Natural Transformation in Campylobacter Species

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Growing cells of Campylobacter coli and C. jejuni were naturally transformed by naked DNA without the requirement for any special treatment. Transformation frequencies for homologous chromosomal DNA were approximately $10^{-2}$ transformants per recipient cell in C. coli and $10^{-4}$ in C. jejuni. Maximum competence was found in the early log phase of growth. Campylobacters preferentially took up their own DNA in comparison with Escherichia coli chromosomal DNA, which was taken up very poorly. Three new Campylobacter spp.-to-E. coli shuttle plasmids, which contained additional cloning sites and selectable markers, were constructed from the shuttle vector pILL550A. These plasmid DNAs were taken up by campylobacters much less efficiently than was homologous chromosomal DNA, and transformation into plasmid-free cells was very rare. However, with the use of recipients containing a homologous plasmid, approximately $10^{-4}$ transformants per cell were obtained. The tetM determinant, originally obtained from Streptococcus spp. and not heretofore reported in Campylobacter spp., was isolated from an E. coli plasmid and was introduced, selecting for tetracycline resistance, by natural transformation into C. coli.

Bacteria have evolved different mechanisms for taking up DNA from the environment and incorporating it into their genomes (for reviews, see references 22 and 24). Such natural transformation may provide a potential advantage for the bacterium, because it enables an individual cell to accumulate advantageous mutations originating from separate individuals or even to acquire genetic material from other species. It has been successfully used in genetic studies and molecular cloning in bacteria, such as in Bacillus (12) and Streptococcus (16) spp.

Natural transformation systems appear to fall into two groups, one typical of gram-positive organisms and the other found in some gram-negative bacteria (22). There is a marked difference in the process of binding and transport of DNA through the envelope of the recipient cell. In Bacillus subtilis, Streptococcus pneumoniae, and S. sanguis, a single competent cell can bind and take up a large number of DNA molecules regardless of their sources. During the uptake process, one strand of the DNA is degraded while the complementary strand is transported into the cell (22, 24). Of the gram-negative bacteria that possess natural transformation systems, Haemophilus species bind and take up DNA possessing a specific 11-base-pair sequence (7). DNA uptake by Neisseria gonorrhoeae also involves recognition of a specific 10-base-pair sequence (11). In both cases, only a few molecules of homologous DNA can be taken up by a competent cell, and heterologous DNA can be taken up only at much lower frequency (10). Natural transformation of plasmid DNA is normally rare in both gram-positive and gram-negative bacteria, because the duplex DNA is nicked and partially degraded during both binding to and entering into the cell (24). The frequency of plasmid transformation can be increased by using a recipient containing a homologous plasmid. Thus, the incoming plasmid can be rescued by the resident plasmid through homologous recombination (12, 16).

In genetic engineering studies, transformation is often initiated by artificial means, e.g., calcium chloride treatment for Escherichia coli and some other gram-negative bacterial species (6), polyethylene glycol-mediated transformation for protoplasts of gram-positive bacteria (4) or whole cells of gram-negative bacteria (5), or electroporation with high-voltage discharge to introduce DNA into a cell (20). These artificial methods are exploited probably because most bacterial species are not naturally transformable and because plasmid and bacteriophage DNAs are believed to be intact after entering into a cell by these artificial pathways.

Campylobacter coli and C. jejuni are gram-negative microaerophilic bacteria, frequently responsible for gastroenteritis in humans. Shuttle vectors which can transfer from E. coli to C. jejuni were constructed by Labigne-Roussel et al. (14), and electroporation of C. jejuni with plasmid DNA was demonstrated by Miller et al. (20). In a preliminary report, several C. coli strains were found to take up DNA from their environment (A. D. E. Fraser and E. M. Riche, Campylobacter IV, abstr. no. 185, p. 333–334, Goterna, Kungalv, Sweden, 1988). In this study, we demonstrate that most C. coli and some C. jejuni strains are naturally competent during the logarithmic phase of growth and that they show strong selectivity for taking up their own DNA. Plasmid and chromosomal DNA transformation was investigated, several cloning vectors were constructed, and the streptococcal tetM gene was transferred by natural transformation into C. coli, where it was found to express tetracycline resistance.

