Variation of the flagellin gene locus of *Campylobacter jejuni* by recombination and horizontal gene transfer

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The capacity of *Campylobacter jejuni* to generate genetic diversity was determined for its flagellar region. Recombination within a genome, as well as recombination after the uptake of exogenous DNA, could be demonstrated. The subunit of the flagellar filament of *C. jejuni* is encoded by two tandem genes, *flaA* and *flaB*, which are highly similar and therefore subject to recombination. A spontaneous recombination within this locus was demonstrated in a bacterial clone containing an antibiotic-resistance gene inserted in *flaA*. A recombinant was isolated in which the antibiotic-resistance gene had been repositioned into *flaB*, indicating that genetic information can be exchanged between the two flagellin genes of *C. jejuni*. The occurrence of recombinational events after the uptake of exogenous DNA by naturally competent bacteria was demonstrated with two mutants containing different antibiotic-resistance markers in their flagellin genes. Double-resistant transformants were formed when purified chromosomal donor DNA was added to a recipient strain, when the two bacterial cultures were mixed under conditions that induce natural competence, or when the two strains were co-cultured. Both mechanisms of recombination may be used by the pathogenic organism to escape the immunological responses of the host or otherwise adapt to the environment.

Keywords: flagellin, genetic recombination, horizontal gene transfer

INTRODUCTION

The flagella of *Campylobacter jejuni*, a bacterium that causes diarrhoea in humans, are now recognized as a major virulence factor (Newell *et al.*, 1985; Morooka *et al.*, 1985; Black *et al.*, 1988; Aguero-Rosenfeld *et al.*, 1990; Pavlovskis *et al.*, 1991). The flagellar filament consists of flagellin, which is an immuno-dominant protein during infection (Lane *et al.*, 1987; Nachamkin & Yang, 1992). Two genes encoding flagellin, *flaA* and *flaB*, are present in the genome of *C. jejuni* and *Campylobacter coli* (Nuijten *et al.*, 1990; Guerry *et al.*, 1990; Fischer & Nachamkin, 1991). Expression of the two genes is differentially regulated. Wild-type (WT) bacteria of *C. jejuni* strain 81116 express only *flaA*, and are fully motile, while *flaB* is

not expressed. However, bacteria expressing *flaB* have been isolated and these are less motile than WT expressing *flaA* (Wassenaar *et al.*, 1994).

The function of the flagellin B flagella, and of the flagellin gene duplication, is not yet known. It has been suggested that the second flagellin gene may serve as a donor, of which parts would be introduced into *flaA* to compensate for mutations, or to increase the immunogenic repertoire of a given *C. jejuni* strain (Guerry *et al.*, 1992; Tompkins, 1992; Alm *et al.*, 1993), in analogy to the fimbrial gene multiplication of *Neisseria gonorrhoeae* (Meyer, 1987). Two approaches were used to detect such exchange of genetic material between the flagellin genes of *C. jejuni*. First, a mutant of strain 81116 was used, in which a kanamycin-resistance gene (Km^R) had been inserted in *flaA*. This mutant, R1, has a decreased motility compared to WT, and produces truncated flagella consisting of flagellin B (Wassenaar *et al.*, 1991). Invasiveness of *C. jejuni* for tissue culture cells depends on the presence of flagella and motility. This requirement was used to enrich for motility

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Abbreviations: mAb, monoclonal antibody; WT, wild-type.

revertants. A revertant was selected from R1 that had regained full motility, and was characterized for DNA recombination between the flagellin genes.

The second approach was based on the fact that *C. jejuni* is naturally competent to take up extracellular DNA and incorporate this into its genome (Wang & Taylor, 1990; Wassenaar *et al.*, 1993). This horizontal gene transfer may result in the formation of new mosaic flagellin genes. To test this, a second flagellin mutant was constructed, with a tetracycline-resistance gene (Tc^R) inserted elsewhere in the *flaB* gene. The formation of Tc^R Km R transformants was tested by incubating chromosomal DNA isolated from the Km R mutant with the Tc^R mutant as a recipient strain and vice versa. Moreover, the formation of double transformants was determined in mixtures of the single mutants under conditions that favour natural transformation and after co-cultivation of the single mutants on the same plate.

