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NOTES

Cytotoxic and Enterotoxic Activities of *Campylobacter jejuni* Are Not Specified by Tetracycline Resistance Plasmids pMAK175 and pUA466

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The 45-kilobase tetracycline resistance plasmids pMAK175 and pUA466 from *Campylobacter jejuni* were examined using curing and mating experiments. However, these plasmids encoded neither cytotoxin production, as determined in Vero cells, nor enterotoxin activity, as determined in Chinese hamster ovary cells.

Two distinct toxic activities have been associated with isolates of *Campylobacter jejuni* and *Campylobacter coli*. We previously described both cytotoxic effects observed in Vero monkey kidney cells and cytotonic effects in Chinese hamster ovary (CHO) cells (1). The choleralike enterotoxin activity has been reported by several workers (2, 5, 6). Preliminary studies have related enterotoxin and cytotoxin production to different manifestations of the disease (3). However, the exact role of these toxins in *Campylobacter*-associated diarrhea continues to be the subject of much speculation.

Lee and co-workers reported that *C. jejuni* CH5 harbored a 46.5-kilobase plasmid (pGK103; 14) which specified both tetracycline resistance (Tc) and enterotoxin production and which was transferable within the genus *Campylobacter* (4). Of 30 enterotoxigenic isolates of *C. jejuni* surveyed by these workers, only 61% of strains harbored demonstrable plasmids (14). Therefore, the presence of the plasmid did not definitively correlate with enterotoxin production in *C. jejuni*.

Tc plasmids from *C. jejuni* and *C. coli* were reported initially by one of us (9) and have been subsequently characterized in more detail (8, 10, 11). They all appeared to be very similar in size, restriction digest patterns, and ability to transfer to other *Campylobacter* species. More recently, a restriction map of the 45-kilobase tetracycline resistance plasmid pUA466 was constructed, and the tetracycline resistance determinant was cloned and expressed in *Escherichia coli* (7). The pUA466 plasmid was isolated from *C. jejuni* CH5 and corresponds to the plasmid designated pGK103 by Walker et al. (14).

The availability of the restriction maps of several *C. jejuni* tetracycline resistance plasmids (7, 8) prompted us to investigate the report that the plasmid present in *C. jejuni* CH5 was able to mediate enterotoxin production; we hoped to map this determinant. To investigate the possible plasmid location of the two toxins, the following experiments were performed. *C. jejuni* UA466 (originally designated CH5) was used to select three derivative strains: UA649, a tetracycline-susceptible strain containing a plasmid in which the Tc determinant had been deleted, and UA650 and UA651, two plasmid-free derivatives of UA466. The plasmid content of the strains was monitored by agarose gel electrophoresis (7, 8). All four strains were tested for cytotoxic and enterotoxic activities, respectively, in Vero and CHO cells (1). The results are shown in Table 1. Strain UA466 possessed both cytotoxic and enterotoxic activities. Moreover, these characteristics remained stable even after UA466 was cured of the Tc plasmid. Similar results were obtained with other Tc plasmids from our collection. *C. jejuni* UA1, which contained plasmid pMAK175, produced both the cytotoxin and enterotoxin, as did UA124, a plasmid-free derivative of UA1.

The Tc plasmids from *C. jejuni* can be transferred to *Campylobacter fetus* subsp. *fetus* ATCC 27374. A mating experiment was performed between *C. fetus* subsp. *fetus* ATCC 27374 and UA466 and UA1, and transconjugants were selected on nalidixic acid (50 μg/ml) and tetracycline (25 μg/ml). The Tc *C. fetus* subsp. *fetus* transconjugants were tested for enterotoxin in CHO cells. No enterotoxic activity was observed with either *C. fetus* subsp. *fetus* ATCC 27374 alone or when it harbored either of the Tc plasmids, pMAK175 or pUA466. Thus, we were unable to demonstrate using curing or mating experiments that either the cytotoxin or the enterotoxin in *C. jejuni* is plasmid mediated.

How can the earlier findings of cotransfer of enterotoxin production and tetracycline resistance (4) be explained? Spontaneous nalidixic acid-resistant (Nal) mutants of *C. jejuni* which can be selected by plating on nalidixic acid-containing agar may arise (12). Mutants of a plasmid-free strain of *C. jejuni* arose at a frequency of 2.5×10^{-8} per cell plated (12). However, we have also observed that Nal mutants may arise more rapidly from some Tc *C. jejuni* strains, with frequencies of 10^{-5} to 10^{-6} per cell plated (L.-K. Ng, N. Chang, and D. E. Taylor, unpublished data). This increased mutation frequency in plasmid-containing

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TABLE 1. Cytotoxic and enterotoxic activity of *C. jejuni* strains[a]

Strain[b]	Plasmid[c]	Tetracycline resistance	Cytotoxin[d]	Enterotoxin[e]
UA466	pUA466	+	+ (4)	+ (40)
UA649	pUA649	−	+ (2)	+ (30)
UA650		−	+ (4)	+ (30)
UA651		−	+ (4)	+ (30)

[a] +, Resistant; −, susceptible.

[b] UA649 was derived from UA466 by loss of a 4.2-kilobase *Acc*I fragment associated with tetracycline resistance. UA650 and UA651 are plasmid-free derivatives of UA466. Construction of all four strains was described in detail previously (7).

[c] Presence or absence of the plasmid was determined by agarose gel electrophoresis as described previously (7, 8).

[d] Cytotoxic activity detected in Vero cells. The reciprocal of the last dilution showing cytoxicity is shown.

[e] Enterotoxic activity (cytotonic activity) detected in CHO cells. The percentage of elongated cells is shown.

strains of *C. jejuni* may depend on the mutagenic activity of insertion sequences or possibly of a transposon residing within the Tc plasmids. Because Tc plasmids transfer at relatively low frequencies, 10^{-6} to 10^{-4} transconjugants per recipient in a 24-h mating experiment (8, 10), spontaneous Nal mutants may be mistaken for true transconjugants if donors and recipients have no other distinguishing features.

A more intriguing explanation should be considered. Tenover and co-workers reported that a Tc plasmid (pFKT1025) showed homology with chromosomal DNA isolated from a tetracycline-susceptible, plasmid-free strain of *C. jejuni* (13). Lee and co-workers reported that the pGK103 plasmid showed homology with chromosomal DNA from several *C. jejuni* strains (4), including an enterotoxigenic, tetracycline-susceptible, plasmid-free strain (14). We have observed that pUA649, the tetracycline-susceptible deletion mutant of pUA466, hybridized with chromosomal DNA from tetracycline-susceptible *C. jejuni* UA650 (L.-K. Ng and D. E. Taylor, unpublished data). Therefore, it appears that *C. jejuni* plasmids may acquire regions of *Campylobacter* chromosomal DNA by some type of recombination mechanism. Our results demonstrate that Tc plasmids pUA466 and pMAK175 do not encode toxin production. It is possible that these determinants are acquired from the chromosome by a recombination process and could be cotransferred with the plasmid. Whether and by what means these plasmids are able to mobilize toxin determinants from a chromosomal location in one *C. jejuni* strain to a second strain remains to be determined.

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