MATERIALS AND METHODS

Strains and culture conditions. The Campylobacter strains used in this study are listed in Table 1. E. coli IM107 (30) was also used. The plasmids used were pUC13 (30), pUA466 (25), pJ13 (kindly provided by V. Burdett [31]), and the shuttle vector pILL550A (kindly provided by A. Labigne-Roussel), which is a derivative of pILL550 (14) containing additional PstI and Smal sites (W. Yan and Y. Wang, unpublished data). Plasmids pUOA11, pUOA13, pUOA15, and pUOA17 were constructed in this study. C. coli and C. jejuni strains were cultured in Mueller-Hinton (MH) broth or on MH agar at 37°C under 7% CO2, and E. coli strains were grown in LB broth or on LB agar at 37°C. When necessary, the medium was supplemented with ampicillin (100 μg/ml), tetracycline
TABLE 1. Campylobacter strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Chromosomal resistance phenotype*</th>
<th>Plasmid content (size [kb])</th>
<th>Plasmid phenotype*</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. coli</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA417</td>
<td>Nal'</td>
<td>-</td>
<td>-</td>
<td>H. Lior (Canada)</td>
</tr>
<tr>
<td>UA417R</td>
<td>Nal' Str'</td>
<td>-</td>
<td>-</td>
<td>This study</td>
</tr>
<tr>
<td>UA420</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>M. A. Karmali (Canada)</td>
</tr>
<tr>
<td>UA585</td>
<td>Ery'</td>
<td>-</td>
<td>-</td>
<td>C. D. Ribeiro (Wales)</td>
</tr>
<tr>
<td>UA724</td>
<td>Ery' Gm' Km'</td>
<td>pUA724 (ca. 30)</td>
<td>-</td>
<td>R. Gomez-Lus (Spain)</td>
</tr>
<tr>
<td>BM2509</td>
<td>Ery'</td>
<td>pIP1433 (47.2)</td>
<td>Km' Te'</td>
<td>P. Courvalin (France [29])</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pIP1445 (4.57)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C. jejuni</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA676</td>
<td>Nal'</td>
<td>-</td>
<td>-</td>
<td>26</td>
</tr>
<tr>
<td>UA466</td>
<td>-</td>
<td>pUA466 (45)</td>
<td>Te'</td>
<td>25</td>
</tr>
<tr>
<td>UA4668</td>
<td>Nal' Str'</td>
<td>pUA466 (45)</td>
<td>Te'</td>
<td>This study</td>
</tr>
<tr>
<td>UA649</td>
<td>-</td>
<td>pUA649 (40.8)</td>
<td>-</td>
<td>pUA466StrO (25)</td>
</tr>
<tr>
<td>UA650</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Plasmid-cured UA466 (25)</td>
</tr>
<tr>
<td>UA697</td>
<td>Ery'</td>
<td>-</td>
<td>-</td>
<td>I. Phillips (United Kingdom)</td>
</tr>
<tr>
<td>C31</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>J. Miller (United States [20])</td>
</tr>
</tbody>
</table>

* Abbreviations: Ery', erythromycin resistance; Gm', gentamicin resistance; Km', kanamycin resistance; Nal', nalidixic acid resistance; Str', streptomycin resistance; Te', tetracycline resistance.

** - Not detected.

* This strain was formerly called C. jejuni SD2.

(12 μg/ml), kanamycin (16 to 32 μg/ml), nalidixic acid (24 μg/ml), and streptomycin (10 μg/ml).

**Plasmids and chromosomal DNA isolation.** Plasmid DNA extractions were carried out as described by Birnboim and Doly (2), followed by cesium chloride-ethidium bromide density gradient centrifugation when necessary. Chromosomal DNA was prepared by the method of Marmur (18).

**Transformation.** Campylobacter transformation was performed either on an agar surface or in a biphasic system. For transformation on MH agar, fresh recipient cells (24-h growth on MH agar) were spread on MH agar at about 5 × 10^7 cells per plate and incubated for 6 h. Aliquots of DNA (ca. 0.2 μg in 5 μl of MH broth, TE buffer, or ligation buffer [17]) were spotted directly onto the inoculated agar without additional mixing or spreading, and incubation was continued for 5 h. For transformation in a biphasic system, cell suspensions (1 × 10^7 to 5 × 10^7 cells per ml of MH broth) were transferred (0.2 ml per tube) to test tubes (10 by 120 mm) containing 1.5 ml MH agar and incubated for 2 to 6 h. DNA samples were added, and incubation was continued for 3 to 5 h. DNase I and MgCl₂ were added to final concentrations of 25 μg/ml and 5 mM, respectively, at various times if needed. E. coli was transformed by the CaCl₂ procedure (6). Transformants were selected on MH or LB agar containing appropriate antibiotics.

