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Correlation between phenotypes of restriction / modification systems and efficiency of transformation in *Campylobacter jejuni*

Masaaki Minami [1, 2, *], Takafumi Ando [2], Michio Ohta [3] and Hidemi Goto [2]

[1] *Department of Bacteriology, Nagoya City University, Graduate School of Medical sciences, Nagoya, Japan.*
[2] *Department of Gastroenterology, Nagoya University, Graduate School of Medicine, Nagoya, Japan.*
[3] *Department of Molecular Bacteriology, Nagoya University, Graduate School of Medicine, Nagoya, Japan.*

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Abstract

We evaluated the effect of restriction and modification genes expressed in *Campylobacter jejuni*, a naturally competent organism, on transformation efficiency. Fourteen strains of *C. jejuni* were tested for sensitivity to 29 restriction enzymes. Strains that showed the same susceptibility pattern by either agarose gel electrophoresis or PFGE were grouped together. Natural transformation between strains within and between groups was assessed using two chromosomal markers, one an artificially introduced gene and the other a selected point mutation. However, no correlation was found between transformation efficiency and sensitivity to the restriction enzymes tested. The activity of the restriction and modification system alone could not explain the failure of transformation to occur or the generation of stable clones in a population of *C. jejuni* with other abilities. It is likely that other factors influence horizontal gene flow in this organism. We suggested that the rule that the restriction and modification system affect horizontal gene flow, based on studies of transfection with *E. coli*, may not apply to the exchange of chromosomal DNA in *C. jejuni*.

Keywords: *Campylobacter jejuni*; Transformation efficacy; Restriction modification; Horizontal gene transfer; Natural competent

1. Introduction

Campylobacter jejuni is a major cause of acute bacterial enteritis in humans worldwide [1]. Epidemiological studies have shown a high degree of phenotypic and genotypic diversity among isolates of *C. jejuni* [2]. The population structure of *C. jejuni* is considered to be weakly clonal, indicating that DNA exchange between strains is frequent but limited [3, 4].

Natural transformation is a potential mechanism of horizontal gene transfer leading to genetic diversity within a population. Natural competence is a physiological state that allows for the uptake of macromolecular DNA from the environment [5]. It is widely present in bacterial species [6]. Competent bacteria can bind DNA and transport it to the cytoplasm, where it can rejoin chromosomes or, in the case of plasmid DNA, replicate freely.

C. jejuni is capable of natural transformation, with a transformation frequency of about 10^{-8} using chromosomal DNA as the DNA source [7]. Natural transformation may contribute to the genetic diversity found among different strains of *C. jejuni*, and the mechanism of transformation has been a subject of study [8].

To prevent contamination of their own DNA, bacteria have evolved defence mechanisms such as restriction enzymes that recognize and cut foreign DNA and methylases that distinguish between their own DNA and foreign DNA. Variations

* Corresponding author: Masaaki Minami
Department of Bacteriology, Nagoya City University, Graduate School of Medical sciences, Nagoya, Japan.

in the presence of restriction modification (R/M) genes or fragments thereof in *C. jejuni* have been previously reported based on Southern blot hybridization [9].

We assessed the expression of such an R/M system by examining the sensitivity of *C. jejuni* chromosomal DNA to several restriction enzymes. Because of the phenotypic diversity observed, we examined the effect on the natural competence of the strain in homologous and heterologous transformations.

2. Material and methods

2.1. Bacterial strains

Fourteen *C. jejuni* strains were used: *C. jejuni* NCTC strains 11168, 81116, and 81176, all of which were obtained from human cases of Campylobacteriosis and are routinely used in laboratory investigations. The other isolates were randomly selected from a collection of *C. jejuni* strains kept in a veterinary drug laboratory. Strain 82107, 99189, 99322, and 99388 were obtained from the feces of diarrhea patients, and 94194, 99346, and 99385 were obtained from the tailpipes of broilers from unrelated poultry flocks. Strain 94194, 99346, and 99385 were collected from the tailpipe of broilers, and strain 99312 was collected from the feces of cattle [9]. Strain 78115 was isolated from a duck, strain 8241 from a cat, and strain 8254 is a human-derived strain from the CDC collection.

2.2. Media and growth conditions

C. jejuni strains were grown on Brucella broth (BB) (Becton, Dickinson and company, USA) agar plates and selective antibiotics (25 μg/ml kanamycin (Km) and 8 μg/ml clarithromycin (CAM)) under microaerophilic conditions at 37°C for 48 hours.

E. coli was maintained in the atmosphere at 37°C using Luria-Bertani (LB) agar medium or LB broth. If necessary, 50 μg/ml ampicillin or 50 μg/ml Km was added to the medium.