METHODS

Bacterial strains and growth conditions. The *Escherichia coli* strain used for cloning and vector isolation was PC2495 [*thi* *dellac-proAB* *phx* *hds* *supE* *recA56* *F'*(*proAB*⁺ *lacIqZ*⁻ Δ *delM15* *traD36*)] (Phabagen Collection, Department of Molecular Cell Biology, University of Utrecht). The origin of the *C. jejuni* strain 81116 and the flagellin mutants R1 (*flaA*::Km R) and R3 (*flaB*::Km R) of this strain has been described elsewhere (Wassenaar *et al.*, 1991). MP1 (*flaB*::Km R) is a motility phenotype revertant derived from R1 (this study). T1 (*flaB*:: Tc^R) is a mutant of 81116 containing a Tc^R gene inserted in its *flaB* gene and is derived from 81116 by electroporation with pIVB::*flaB*:: Tc^R , a Bluescript vector which contains *flaB* sequences interrupted by a Tc^R gene (see below). All strains were cultured at 42 °C on saponin plates (Nuijten *et al.*, 1989) supplemented with 30 µg kanamycin ml $^{-1}$, or 20 µg tetracycline ml $^{-1}$, when appropriate, under microaerobic conditions. Bacterial motility was confirmed by colony size on thioglycollate plates containing 0.4% agar (Caldwell *et al.*, 1985).

Construction of pIVB::*flaB*:: Tc^R . The Tc^R gene that was inserted into *flaB* was originally derived from a WT *Campylobacter* vector. It was isolated from pUOA15 (Wang & Taylor, 1990) by partial *Sau3A* digestion. A 5 kb fragment conferring Tc^R to *E. coli* was cloned into the *Bam*HI site of pBluescript II KS(+) (Stratagene). This insert was then introduced into the *Nde*I site located in the 3'-end of the *flaB* gene present on pIVB3-300 (Nuijten *et al.*, 1990) by blunt-end ligation after filling in the sticky ends of the restriction fragments with DNA polymerase.

Electroporation of *C. jejuni* 81116 with pIVB::*flaB*:: Tc^R . *C. jejuni* 81116 was cultured on Mueller-Hinton (MH) agar plates and electrocompetence was induced as described by Wassenaar *et al.* (1993). Vector DNA pIVB::*flaB*:: Tc^R was isolated from *E. coli* PC2495 by the alkaline lysis procedure and purified by CsCl density centrifugation (Sambrook *et al.*, 1989). A sample of 5 µg DNA was transformed into 10 10 electrocompetent bacteria according to Wassenaar *et al.* (1991) with one modification to increase the yield of transformants: after the pulse the bacteria were left to recover on MH agar plates for 24 h in a microaerobic atmosphere before they were harvested and plated out on Tc selective saponin plates. This resulted in seven Tc^R transformants. Chromosomal DNA was isolated from five transformants, digested with *Eco*RV and hybridized with the 5 kb Tc

specific fragment of pUOA15 and with the flagellin specific insert of pIVB3-300. A mutant, T1, was selected which contains the Tc insert in its *flaB* gene, as was concluded from the hybridization (results not shown).

Invasion of R1 into tissue cultured cells. Bacterial invasion into INT-407 cells was performed as described elsewhere (Wassenaar *et al.*, 1991). Briefly, intestinal cells 407 were cultured to a confluent monolayer ($\sim 10^6$ cells per well) and loaded with a bacterial suspension ($\sim 10^9$ bacteria) of R1. Bacteria were allowed to invade the cells for 30 min at 37 °C in 5% CO $_2$:95% air. After the monolayer was washed five times, it was incubated with medium containing 250 µg gentamycin ml $^{-1}$. This treatment protects internalized bacteria only, as the antibiotic does not enter the cells. After washing, the cells but not the bacteria were lysed with 0.5% Triton X-100. Internalized bacteria were recovered by culturing on saponin plates. The motility revertant MP1 of R1 was selected from recovered bacteria by the swarming appearance of the colony on saponin plates. Motility was confirmed on thioglycollate agar plates.

Western blot analysis. Western blot analysis of whole bacterial protein extracts was performed as described by Wassenaar *et al.* (1991). Monoclonal antibodies (mAbs) CF1 and CF17, which were raised against flagellin of *C. jejuni* 81116 (Newell, 1986), were used in dilutions of 1:500.

