resistance at the level of protein synthesis rather than promoting the efflux of tetracycline out of the cell (4). The mechanism of Tc^r in campylobacters is currently unknown; our hypothesis is that although the proteins are encoded by different genes, the functions of the proteins may be similar. However, our studies to determine the location of the *tet*-encoded protein in both maxicells and in native *C. jejuni* cells by fractionating membranes on sucrose gradients using a variety of techniques (1, 23) have been unsuccessful.

In summary, although we have been able to demonstrate that a cloned *tet* resistance gene from *C. jejuni* produces a protein similar in size to that produced by the *tetM* determinant, a probe isolated from the cloned campylobacter *tet* determinant failed to show homology to several organisms known to contain the *tetM* gene. Furthermore, purified plasmid DNA of known campylobacter *tet* R factors only demonstrated homology to the *tetM* determinant when tested under conditions of low stringency. From this data we conclude that the campylobacter *tet* gene is not related to the *tetM* determinant nor is it related to the *tetL* or *tetN* determinants. Further studies to characterize the function and location of the *tet*-encoded protein are under way.

ACKNOWLEDGMENTS

We gratefully acknowledge the excellent technical expertise of S. Williams and the helpful advice of S. Moseley, S. Lory, and M. Blaser.

This project was supported by the Medical Research Service of the Veterans Administration.

LITERATURE CITED

1. **Ames, G. F.-L., C. Prody, and S. Kustu.** 1984. Simple, rapid, and quantitative release of periplasmic proteins by chloroform. J. Bacteriol. **160**:1181–1183.
2. **Blaser, M. J., and L. B. Reller.** 1981. Campylobacter enteritis. N. Engl. J. Med. **305**:1444–1452.
3. **Buck, G. E., and M. T. Kelly.** 1982. Susceptibility testing of *Campylobacter fetus* subsp. *jejuni*, using broth microdilution panels. Antimicrob. Agents Chemother. **21**:274–277.
4. **Burdett, V.** 1986. Streptococcal tetracycline resistance mediated at the level of protein synthesis. J. Bacteriol. **165**:564–569.
5. **Burdett, V., J. Inamine, and S. Rajagopalan.** 1982. Heterogeneity of tetracycline resistance determinants in *Streptococcus*. J. Bacteriol. **149**:995–1004.
6. **Burdett, V., J. Inamine, and S. Rajagopalan.** 1982. Multiple tetracycline resistance determinants in *Streptococcus*, p. 155–158. *In* D. Schlessinger (ed.) Microbiology—1982. American Society for Microbiology, Washington, D.C.
7. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R factor DNA. Proc. Natl. Acad. Sci. USA **69**:2110–2114.
8. **Dougan, G., M. Saul, A. Twigg, R. Gill, and D. Sherrat.** 1979. Polypeptides expressed in *Escherichia coli* K-12 minicells by transposition elements Tn*1* and Tn*3*. J. Bacteriol. **138**:46–54.
9. **Gootz, T. D., F. C. Tenover, S. A. Young, K. P. Gordon, and J. J. Plorde.** 1985. Comparison of three DNA hybridization methods for the detection of the aminoglycoside 2''-*O*-adenylyltransferase gene in clinical bacterial isolates. Antimicrob. Agents Chemother. **28**:69–73.
10. **Harris, N. V., D. Thompson, D. C. Martin, and C. M. Nolan.** 1986. A survey of campylobacter and other bacterial contaminants of pre-market chicken and retail poultry and meats, King County, Washington. Am. J. Public Health **76**:401–406.
11. **Harris, N. V., N. S. Weiss, and C. M. Nolan.** 1986. The role of poultry and meats in the etiology of *Campylobacter jejuni/coli* enteritis. Am. J. Public Health **76**:407–411.
12. **Karmali, M. A., S. DeGrandis, and P. C. Fleming.** 1981. Antimicrobial susceptibility of *Campylobacter jejuni* with special reference to resistance patterns of Canadian isolates. Antimicrob. Agents Chemother. **19**:593–597.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
14. **Levy, S. B.** 1984. Resistance to the tetracyclines, p. 191–240. *In* L. E. Bryan (ed.), Antimicrobial drug resistance. Academic Press, Inc., New York.
15. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Martin, P., P. Trieu-Cuot, and P. Courvalin.** 1986. Nucleotide sequence of the *tetM* tetracycline resistance determinant of the streptococcal conjugative shuttle transposon Tn*1545*. Nucleic Acids Res. **14**:7047–7058.
17. **Michel, J., M. Rogol, and D. Dickman.** 1983. Susceptibility of clinical isolates of *Campylobacter jejuni* to sixteen antimicrobial agents. Antimicrob. Agents Chemother. **23**:796–797.
18. **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.
19. **Roberts, M. C., and G. E. Kenny.** 1986. Dissemination of the *tetM* resistance determinant to *Ureaplasma urealyticum*. Antimicrob. Agents Chemother. **29**:350–352.
20. **Roberts, M. C., L. A. Koutsky, K. K. Holmes, D. J. LeBlanc, and G. Kenny.** 1985. Tetracycline-resistant *Mycoplasma hominis* strains contain *tetM* sequences. Antimicrob. Agents Chemother. **24**:141–143.
21. **Sancar, A., A. M. Hack, and W. D. Rupp.** 1979. A simple method for identification of plasmid-coded proteins. J. Bacteriol. **137**:692–693.
22. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
23. **Strom, M. S., and S. Lory.** 1986. Cloning and expression of the pilin gene of *Pseudomonas aeruginosa* PAK in *Escherichia coli*. J. Bacteriol. **165**:367–372.
24. **Svedham, A., B. Kaijser, and E. Sjögren.** 1981. Antimicrobial susceptibility of *Campylobacter jejuni* isolated from humans with diarrhea and healthy chickens. J. Antimicrob. Chemother. **7**:301–305.
25. **Taylor, D. E.** 1986. Plasmid-mediated tetracycline resistance in *Campylobacter jejuni*: expression in *Escherichia coli* and identification of homology with streptococcal class M determinant. J. Bacteriol. **165**:1037–1039.
26. **Taylor, D. E., R. S. Garner, and B. J. Allan.** 1983. Characterization of tetracycline resistance plasmids from *Campylobacter jejuni* and *Campylobacter coli*. Antimicrob. Agents Chemother. **24**:930–935.
27. **Tenover, F. C., T. D. Gootz, K. P. Gordon, L. S. Tompkins, S. A. Young, and J. J. Plorde.** 1984. Development of a DNA probe for the structural gene of the 2''-*O*-adenyltransferase aminoglycoside modifying enzyme. J. Infect. Dis. **150**:678–687.

28. **Tenover, F. C., S. Williams, K. P. Gordon, C. Nolan, and J. J. Plorde.** 1985. Survey of plasmids and resistance factors in *Campylobacter jejuni* and *Campylobacter coli*. Antimicrob. Agents. Chemother. **27**:37–41.
29. **Thayer, R. E.** 1979. An improved method for detecting foreign DNA in plasmids of *Escherichia coli*. Anal. Biochem. **98**:60–63.
30. **Vanhoof, R., B. Gordts, R. Dierickx, H. Coignau, and J. P. Butzler.** 1980. Bacteriostatic and bacteriocidal activities of 24 antimicrobial agents and *Campylobacter fetus* subsp. *jejuni*. Antimicrob. Agents Chemother. **18**:118–121.
31. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**:259–268.
32. **Wahl, G., M. Stern, and G. R. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. USA **76**:3683–3687.