2.3. *C. jejuni* chromosomal and plasmid DNA preparation

Chromosomal DNA of *C. jejuni* was extracted using the Wizard Genomic DNA purification kit (Promega, WI, USA) according to the manufacturer's instructions. Chromosomal DNA was adjusted to a standard concentration of 1 μg/ml (Nano drop 3.0; Laboratory & Medical supplies, Tokyo). Plasmid DNA of *C. jejuni* was extracted using the QIAGEN plasmid purification mini kit (QIAGEN, VA, USA) according to the manufacturer's instructions. The plasmid DNA extracted with this kit was embedded in agarose and analyzed by PFGE.

2.4. Restriction endonuclease digestion of *C. jejuni* chromosomal DNA

Each strain was evaluated for resistance of chromosomal DNA to restriction by the following enzymes: ApaI, ApoI, BamHI, BfaI, BglII, DraI, EcoRI, EcoRV, HindIII, HpyCH4III, HpyCH4IV, HpyCH4V, Hpy188I, KpnI, DraI, EcoRI, EcoRV, HindIII, HpyCH4III, HpyCH4IV, HpyCH4V, Hpy188I, KpnI, MboI, MfeI, MseI, NheI, NlaIII, PstI, PvuI, RsaI, Sau3AI, SmaI, SpeI, SspI Tsp509I, XbaI (all from New England Biolabs (MA, USA)). For each reaction, 1 μg of DNA was incubated with 5 U of restriction enzyme in the appropriate buffer for 16 hours at optimal temperature. After incubation, electrophoresis was performed on a 1% agarose gel and the digestion patterns were compared. After electrophoresis, the gels were stained with ethidium bromide and photographed. All enzymes that apparently did not digest the DNA were repeated by pulsed-field gel electrophoresis (PFGE) analysis, which confirmed the low frequency of digestion according to published protocols [10]. For the isolation of DNA, *C. jejuni* isolates were grown on BB agar medium at 37°C for 3 days in a microaerobic environment. The bacteria were harvested and resuspended in 900 μl of cold saline and treated with 100 μl of formaldehyde for 1 hour on ice to inactivate the endogenous nuclease. The bacterial suspension was then washed three times with 1 ml of cold saline and resuspended in 1 ml of saline. The optical density of the bacterial suspension was then adjusted to 1.9 at 405 nm, and the bacteria were embedded in low melting point (LMT) agarose blocks and subsequently placed in a lysis solution containing 0.5 mg/ml proteinase K and 0.5% lauroyl sarkosyl in 0.25 M EDTA (pH 9.0). Slices of the blocks were washed at least three times with phenylmethylsulfonyl fluoride solution (1 mM) for 4 hours, washed with TE buffer, and then digested with 50 U of restriction enzyme in fresh buffer. These were incubated overnight at the appropriate temperature. The genomic DNA of *C. jejuni* was subjected to Contour-clamped homogeneous electric (CHEF) electrophoresis on 1% agarose gels for 24 hours at 14°C and 175-185 V using an instrument sold by LKB Instruments. Inc. equipment (Victiria, Australia) was used. The pulse time was varied from 20 to 45 seconds and fragments of various sizes were examined. The concatamer of bacteriophage λ was used as a size marker. After electrophoresis, the gels were stained with ethidium bromide as before.

2.5. Construction of CAM -resistant *C. jejuni* mutants

We selected a spontaneous CAM-resistant mutant strain of *C. jejuni* by plating a large number (approximately 10^{10}) of bacteria on BB agar medium containing 8 µg/ml CAM. In addition, the colonies were cultured on plates supplemented with CAM to confirm their resistance. As a result, only four strains (81116, 8254, 8214, and 99385) formed resistant mutants.

2.6. Construction of Km-resistant *C. jejuni* mutants

The vector pGEMT (Promega) was used to clone the *flaB* gene of *C. jejuni* strain 81116 to obtain the plasmid pGEMTflaB. The 637 bp fragment of the *flaB* ORF of strain 81116 was replaced by specific primers (sense: 5'-AGCAGCAGATGATGCTTCA -3', positions 120-138 of the *flaB* sequence; antisense: 5'-GTCTTGAGAAGTAGTTCCT -3', positions 756-738 of *flaB*). Cloning into vectors was performed in *E. coli* DH5α.

The 1.27 kb EcoRI-flanked *aphA* gene of pUC4K was cloned into the unique EcoRI site of this insert to obtain pGEMTflaB::Km. The nucleotide sequence of the constructed plasmid was confirmed by direct nucleotide sequence analysis. The plasmid pGEMTflaB::Km isolated from *E. coli* was used as donor DNA to transform *C. jejuni* strains as described previously [11]. Transformants selected on BB agar plates containing 25 µg/ml Km were obtained for strain 81116, 8254, 8214, and 99385.