PCR analysis. PCR reactions were performed on single *C. jejuni* colonies. A toothpick stab of cells was suspended in 100 µl 10 mM Tris/HCl, 1 mM EDTA, pH 8.0, and 5 µl was used in a 100 µl reaction mix for amplification. The following primers were used: primer A1 (5'-CATATGAAAGCATCAAAA-3') is located upstream of *flaA* at position -317 to -300 (the initiating ATG-codon starts at +1); primer B1 (5'-TTAATGTTAAAGCAGCAG-3') is located within the coding region of *flaA* at nucleotide position +1576 to +1593 (corresponding with position -329 to -312 of *flaB*); and primer C (5'-ACCAACACCTATATCTCC-3') is complementary to both flagellin genes, at position +931 to +948. PCR reactions were performed using *Taq* DNA polymerase (Promega) with 35 cycles of 1 min at 90 °C, 2 min at 45 °C and 3 min at 72 °C.

Sequence analysis. The partial nucleotide sequence of the PCR products was determined by dideoxy sequencing using T7 DNA polymerase (Pharmacia sequencing kit) and 5 µCi [32 P]dATP (185 kBq; 3000 Ci mmol $^{-1}$). Prior to sequencing the PCR products were excised from agarose gel and purified with Gene-Clean (Bio-101). Purified PCR product (1 µg) was denatured by incubation with NaOH and precipitated with ethanol. For the sequence reaction the PCR primer C and two nested primers were used: primer A2 (5'-CGTATTAACACA-AATGTTACAGCCATTG-3') is identical to position +10 to +36 of *flaA* and nested primer B2 (5'-AGGATAAACACCA-ACATCGGTGCATTA-3') is identical to nucleotides +10 to +36 of *flaB*.

Natural transformation. For natural transformation of *C. jejuni* 81116, mutant R1 and mutant T1 with *Campylobacter* chromosomal DNA, the acceptor strain was cultured overnight on saponin plates, harvested in MH broth and diluted in MH broth to a suspension of 10 8 c.f.u. ml $^{-1}$. This suspension was incubated at 37 °C for 3 h under microaerophilic conditions to induce competence. Transformation was carried out in biphasic medium in a capped and pierced microcentrifuge tube containing 1 ml MH agar which was overlaid with 200 µl competent cells. Chromosomal DNA (10 µg dissolved in water) was added and incubated for 2 h at 37 °C under microaerophilic conditions. Transformation was terminated by the addition of 50 µg DNase I

(Bovine Pancreas DNase I, grade II; Boehringer). In the controls 50 µg DNase was added prior to the DNA. Cells were harvested and plated out on saponin plates supplemented with the selective antibiotic to determine the number of transformants.

For transformation in mixed cultures, bacteria of each strain were cultured, harvested and diluted in MH broth as described above. Equal amounts (200 µl each) of each strain were mixed and overlaid on 1 ml MH agar in a microcentrifuge tube as above. In the controls, cells of each strain were diluted in PBS (0.145 M NaCl; 0.15 M sodium phosphate) and mixed together in equal amounts. When indicated, 50 µg DNase I was added to the 400 µl cell suspension. The mixture was left for 5 h at 37 °C under microaerobic conditions, and dilutions were plated on selective saponin plates to determine the number of transformants.

To monitor natural transformation during co-cultivation, T1 and R1 were grown on saponin plates separately and equal amounts of bacterial suspensions (about 10^8 c.f.u.) were mixed and cultured on saponin plates for 48 h. After this incubation, the bacterial lawn was harvested in 1 ml PBS and dilutions were again plated out. The number of T1 and R1 was determined on plates containing Tc and Km, respectively, and the number of double-resistant transformants on plates containing Tc and Km.

RESULTS

Intra-genomic recombinations between *flaA* and *flaB*

Motile bacteria containing an active *flaA* gene are more invasive for tissue culture cells than non-motile bacteria. This property was used in an invasion assay to select fully motile variants originating from the non-motile mutant R1, which contains a Km^R gene inserted into its *flaA* gene. Of the 3×10^2 c.f.u. recovered from the cells after invasion, one colony had the swarming appearance on saponin plates typical for WT. The colony shape of this clone was also similar to WT in thioglycollate plates (results not shown). However, these motile bacteria, designated MP1 for motility phenotype 1, were still kanamycin resistant.