**DNA labeling and uptake experiments.** ³²P-labeled transforming DNA was prepared in vitro by either nick translation, end labeling, or the random primer labeling method. Nick translation was performed by the method of Maniatis et al. (17), except that only 1/50 of the amount ofDNase I was used and DNA was preincubated with DNase I at 15°C for 30 min before DNA polymerase I, [α-³²P]dATP, dCTP, dGTP, and dTTP were added. End-labeled DNA was prepared by filling in with DNA polymerase I (Klenow fragment) in the presence of deoxynucleoside triphosphates. Plasmid DNA was linearized by the restriction enzyme XbaI for end filling. The reaction (typically 0.5 μg of DNA in 20 μl of mixture) was carried out at room temperature for 20 min. Random primer labeling was performed as described previously (9), except that following the labeling, 0.1 mM cold deoxynucleoside triphosphates and 3 U of DNA polymerase I (Klenow fragment) were added and the reaction was continued at room temperature for 30 min. All the labeled DNA samples were collected by ethanol precipitation with 1 μg of tRNA and were suspended in MH broth.

³²P-DNA uptake was performed by using the biphasic system. Typically, the competent cells were incubated with [³²P]DNA (0.1 μg/ml). At various times, DNase I and MgCl₂ were added for 1 min. The cells were centrifuged, washed twice with TE buffer, dissolved in lysing solution (TE, 1% sodium dodecyl sulfate), and then counted in a liquid scintillation counter. Competition for DNA uptake was carried out by using a procedure similar to that described by Scocca et al. (21).

**Detection of DNA hydrolysis.** The production of DNase by campylobacters was detected as described previously (15).

**Chemicals.** Restriction endonucleases and enzymes involved in nucleic acid metabolism were purchased from Boehringer Mannheim Canada Ltd., Dorval, Que., Canada. [α-³²P]dATP was purchased from Du Pont, NEN Research Products, Boston, Mass.; MH medium was purchased from Oxoid Ltd., Basingstoke, England; and antibiotics and chemicals were purchased from Sigma Chemical Co., St. Louis, Mo.

**RESULTS**

**Isolation from mutants.** A spontaneous Str' mutant of the Nal' C. coli UA417 (designated UA417R) was isolated by plating fresh UA417 cells onto MH agar containing streptomycin (10 μg/ml). The mutation frequency for Str' in this strain was less than 10⁻⁹. We were unable to isolate a spontaneous Str' mutant from C. jejuni UA466. Therefore, some UA466 Str' mutants were first isolated by transformation with UA417R DNA as the donor. TheUA466R (Nal' Str') strain was then isolated from one of the Nal' Str' transformants by spontaneous mutation. Mutation frequencies for Nal' in most C. coli and C. jejuni strains were about 5 × 10⁻⁹ (28).

**Natural competence of C. coli and C. jejuni and transformation of chromosomal DNA.** All five C. coli strains and three of six C. jejuni strains tested were naturally competent
for DNA uptake (Table 2). Transformation frequencies of two chromosomal markers (Nal' and Str') were about 10^-3 per cell in C. coli and 10^-4 per cell in C. jejuni with saturating DNA. C. coli UA420 and BM2509 could not be transformed to Nal' by UA417R DNA. C. coli UA585 was highly competent in this preliminary study and was chosen for most of the transformation experiments. C. coli UA585 cells could be transformed to Nal' by C. jejuni UA466R DNA at about 20% efficiency compared with homologous DNA transformation, but these interspecies Nal' transformants grew more slowly than either parent. C. jejuni UA466 could be transformed to Str' by C. coli UA417R DNA at 1% efficiency; however, these transformants exhibited normal growth rates.

Transformation frequency increased as donor DNA concentration increased, and the saturation level of transforming DNA was about 1 μg/ml when cells were incubated with DNA for 30 min (Fig. 1). The transformation efficiency obtained in the sample at a concentration of 0.01 μg of DNA per ml (4 × 10^6 transforms per μg of DNA) was greater than that obtained at a concentration of 1 μg of DNA per ml (8 × 10^4 transforms per μg of DNA). The transformation frequency also increased as the incubation time with DNA increased, and no transformants were obtained when DNase I and MgCl₂ were added to 0 min (Fig. 2).