2.7. Natural transformation study of strain with chromosomal DNA

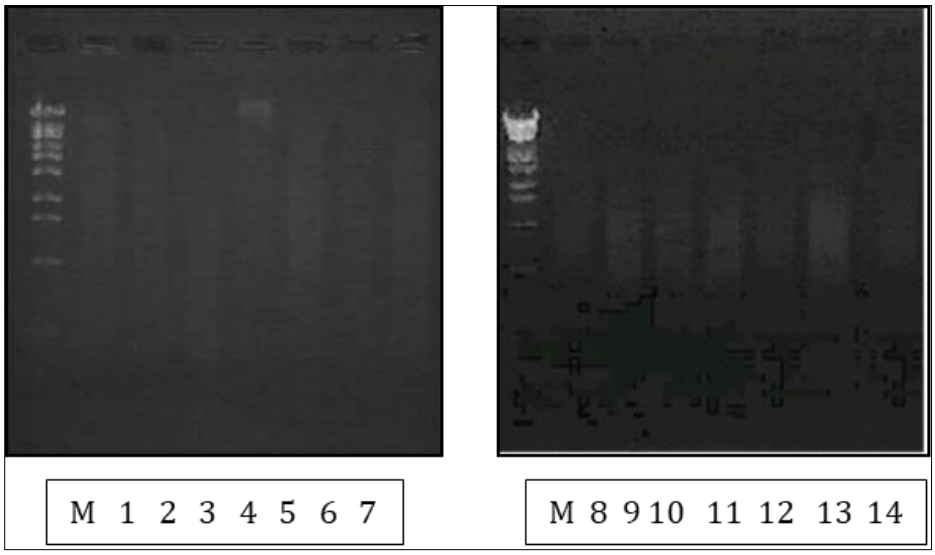
Natural transformation of *C. jejuni* strains using *C. jejuni* chromosomal DNA was performed as follows. Recipient cells were grown overnight at 37°C on plates in a microaerophilic environment. Bacteria were scraped from four plates, resuspended, and diluted to 10^9 CFU/ml. 100 µl of this bacterial suspension was added to a microtube containing 0.5 ml of BB-agar, and the bacteria were left to become competent for 3 hours at 37°C in microaerobic conditions. DNA dissolved in 5µl of water was added and incubation was continued at 37°C for 4 hours. Bacteria were collected and plated on selective BB agar plates. To ensure the absence of naturally occurring resistant mutants, DNA was omitted, and negative controls were included, and viable bacterial counts were measured on non-selective plates. Transformation frequency was determined and calculated as the number of transformants per µg of donor DNA per 10^8 recipient CFUs.

3. Results

3.1. Diversity of restriction enzyme sensitivity

The sensitivity of *C. jejuni* chromosomal DNA from 14 strains to several restriction endonucleases was examined. The results showed that all the strains were sensitive to 12 restriction enzymes: BfaI, DraI, HindIII, HpyCH4V, Hpy188I, HpyCH4III, MseI, NlaIII, RsaI, Sau3AI, SspI, and Tsp509I. When tested on a 1% agarose gel, as in the case of MfeI shown in Figure 1, many strains did not seem to be digested by the particular enzyme. For some enzymes, both apparently resistant and sensitive strains were identified.

A.



B.

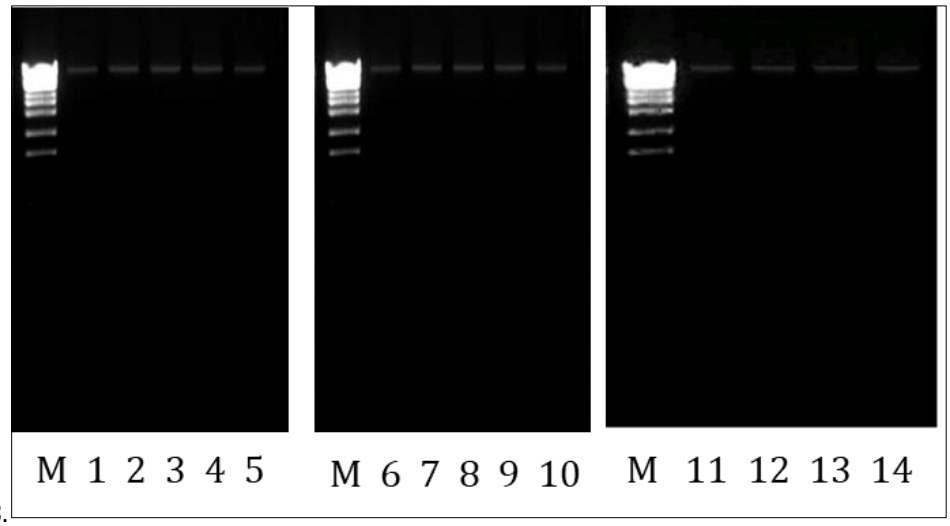


Figure 1 Analysis of digested chromosomal DNA by agarose gel electrophoresis. (A) HiindIII digestion. Digested DNA migrates into the gel as a smear. (B) MfeI digestion. All *C. jejuni* DNA remains in the top of the gel, apparently non-digested. Marker DNA is indicated by M. Numbers indicate strains 78115 (1), 8241 (2), 8254 (3), 82107 (4), 11168 (5), 81116 (6), 81176 (7), 94194 (8), 99189 (9), 99312 (10), 99322 (11), 99346 (12), 99385 (13) and 99388 (14)