The flagellin content of MP1 was analysed on Western blots, using mAbs CF1 and CF17. mAb CF17 binds to a conserved epitope present on flagellins A and B, whereas mAb CF1 recognizes an epitope which is present on flagellin A only, situated between amino acids 259 and 275 (nt 775–825) (Nuijten *et al.*, 1991). The parental strain R1 and the derived MP1 mutant produce flagellin that is recognized by monoclonal CF17 but not by CF1 (Fig. 1). WT and mutant R3, which were included as controls, produce flagellin that can be detected with both CF17 and CF1. Mutant R3 contains a Km^R insertion in *flaB* and is fully motile by means of flagellin A flagella (Wassenaar *et al.*, 1991). These results show that the flagellin protein of MP1 lacks the flagellin-A-specific epitope recognized by mAb CF1.

To obtain information on the organization of the flagellin genes of MP1, PCR analysis was performed on the genomic DNA, with *flaA*- and *flaB*-specific oligonucleotides as primers. As expected, the *flaA*-specific primer set A1 + C resulted in an extended PCR product III with R1 compared to fragment I of WT, due to the

Km^R insertion. In contrast, these primers resulted in a PCR product I of MP1 that was identical in length to that of WT (Fig. 2). Conversely, the PCR product IV of MP1 obtained with the *flaB*-specific primer set B1 + C was identical in length to the Km^R-containing *flaA* product III of R1. These results indicate that the Km^R gene of MP1 is inserted in *flaB*. The lack of binding of mAb CF1 to flagellin of MP1 suggests that during a recombinational event in R1, which led to MP1, *flaA* sequences had been removed from their original context.

The PCR fragments obtained from MP1 (Fig. 2) were used to determine the nucleotide sequences of the 5'-end of the flagellin genes where *flaA* and *flaB* are different and can be distinguished (Nuijten *et al.*, 1990; GenBank/EMBL Data Bank accession number J05635) (Fig. 3). The results show that the recombination event upstream of the Km^R gene had occurred between nt 90 and nt 156, since in this region the DNA sequence of MP1 changes from *flaA* to *flaB* as compared to WT. The recombination event downstream of the Km^R gene should have occurred downstream of nt 825, positioning the CF1-binding epitope in the second, interrupted, gene of MP1. This epitope is not expressed in MP1 (Fig. 2). The site of recombination downstream of the Km^R gene could further be deduced from the size of the PCR product obtained with primers B1 and C on MP1. Primer B1 is situated within the coding region of the WT *flaA* at nt 1576. This part of *flaA* seems to be conserved in MP1. Thus, the recombination site has occurred between nt 825 (after the CF1 epitope) and 1576. However, the exact position of this cross-over event could not be determined, since the flagellin genes of *C. jejuni* 81116 are identical between nt 825 and 1527.

Two different recombinational events could theoretically result in the formation of MP1 (Fig. 3): two double cross-over recombinations within the genome of R1 (intra-genomic recombination) or four independent single cross-overs between two genomes (inter-genomic recombinations). In our view MP1 originated from an intra-genomic recombination. The second model allows for duplications of *flaA*-specific sequences that would replace *flaB*-sequences without being lost in their original position, or vice versa. Such duplications are not present within the distinguishable 5' part of the *fla* genes. It is very unlikely that the four independent single cross-overs of the second model have occurred at homologous positions. A more likely origin of MP1 is an intra-genomic recombination with two double cross-overs in the R1 genome. However, as shown below, inter-genomic recombinations do occur in the flagellin locus of *C. jejuni*.

Horizontal gene transfer

To determine whether genetic exchange between flagellin sequences does occur between members of a bacterial population, a second antibiotic-resistance gene was introduced into the flagellin region of *C. jejuni* 81116. This Tc^R gene, isolated from a natural *Campylobacter* plasmid, was cloned into the 3'-region of *flaB*, whereas the Km^R gene in R1 had been inserted in the 5'-region of *flaA*. The two

Fig. 1. Western blots of total bacterial protein extracts of WT, R1, MP1 and R3 reacted to mAb CF1, specific for flagellin A (a), and mAb CF17, specific for flagellins A and B (b).

Fig. 2. (a) Schematic representation of the flagellin genes of WT, R1 and MP1. The *flxA* gene of R1 was interrupted by a Km^R gene. In MP1 the Km^R gene appeared to be inserted in the downstream flagellin gene. The position of the PCR products I, II, III and IV, and the position of the CF1 epitope are indicated. (b) PCR fragments of WT, R1, and MP1 obtained with primer sets A1 + C and B1 + C. The lengths of the bands I to IV are given in kb.

mutants T1 and R1 were used to monitor the formation of double-resistant mutants of *C. jejuni*.