Transformation of both Nal' and Str' markers was also performed. The transformation frequencies of UA585 were approximately 1.2 × 10^-3 for the Nal' marker, 4 × 10^-4 for the Str' marker, and about 2 × 10^-7 for the Nal' Str' cotransformants. The results indicated that these sites were unlinked in this transformation system.

DNA hydrolysis in these Campylobacter strains was examined. There was no clear correlation between their own DNase activity and competence, and production of a small amount of extracellular DNase activity did not appear to interfere with chromosomal DNA transformation when the donor DNA was suspended in TE buffer (Table 2). Addition of 10 mM MgCl₂ to donor DNA did not affect the chromosomal transformation frequency in UA585, which had little DNase activity, but reduced the transformation frequencies in UA417 and UA466 to about 40% (data not shown), both of which showed some DNase activity.

Influence of growth phase on competence. To study the development of competence, we performed the biphasic transformation procedure with different growth phases of C. coli UA585 cultures as recipients and UA417R DNA as the donor. UA585 cells were competent constitutively throughout their growth cycle (Fig. 3). A maximum number of transformants was obtained after 6 h of incubation, but the transformation frequency (2 × 10^-4 per cell) was lower than that of 2-h samples (5 × 10^-4 per cell). This indicated that

![Graph](http://jb.asm.org/Downloaded from http://jb.asm.org/)

**FIG. 1.** Dependence of transformation frequency on the concentration of donor DNA. The recipient was C. coli UA585, and the donor was C. coli UA417 DNA. Transformation was performed in a biphasic culture system. DNA was added at the indicated concentrations for 30 min before DNase I and MgCl₂ were added. Nal' transformants were selected after 3 h of incubation.

![Graph](http://jb.asm.org/Downloaded from http://jb.asm.org/)

**FIG. 2.** Dependence of transformation frequency on the incubation time with donor DNA. The recipient was UA585 (5 × 10^8/ml), and the donor was UA417 DNA (1 μg/ml). DNase I and MgCl₂ were added at the indicated times after DNA was added. Nal' transformants were selected after 3 h of incubation.

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**TABLE 2. Transformation of different strains of C. coli and C. jejuni with homologous chromosomal DNA**

<table>
<thead>
<tr>
<th>Recipient</th>
<th>No. of transformants/spot (ca. 3 × 10^10 cells)</th>
<th>DNA hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nal'</td>
<td>Str'</td>
</tr>
<tr>
<td>C. coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA417</td>
<td>NA</td>
<td>3,500</td>
</tr>
<tr>
<td>UA420</td>
<td>0</td>
<td>3,000</td>
</tr>
<tr>
<td>UA585</td>
<td>6,000</td>
<td>4,000</td>
</tr>
<tr>
<td>UA724</td>
<td>1,000</td>
<td>1,000</td>
</tr>
<tr>
<td>BM2509</td>
<td>0</td>
<td>4,000</td>
</tr>
<tr>
<td>C. jejuni</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA67</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>UA466</td>
<td>300</td>
<td>1,000</td>
</tr>
<tr>
<td>UA649</td>
<td>200</td>
<td>800</td>
</tr>
<tr>
<td>UA580</td>
<td>150</td>
<td>600</td>
</tr>
<tr>
<td>UA697</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C31</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Transformation was performed on MH agar (see Materials and Methods). The donor DNA (0.2 μg in 5 μl of TE buffer) was from C. coli UA417R for transformation of C. coli strains or from C. jejuni UA466R for transformation of C. jejuni strains. The cells within the DNA spot were scraped up and spread on selective media with or without dilution. All experiments were repeated at least twice.

* Symbols: ++, strong positive; +, weak positive; ±, very weak reaction after 2 to 3 days of incubation; −, negative.

* NA, Not applicable.

The results indicate that transformation frequencies of two chromosomal markers (Nal' and Str') were about 10^-3 per cell in C. coli and 10^-4 per cell in C. jejuni with saturating DNA. C. coli UA420 and BM2509 could not be transformed to Nal' by UA417R DNA. C. coli UA585 was highly competent in this preliminary study and was chosen for most of the transformation experiments. C. coli UA585 cells could be transformed to Nal' by C. jejuni UA466R DNA at about 20% efficiency compared with homologous DNA transformation, but these interspecies Nal' transformants grew more slowly than either parent. C. jejuni UA466 could be transformed to Str' by C. coli UA417R DNA at 1% efficiency; however, these transformants exhibited normal growth rates.