As agarose gel electrophoresis is not suitable for detecting large DNA fragments that are not digested frequently, PFGE was then performed to see if the chromosomal DNA was indeed resistant to a particular restriction enzyme. The MfeI chromosomal digests that appeared to be uncleaved in the 1% agarose gel were actually digested when analyzed by PFGE (Fig. 2). Similarly, all strains were digested by ApaI,

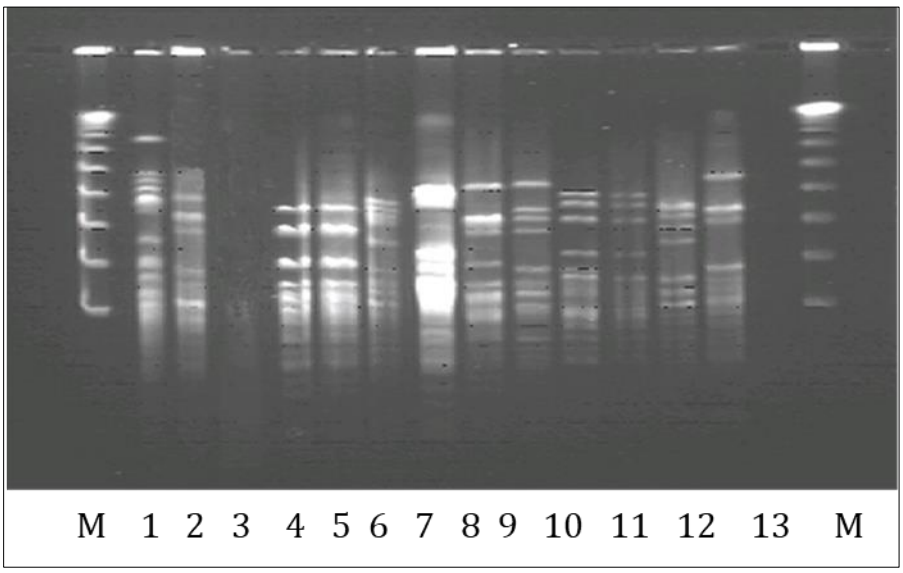


Figure 2 Pulsed field gel electrophoresis of chromosomal DNA digested with MfeI that appeared not digested on agarose gel electrophoresis. Marker DNA is indicated by M. Numbers indicate strains 78115 (1), 8241 (2), 8254 (3), 82107 (4), 11168 (5), 81116 (6), 81176 (7), 94194 (8), 99189 (9), 99312 (10), 99322 (11), 99346 (12), and 99385 (13)

ApoI, BamHI, BglII, EcoRI, NheI, PvuI, SpeI, and XbaI as revealed by PFGE. all strains except 81116 were sensitive to SmaI by PFGE. All strains, except for strain 99322, were sensitive to KpnI by PFGE. These findings are summarized in Table 1. The strains sharing sensitivity were classified into four groups with distinguishable

restriction enzymes. The largest group consisted of 10 strains, including 11168; three groups contained two, one, and one strain, which showed individual resistance patterns. In contrast to some of the MfeI patterns in Figure 2, PFGE patterns obtained using SmaI and KpnI (commonly used for genotyping *C. jejuni* [10, 12]) show that our strains are genetically very diverse, even within groups with common restriction resistance. We interpret strains within such a

group have identical DNA modification phenotypes and probably identical genotypes. The presence or absence of expression of genes encoding restriction enzymes could not be assessed due to the lack of specific probes for these genes.

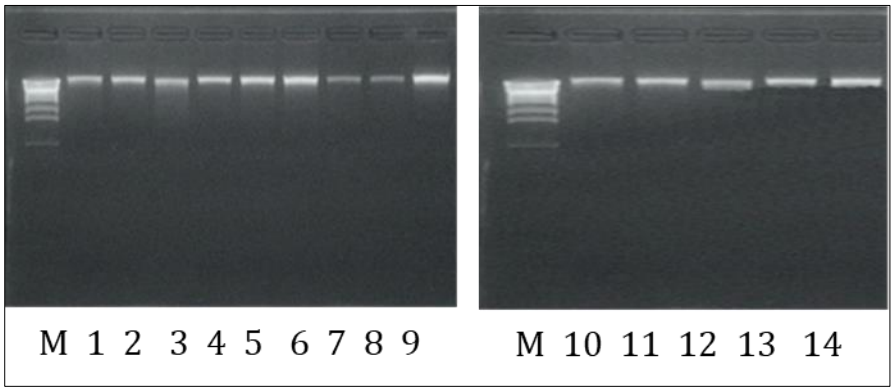
3.2. Presence of plasmids in *C. jejuni* strains

Since the R/M gene is often present on plasmids, at least in *E. coli*, we examined the plasmid content of the *C. jejuni* strains we used. As a result, a single large band with a size of about 280 kbp was identified on the PFGE gel from all the strains included in this study (Figure 3), but no smaller band was present. Analysis of the plasmid preparations on a regular agarose gel did not reveal any small plasmids. This large band could not be seen in the chromosomal DNA extracted by PFGE, which may indicate that the copy number of this presumed plasmid is low. Since there was no difference in the content of the plasmid among the strains, it was expected that there would be no difference in the content of the R/M gene through the plasmid.