In the first set of experiments, 10 µg chromosomal DNA of T1 was mixed with competent R1 bacteria under conditions that allow transformation to occur in WT (Wassenaar *et al.*, 1993). Transformants were selected on plates containing Km and Tc. By analogy, R1-derived chromosomal DNA was mixed with competent T1 cells. As a control, competent WT cells were transformed with DNA of T1 or R1, and in this case transformants were selected on Tc and Km plates, respectively. The results show that T1 DNA can indeed transform R1 and vice versa, with a frequency slightly below that of WT (Table 1). The addition of DNase together with DNA largely prevented the formation of double-resistant transformants; the omission of donor DNA totally prevented

their formation, indicating that they arise from the transfer of DNA. Southern blots of genomic DNA of the double-resistant transformants hybridized with probes specific for the Km or Tc cassette confirmed the presence of both markers in the flagellin locus of these transformants (results not shown).

Double-resistant mutants were also formed when cultures of T1 and R1 were mixed in a biphasic medium, which permits natural transformation (Wang & Taylor, 1990). In contrast, mixed cultures suspended in PBS, which does not allow natural transformation, did not result in any detectable double-resistant mutants. Natural transformation in biphasic MH medium was determined with 8×10^7 c.f.u. R1 mixed with 8×10^7 c.f.u. T1, which resulted after 5 h in 1.2×10^{-5} $Tc^R Km^R$ mutants per total c.f.u. The formation of $Tc^R Km^R$ mutants is 1000-fold

Fig. 3. (a) Part of the nucleotide sequence of the flagellin genes of WT (GenBank/EMBL Data bank accession number J05635), R1 and MP1 from nt 1 to nt 90 (amino acids 1–30) and nt 157–nt 180 (amino acids 52–59). The nucleotide sequences of *flaA* and *flaB* are identical between nt 91 and 156 and are not shown. *flaB* sequences are given in italics. The Km^R insert (lower case) of R1 was inserted in the *Bg*/II site (underlined in WT). The obtained sequence of MP1 suggests that the cross-over event upstream of the Km^R gene had taken place between nt 90 and 156. (b) Two possible recombinational events can result in the formation of MP1: an intra-genomic recombination, requiring two double cross-overs within the genome of R1 (top); or inter-genomic recombinations, requiring four single cross-overs between two genomic molecules (bottom). For clarity, *flaA*, *flaB* and the intergenic region are not drawn to scale. Note that the donor DNA of R1 in the lower part of the figure is drawn from right to left. The direction of transcription is indicated by arrows.

Table 1. Transformation of naturally competent cells with isolated DNA

Natural transformation was carried out in biphasic MH medium as described in Methods. The number of transformants was determined as c.f.u. on saponin plates supplemented with Km (WT transformed with R1 DNA), Tc (WT transformed with T1 DNA) and Km and Tc (R1 transformed with T1 DNA and vice versa). The results of one single experiment are shown. Variation between two independent experiments was less than a factor 10 but the differences did not allow mean values to be calculated.

Acceptor strain	Donor DNA	No. of transformants per no. of acceptor cells
R1	T1	7.3×10^{-5}
WT	T1	2.0×10^{-4}
T1	R1	6.0×10^{-5}
WT	R1	4.3×10^{-4}
T1*	R1	1.4×10^{-7}

*DNase I was added prior to the addition of DNA.

lowered by the addition of 50 μ g DNase I, which resulted in 2.5×10^{-8} transformants per total c.f.u. This suggests that the mutants were the result of transformation, rather than conjugation. The number of transformants obtained in mixed cultures is a factor of 5–6 lower than obtained after transformation with 10 μ g DNA (Table 1), which may be a reflection of the amount of free DNA present in the mixed cultures.

Finally, it was tested whether transformation also occurs when Tc^R and Km^R mutants are cultivated together. The two mutants were mixed together in equal quantities (6.3×10^5 c.f.u. T1 and 2.5×10^5 c.f.u. R1) and co-cultivated on saponin plates for 48 h. The bacteria were then harvested, diluted and replated on three types of saponin plates: plates containing Tc resulted in 4.0×10^9 c.f.u. T1; plates containing Km resulted in 4.6×10^9 c.f.u. R1; and plates containing Tc and Km resulted in 1.6×10^3 c.f.u. newly formed double-resistant mutants. The addition of 50 μ g DNase to the cell suspension prior to plating and cultivation decreased the number of formed $Tc^R Km^R$ mutants to 3 c.f.u. These results show that during conditions of growth, the frequency of genetic transfer between mutants derived from the same strain is about 1.8×10^{-7} mutants per total c.f.u.