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the early-log-phase bacteria were slightly more competent than the late-log-phase cells.

Construction of shuttle vectors. To study plasmid transformation and to improve techniques for molecular cloning in Campylobacter spp., we constructed three shuttle vectors by using pILL550A as the parent (Fig. 4). Plasmid pUOA13 consisted of the entire pUC13 plasmid (cut at the unique AatII site) and an EcoRI-SalI fragment of pILL550A which contains a replication origin of C. coli plasmid pIP1445, a Km gene, and an origin of transfer (oriT) of the RK2 plasmid (14). This plasmid encodes Ap' and Km' and the ability to complement a defective ß-galactosidase in E. coli, whereas only Km' is expressed in Campylobacter spp. Plasmid pUOA15 was derived from pUOA13 by replacing the Km' gene with the tetO gene from pUOA3 (25). The tetO determinant is expressed in both E. coli and Campylobacter spp. Plasmid pUOA17 was obtained by deleting the ClaI-ScaI fragment from pUOA13, thus removing part of the E. coli Ap' gene.

[^32]P]DNA uptake and competition studies. Figure 5 shows the kinetics of irreversible uptake of homologous (C. coli UA417), heterologous (E. coli), or plasmid (pUOA13) DNA by C. coli UA585 cells. Uptake of C. coli chromosomal DNA continued to increase up to 28 min of incubation, whereas E. coli and plasmid pUOA13 DNAs were taken up in barely detectable amounts. Similar results were obtained when DNA labeled by end filling or the random primer labeling method was used as the donor (data now shown).

When unlabeled C. coli, C. jejuni, and E. coli DNAs were used to compete with[^32]P]DNA for uptake, we found that C. coli and C. jejuni DNAs competed for uptake with approximately equal efficiency, whereas E. coli DNA did not interfere with the uptake of C. coli DNA (Fig. 6).

Transformation of plasmid DNA. The frequency of trans-

\[ \text{FIG. 3. Effect of growth phase on the competence of C. coli UA585. Transformation was performed in the biphasic system. UA417R DNA (in TE buffer) was added to 1 \mu g/ml at the indicated time intervals, and the cell number was counted by plating onto MH agar. Str' transformants were selected after 4 h of incubation. Symbols: O, CFU per milliliter; \Delta, number of transformants per milliliter.} \]

\[ \text{FIG. 4. Restriction maps of the shuttle vectors pUOA13, pUOA15, and pUOA17. Symbols: , fragment containing Km gene or tetO gene; ---, DNA sequence from the Campylobacter plasmid pIP1445; ----, oriT DNA; ---, pUC13 DNA. Numbers represent kilobase pairs.} \]
formation of plasmid pUOA17 (8.3 kilobase pairs [kb]) and pILL550A (8.6 kb) into plasmid-free UA585 cells (Table 3) was about 1,000 times lower than that of the chromosomal markers (Table 2), and we were unable to obtain plasmid transformants (shuttle vectors) by using C. coli UA417 or C. jejuni UA649 as the recipient. When strain UA585 containing a homologous plasmid was used as the recipient, transformation frequencies of the shuttle plasmids were increased 100-fold, although they were still 10 times lower than that of chromosomal markers (Table 3). We were unable to transform the 45-kb plasmid pUA466 into plasmid-free C. coli or C. jejuni strains. However, Tc-resistant transformants could be obtained by transformation of pUA466 into C. jejuni UA649 which contains a 4.2-kb deletion of plasmid pUA466 and in which most of the tetO determinant has been deleted (25). In this strain the transformation frequency of Tc was close to that of chromosomal markers. Also, in this natural transformation system the transforming activity of pUOA17 when it was isolated from E. coli was not significantly lower than that obtained when it was isolated from Campylobacter spp. (Table 3).