3.3. Effect of R/M phenotype on natural transformation

We tested whether the sensitivity pattern of restriction enzymes affects the efficiency of natural transformation. As a marker for transformation, CAM-resistant mutants were

A.



B.

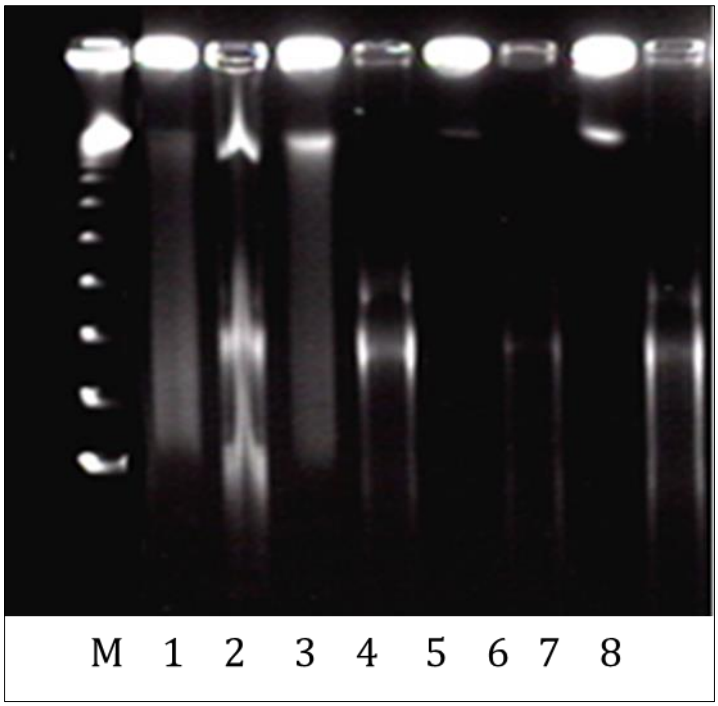


Figure 3 Possible plasmid content of *C. jejuni.* DNA was extracted from all 14 *C. jejuni* strains included in this study using a QIAGEN plasmid purification kit and analyzed by agarose gel (A) and PFGE gel (B). The PFGE gel shown included undigested chromosomal DNA (odd numbers) preceding plasmid DNA (even numbers) for each sample, with (M)

Marker; (1) and (2) 81116, (3) and (4) 81176, (5) and (6) 11168, (7) and (8) 8254. The arrow indicates a band of approximately 280 kbp

isolated and Km-resistant mutants were constructed. The undigested chromosomal DNA of these mutants was used for transformation experiments. Self-transformation (transformation of DNA from homologous mutants into wild-type strains) showed that the transformation efficiency of CAM-resistant mutants was higher than that of Km-resistant mutants (Table 1). Since CAM resistance is the result of point mutation, whereas Km resistance is the result of gene insertion, the difference in transformation efficiency can be attributed to the higher efficiency of homologous recombination in the case of point mutation. In both experiments, all the resistant colonies obtained were the result of transformation, since no resistant colonies were formed when no DNA was added. Comparing self and heterologous transformation, heterologous transformation was less efficient in three out of four isolates, but strain 99385 belonging to group I showed low but equal transformation efficiency in self and heterologous transformation (Table 2). The efficiency of natural transformation between strains within a group with the same DNA modification pattern (e.g., 99385 and 81116) was not significantly higher than that between strains with different DNA modification patterns. The same transformation efficiency was not observed between strains within the same group. If the strain expresses a particular restriction enzyme to which the donor DNA is susceptible (and is resistant to that restriction enzyme), it is likely that the

Table 1 Diversity in restriction sensitivity of 14 strains of *C. jejuni*

Restriction Eznyme	11168	81176	99312	99346	99385	99388	94194	82107	78115	99189	8214	8254	81116	99322
SmaI	+	+	+	+	+	+	+	+	+	+	+	+	-	+
KpnI	+	+	+	+	+	+	+	+	+	+	+	+	+	-
EcoRI	-	-	-	-	-	-	-	-	-	-	+	+	-	-
Other RE	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Group	I										II		III	IV

All strains were sensitive to the following enzymes: ApaI, ApoI, BamHI, BfaI, BglII, EcoRI, DraI, HindIII, HpyCH4V, Hpy188I, HpyCH4III, MfeI, MseI, NheI, NlaIII, PvuI, RsaI, Sau3AI, SspI SpeI, Tsp509I, XbaI. Strains are grouped according to their shared sensitivity characteristics.