DISCUSSION

A motility revertant of flagellin mutant R1, MP1, was selected after invasion of bacteria into tissue cultured cells. MP1 originated from R1 by a spontaneous recombination of the flagellin genes, which had repositioned the Km^R gene insert into the downstream flagellin gene. Recently, spontaneous recombinations occurring in the flagellin region were also described for *C. coli* (Alm *et al.*, 1993), which has a flagellin gene organization similar to *C. jejuni* 81116. In this case, a single intra-genomic cross-over resulted in a deletion mutant expressing a chimerical, functional flagellin protein, composed of flagellin A and B parts. Moreover, from an R1-like *flaA flB*⁺ *C. coli* mutant motile Km^R transformants were isolated that were

ascribed to inter-genomic recombinations after natural transformation, removing the Km^R gene from *flaA* and inserting a new Km^R gene in *flaB* (Alm *et al.*, 1993), as depicted in the lower half of Fig. 3(b). Although such an event could also explain the formation of MP1, we favour the simpler explanation of two double cross-over recombinations within one genome (top half of Fig. 3b), for the following reason: no duplications of specific *flaA* or *flaB* sequences were found in the flagellin locus of MP1. This indicates that the two cross-over events took place in the same position in the two genes (the exact positions could not be determined due to the strong homology of the genes, see Fig. 2). It is unlikely that this occurs in four independent single cross-overs between two genomes; two double cross-over recombinations within one genome does not allow sequence duplications.

The occurrence of recombinations between the duplicated flagellin genes of *C. jejuni* can be of importance for its virulence: variation in the immunogenic properties of flagella may be an advantage for the bacteria to evade the host's immune response, as is shown for other organisms (recently reviewed by Brunham *et al.*, 1993). The best studied example is the antigenic variation of pili (fimbriae) of *N. gonorrhoeae*. Pilin variation involves the rearrangement of a partial *pil* gene from one of the many silent loci into an expression locus (Gibbs *et al.*, 1989; Meyer *et al.*, 1990). In comparison, the flagellin variation of *C. jejuni* is limited as there are only two flagellin genes present. Another difference is that the silent *pil* genes of *N. gonorrhoeae* do not contain promoter sequences and are incomplete. In contrast, the complete *flaB* gene of *C. jejuni* is preceded by promoter sequences and can be expressed under certain circumstances (Wassenaar *et al.*, 1994).

Bacteria expressing flagellin B flagella are less motile, and are less able to invade tissue culture cells *in vitro* (Wassenaar *et al.*, 1994). Therefore, the two flagellin genes present in *C. jejuni* are probably not alternately expressed during infection, when motility is important. However, as is shown in this study, the incorporation of epitopes of flagellin B into flagellin A can result in fully motile flagella with altered antigenic properties. If such epitopes are surface exposed along the flagellar filament, as was suggested by Nuijten *et al.* (1990), and if the immune response reacts selectively to flagella, the formation of such recombinants *in vivo* would be advantageous for the bacteria.

A second mechanism to produce antigenic variation in bacteria is the uptake of DNA by natural transformation followed by recombination. Natural transformation is also described for *N. gonorrhoeae*, and causes fimbrial variation at higher frequencies than intra-genomic recombinations (Seifert *et al.*, 1988; Meyer *et al.*, 1990). In this case, the uptake of DNA is dependent on specific sequences (Goodman & Scocca, 1988). This paper describes the occurrence of recombinations between the DNA of two *C. jejuni* mutants after natural transformation. Two antibiotic-resistance markers were introduced into the flagellin locus to monitor such recombinations. The results indicate that transfer of DNA is very efficient

between mutants derived from the same *C. jejuni* strain. It has been suggested that specific sequences are required for DNA uptake during experimentally induced natural transformation (Taylor, 1992). Although transfer of DNA between different strains or species is less efficient, it does occur (Wassenaar *et al.*, 1993). The presence of purified DNA is not required for transformation, as a high number of transformants were obtained from two mutants during co-culturing under laboratory conditions. The frequency of genetic transfer under environmental conditions needs to be determined, but, in analysing *Campylobacter* strains arising from multiple strain infections, the possibility of genetic transfer must be taken into account.

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