Cloning and expression of streptococcal tetM gene in C. coli. The tetM gene was originally cloned into the E. coli vector pACYC177 at the HincII site (pJJ3) (3). The gene has never been demonstrated in Campylobacter spp. (23). The 5-kb HincII fragment containing the tetM determinant is very unstable when cloned in a high-copy-number E. coli plasmid such as the pUC series of vectors (13; Y. Wang, and D. E. Taylor, unpublished observations). A stable tetM-pUC13 clone was obtained by cleaving pJJ3 with HincII, partially digesting it with BAL 31, and inserting it into the Smal site of pUC13 (termed pUA11; 6.9 kb). The plasmid pUA11 (0.3 µg) was linearized with HincII and ligated with Smal-cut pILL550A (0.3 µg), and the mixture was used to transform UA585(pUA11) cells on MH agar. Two Tc colonies were obtained from some of the DNA-treated cells. Plasmid DNA was isolated from these two transformants and verified by Clal cutting, which has one site in the tetM gene (19) and another site in the vector pILL550A (Fig. 4). The MICs of tetracycline for both clones were 256 µg/ml.

**DISCUSSION**

In this study we have demonstrated that most C. coli and some C. jejuni strains are naturally competent for DNA uptake. Transformation with both Nal and Str markers indicates that C. coli is able to take up only a very limited number of DNA molecules at any given time. The frequency of Nal' Str' cotransformants (2 × 10⁻¹⁰) is close to the product of the frequencies of Nal' transformants and Str' transformants (1.2 × 10⁻⁹ multiplied by 4 × 10⁻⁴ = 4.8 × 10⁻⁸); therefore, each competent cell probably takes up an average of two DNA molecules.

The results obtained from both [³²P]DNA uptake and competition experiments demonstrate that the DNA uptake system of C. coli is specific for DNA derived from this and closely related species. Such a mechanism is very similar to that seen in Haemophilus (21) and Neisseria (8) species. Although direct evidence for a recognition sequence is still lacking, our results favor the view that a specific recognition sequence is present in both C. coli and C. jejuni. The possibility that DNA recognition involves interaction with
modified residues (21) is unlikely, because the transformation efficiencies of plasmid DNA isolated from E. coli and C. jejuni were similar (Table 3). The possibility that homologous recombination is required during the DNA uptake process (24) also appears unlikely, because C. jejuni cells take up C. jejuni DNA as efficiently as they take up C. coli DNA (data not shown), yet the two DNAs share only a limited amount of homology (approximately 32 to 48% [1]). Furthermore, the frequency of transformation of the shuttle plasmid into C. coli cells containing a homologous plasmid was still much lower than that of homologous chromosomal DNA.

It has been demonstrated that a restriction-modification system exists in C. jejuni C31 (14, 20), which acts on the EcoRI recognition site. DNA isolated from Campylobacter spp. was resistant to EcoRI digestion and became susceptible if the same DNA was transferred and isolated from E. coli. Moreover, similar results were obtained in electroporation experiments in which pILL550 DNA isolated from E. coli transformed C. jejuni poorly (20). In contrast, during natural transformation of Campylobacter spp., the transforming activity of the plasmid DNA isolated from E. coli was about equivalent to that of DNA isolated from Campylobacter spp. This result suggests that the incoming DNA was protected from the action of restriction endonucleases, and it is consistent with the protection of transforming DNA from cellular nucleases observed in other natural transformation systems (22).

The frequencies of plasmid transformation were about 1,000 times lower than those of chromosomal markers with the use of the highly competent UA585 strain and small plasmids (e.g., pUA17 [8.3 kb]). We were unable to transform UA417 or UA466 with plasmid DNA, possibly because these two strains have some extracellular DNase activity (Table 2). Such low transformation frequencies may be due to very inefficient uptake of the shuttle plasmid by Campylobacter spp. (Fig. 5), combined with partial digestion of the donor DNA during uptake, which has been noted in all other well-characterized natural transformation systems (24). The damage to transforming plasmid DNA can be compensated for by the use of recipients containing a homologous plasmid; thus, the incoming plasmid can be rescued by the resident plasmid through homologous recombination (24). Our results showed that transformation frequencies of the shuttle plasmids increased to about 100-fold in this system.

Natural transformation of Campylobacter spp. should be useful in chromosome mapping, as well as in the development of improved methods for gene replacement mutagenesis. Workers in our laboratory are constructing additional vectors as well as developing genetic mapping technique for Campylobacter spp., which could be applied to the study of the biology and pathogenesis of these microorganisms.

ACKNOWLEDGMENTS

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LITERATURE CITED