Table 2 Autologous and heterologous transformation of 4 *C. jejuni* strains using two markers

		Number of transformants of *C. jejuni* obtained with DNA from							
Recipient		**81116 Km**	**81116 CAM**	**8254 Km**	**8254 CAM**	**8241 Km**	**8241 CAM**	**99385 Km**	**99385 CAM**
Strain	**Group**								
81116	III	**106**	**815**	65	217	77	176	61	130
8254	II	45	110	**102**	**783**	40	198	54	189
8241	II	11	48	9	34	**23**	**137**	7	24
99385	I	10	36	9	46	11	43	**12**	**31**
11168	I	0	0	0	0	0	0	0	0
81176	I	3	12	0	1	0	1	0	1
99312	I	2	20	3	31	6	34	5	27
99346	I	0	2	1	1	0	2	0	2
94194	I	0	0	0	0	0	0	0	0
82107	I	0	0	0	1	0	1	0	1

78115	I	0	0	0	0	0	0	0	0
99189	I	1	1	0	0	0	1	0	1
99322	IV	0	3	0	6	0	3	2	3
99388	I	0	25	0	9	0	20	0	18

Incoming DNA during transformation will be digested prior to recombination. This may result in a shorter DNA fragment available for recombination; a shorter DNA size may result in a lower recombination efficiency. On the other hand, free popping ends may increase the efficiency of transformation. We were not able to verify which effect is stronger, because the enzyme that is frequently cleaved, to which only certain strains are resistant, has not been identified. To determine how efficiently fragmented DNA can be transformed, we tested the effect of digesting chromosomal DNA prior to transformation using enzymes to which both the recipient and donor are sensitive. 81116 and 8241 CAM-resistant DNA were digested with HindIII and transformed. The efficiency was compared with that of undigested DNA. Pre-incubation of the donor DNA with HindIII effectively reduced the transformation efficiency, as transformants were no longer detected. As a control, donor DNA was treated with EcoRI; EcoRI is sensitive to donor DNA but produces only large fragments. In this case, the transformation efficiency was comparable to that of undigested DNA, indicating that the large size of digested DNA can be transformed and recombined.

4. Discussion

Our study was conducted to determine the efficiency of transformation of various *C. jejuni* strains and relate this to R/M sensitivity. Although the natural transforming ability of *C. jejuni* has been reported in 1990 [7], the transforming ability varies among strains [11]. Frequent DNA exchange among naturally competent strains results in weak clonal populations, as has been found in *C. jejuni* [3, 4]. On the other hand, genetically stable clones of *C. jejuni* have also been identified [13, 14, 15]. It has been suggested that 81116 strain is digested by HindIII, not by EcoRI. The transformation frequency was determined on the basis of antibiotics-resistant colonies per mg DNA per 10^8 recipient CFU. stable clones are the result of lack of competence due to restriction/modification activity [16]. Thus, the general dogma that the R/M system exists to restrict phage entry (based primarily on observations in *E. coli*) is extended to the point that the R/M system also restricts the uptake of DNA by competent organisms. The diversity in R/M content among strains of *C. jejuni* was revealed by a subtractive hybridization study comparing the gene content of strain 81116 and 11168 [9]. In this study, at least five gene fragments derived from the R/M gene, present in strain 81116 but absent in strain 11168, were identified, and Southern blot hybridization confirmed the variability in the presence of these gene fragments in other strains [9]. We decided to study the phenotype by expression of the R/M gene, not by its content. This resulted in resistance to the restriction enzyme in question. We assumed that strains that were resistant to a particular restriction enzyme expressed at least the modifying enzyme associated with that restriction enzyme, and in some cases also the restriction enzyme. Strains that were sensitive to a particular restriction enzyme were interpreted as not expressing that restriction enzyme or the associated modifying enzyme. Since we did not examine the R/M genotypes of the strains, we cannot rule out the possibility that the modifier enzyme genes are present but not expressed. We also did not distinguish between strains that were frequently cleaved by restriction enzymes (visible in agarose gel electrophoresis) and those that were less frequently cleaved (visible in PFGE), which could be the result of partial methylation. In this way, strains with the same sensitivity pattern were grouped and the transformation efficiency of strains belonging to the same and different groups was compared. Note that we did not evaluate conjugation, an alternative mechanism of gene transfer between bacteria. Although we assume that the presence and expression of methylation genes and restriction genes are coupled (when a restriction gene is expressed, at least a modifier gene must be expressed), there is evidence that this is not necessarily the case: At least one of the polymorphic tracts identified in the genome of *C. jejuni* 11168 separates a restriction gene and its modifier gene (CJ0031/0032) [17, 18]. Here, certain polymorphic variants turn off the expression of the downstream gene that is the restriction gene without affecting the expression of the modifier gene [18]. It is currently unknown whether other *C. jejuni* strains also have R/M genes with polymorphic sections. Multiple factors affect the efficiency of the transformation measured here: the efficiency of DNA uptake may vary between strains depending on the gene content [8]. The half-life of the DNA in the recipient strain is affected by the expressed restriction enzyme attacking this DNA. Recombination efficiency may be affected by gene homology (especially in the case of fla:Km constructs) and the size of the recombination fragments, which may also be affected by the frequency of digestion by the endonuclease expressed by the recipient strain. Since all the markers used were chromosomally encoded, the effect of incompatibility, which is often seen in plasmids, was eliminated. However, since the R/M gene is often encoded by a plasmid, we checked for the presence of a plasmid. This was because a large and distinct band was observed in all strains. The nature of this band is currently unknown. The nature of this band is

currently unknown, because the plasmid is not described in *C. jejuni* 11168, whose genome has been completely sequenced [17].

Table 3 Heterologous transformation from restriction enzyme sensitive donor to restriction enzyme sensitive or resistant *C. jejuni* strains

Donor strain	**Undigested 8254CAM Resistant DNA**	***HindIII* treated 8254CAM Resistant DNA**	***EcoRI* treated 8254 CAM Resistant DNA**
81116	1000	0	830

However, we cannot deny the possibility that large plasmids with low copy numbers exist in this and other strains. Although any of the factors mentioned above could affect transformation efficiency, the limiting effect of the R/M system is expected to override these, or at least be significant enough to be detectable. However, we were not able to correlate transformation efficiency with sensitivity to the restriction enzymes tested. The general assumption that lack of conservation of R/M enzymes between donor and recipient strains is a barrier to transformation may not hold true for *C. jejuni*; strains with identical R/M patterns were not more efficient at transformation than strains with different R/M patterns. However, because the R/M characterization of the strains in this study was incomplete, we cannot rule out the possibility that strains within one group may have differences in R/M enzymes that we have not evaluated. It is also possible that there are undiscovered R/M genes in the strains we studied. If the incoming DNA is not protected by modifications, it could be digested by endonucleases expressed in the recipient strains and eventually be completely degraded by exonucleases. However, it is also possible that endonucleases generate DNA fragments that increase the recombination efficiency of the incoming DNA, thus increasing the overall efficiency of the observed transformation. In *E. coli*, DNA ends generated by restriction enzymes have been shown to promote recombination [19]. Therefore, we tested the effect of pre-digesting chromosomal DNA prior to transformation using enzymes to which both donor and recipient are sensitive. The results showed that digestion with enzymes with high cleavage frequency reduced the transformation efficiency below the detection level. This suggests that either the pre-digested DNA does not enter the recipient cells, the digested DNA is rapidly degraded by exonucleases, or the fragments generated by HindIII are too short for homologous recombination. Previous studies have shown that relatively short homologous sequences (<100 bp) are sufficient for recombination after electroporation, so it is unlikely that the fragments generated by HindIII are too short to be recombined [19]. This is unlikely, since previous studies have shown that relatively short homologous sequences (<100 bp) are sufficient for recombination after electroporation [20]. It is also possible that exonucleases are likely to destroy fragmented DNA after uptake, and the possibility that DNA uptake serves as food cannot be ruled out. The question arises as to what the role of the R/M gene is and why there is diversity among strains in genotype [9] and phenotype in naturally competent species. In general, R/M genes are considered to be "gatekeepers" that allow incoming DNA only if it occurs in a strain with the same R/M gene. Not all strains of *C. jejuni* are equally competent [11], and inactivation of a single gene involved in the DNA uptake machinery can render a strain non-competent [8]. This result suggests that the effect of R/M expression may be of limited importance in predicting transformation efficiency and hence gene flow between strains. As it has been shown that multiple genes are required for natural competence [8], it is unlikely that such a complex of genes would be maintained in a bacterial population and only prevented by a single restriction/modification gene. If it were preferable for a strain not to accept foreign DNA, then mutation of the transforming gene family would be simpler than acquisition of the R/M gene. Perhaps the R/M gene has a different role (such as the effect of DNA modification on gene expression), or it is evolutionarily neutral (a selfish gene). The results of this study do not support the general hypothesis that restriction/modification genes induce gene flow within *C. jejuni* populations by promoting gene exchange between strains with similar R/M sensitivity patterns. This may be because the small number of strains studied may have overlooked R/M genes that were present but not evaluated, and because we were only able to evaluate a limited combination of DNA exchange between strains.

5. Conclusion

In this study, no correlation was found between transformation efficiency and sensitivity to the restriction enzymes in *C. jejuni* like other bacteria. The function of the R/M gene in bacterial gene exchange is not fully understood and further studies on these aspects are desirable.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

All authors declare no conflict of interest of regarding the publication of this paper.

References

[1] Tauxe RE. Epidemiology of Campylobacter jejuni infections in the United States and other industrialized nations. In: Campylobacter jejuni. Current Status and Future Trends. Washington DC. ASM press. 1992; 9-19.

[2] Wassenaar TM, Blaser MJ. Pathophysiology of Campylobacter jejuni infections of humans. Microbes and Infection. 1999; 1: 1023-1033.

[3] Dingle KE, Colles FM, Wareing DR, Ure R, Fox AJ, Bolton FE, Bootsma HJ, Willems RJ, Urwin R, Maiden MC. Multilocus sequence typing system for Campylobacter jejuni. Journal of Clinical Microbiology. 2001; 39: 14-23.

[4] [4] Meinersmann RJ, Patton CM, Evins GM, Wachsmuth IK, Fields PI. Genetic diversity and relationships of Campylobacter species and subspecies. International Journal of Systematic Evolutionary Microbiology. 2002; 5: 1789-1797.

[5] Dubnau D. DNA uptake in bacteria. Annual Reviews of Microbiology. 1999; 53: 217-244.

[6] Lorenz MG, Wackernagel W. Bacterial gene transfer by natural genetic transformation in the environment. Microbiological Reviews. 1994; 58: 563-602.

[7] Wang Y, Taylor DE. Natural transformation in Campylobacter species. Journal of Bacteriology. 1990; 172: 949-955.

[8] Wiesner RS, Hendrixson DR, DiRita VJ. Natural transformation of Campylobacter jejuni requires components of a type II secretion system. Journal of Bacteriology. 2003; 185: 5408-5418.

[9] Ahmed IH, Manning G, Wassenaar TM, Cawthraw S, Newell DG. Identification of genetic differences between two Campylobacter jejuni strains with different colonization potentials. Microbiology. 2002; 148: 1203-1212.

[10] Michaud S, Menard S, Gaudreau C, Arbeit RD. Comparison of smaI-defined genotypes of Campylobacter jejuni examined by kpnI: a population-based study. Journal of Medical Microbiology. 2001; 50: 1075-1081.

[11] Wassenaar TM, Fry BN, van der Zeijst BAM. Genetic manipulation of Campylobacter evaluation of natural transformation and electro-transformation. Gene. 1993; 132: 131-135.

[12] Wassenaar TM, Newell DG. Genotyping methods of Campylobacter: a review. Applied and Environmental Microbiology. 2000; 66: 1-9.

[13] Manning G, Duim B, Wassenaar TM, Wagenaar JA, Ridley A, Newell DG. Evidence for a genetically stable clone of Campylobacter jejuni. Applied and Environmental Microbiology. 2001; 67: 1185-1189.

[14] Wassenaar TM, Lastovica AJ, Duim B, Wagenaar JA, Fry BN. Genetic characterization of Campylobacter jejuni O:41 in relation to Guillain Barre Syndrome. Journal of Clinical Microbiology. 2000; 38: 874-876.

[15] Duim B, Godschalk PC, van den Braak N, Dingle KE, Dijkstra JR, Leyde E, van der Plas J, Colles FM, Endtz HP, Wagenaar JA, Maiden MC, van Belkum A. Moleclar evidence for dissemination of unique Campylobacter jejuni clones in Curacao, Netherlands Antilles. Journal of Clinical Microbiology. 2003; 41: 5593-5597.

[16] De Boer P, Wagenaar JA, Achterberg RP, van Putten JPM, Schouls LM, Duim B. Generation of Campylobacter jejuni genetic diversity in vivo. Molecular Microbiology. 2002; 44: 351-359.

[17] Parkhill J, Wren BW, Mungall K, Ketley JM, Churcher C, Basham D, Chillingworth T, Davies RM, Feltwell T, Holroyd S, Jagels K, Karlyshev AV, Moule S, Pallen MJ, Penn CW, Quail MA, Rajandream MA, Rutherford KM, van Vliet AH, Whitehead S, Barrell BG. The genome sequence of the food-borne pathogen Campylobacter jejuni reveals hypervariable sequences. Nature. 2000; 403: 665-668.

[18] Wassenaar TM, Wagenaar JA, Rigter A, Fearnley C, Newell DG, Duim B. Homonucleotide stretches in chromosomal DNA of Campylobacter jejuni display high frequency polymorphism as detected by direct PCR analysis. FEMS Microbiology Letters. 2002; 212: 77-85.

[19] Price C, Bickle TA. A possible role for DNA restriction in bacterial evolution. Microbiological Sciences. 1986; 3: 296-299.

[20] Wassenaar TM, Fry BN, Van der Zeijst BAM. Variation of the flagellin gene locus of Campylobacter jejuni by recombination and horizontal gene transfer. Microbiology. 1995; 141: 95-1.