Identification of genetic differences between two Campylobacter jejuni strains with different colonization potentials

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The consumption of poultry meat contaminated with Campylobacter jejuni is considered to be a risk factor for human campylobacteriosis. The development of targeted strategies to control campylobacters in broilers would benefit from knowledge of those bacterial factors important in colonization of the avian gut. During preliminary studies it was noted that C. jejuni NCTC 11168 was a poorer colonizer of chickens than strain 81116. This poor colonization could not be fully restored by in vivo passage, suggesting that it was a genetically endowed property of strain 11168. As the genome sequence is available for this strain, the technique of subtractive hybridization was used to identify gene fragments of strain 81116 not present in strain 11168. After two screening cycles, 24 out of 42 clones were identified as having DNA inserts specific for strain 81116. Six of these 24 clones contained gene fragment inserts with similarities to restriction–modification enzymes found in other bacteria. Two inserts had similarity to arsenic-resistance genes, whereas four others had similarities to cytochrome c oxidase III, dTDP-glucose 4,6-dehydratase, γ-glutamyl transpeptidase and an abortive phage-resistance protein. At least some of these genes may be involved with colonization. A further six inserts had weak similarities to hypothetical proteins or to proteins with assigned functions from strain 11168. The remaining six clones had gene-fragment inserts with no database matches. Southern-blot analysis confirmed that strain-dependent variation existed for each of these DNA inserts. These results indicate that subtractive hybridization can successfully identify genes that are absent from the only C. jejuni strain for which the genome sequence is currently available.

Keywords: subtractive hybridization

INTRODUCTION

Campylobacter jejuni is a major cause of human acute bacterial enteritis worldwide (Tauxe, 1992). The sources and routes of infection are debatable, but the consumption of contaminated poultry meat is considered to be a significant risk factor. Surveys have shown that broilers frequently carry large numbers of these organisms in their intestinal contents (Newell & Wagenaar, 2000). This carriage is both chronic and asymptomatic. The spillage of gut contents during processing can contaminate the retail poultry product and the abattoir environment (Newell et al., 2001). Currently, the reduction or elimination of campylobacters in the food chain is largely focussed at the poultry farm level. Unfortunately, enhanced biosecurity has largely failed to prevent campylobacter entering the broiler house from the environment. In part, this failure is a reflection of the low infectious dose, rapid bacterial growth within the avian gut and subsequent rapid transmission to adjacent animals. All of the evidence suggests that C. jejuni has evolved to efficiently colonize the avian gut as a commensal. Therefore, a better...
understanding of the colonization determinants involved in this process may ultimately lead to more targeted and effective control strategies, such as probiotic administration or vaccination.

To date, only a few potential colonization factors, specific for the avian host, have been identified using defined mutants in experimental oral models of chick colonization. A role for the flagellin genes in chicken colonization has been demonstrated (Wassenaar et al., 1993; Nachamkin et al., 1993); however, non-motile and aflagellate bacteria are still able to colonize (Wassenaar et al., 1993). Other genes apparently important in avian-gut colonization identified so far include sod (Purdy et al., 1999), racR (Bras et al., 1999), pgH (A. Karlyshev, unpublished data) and cadF (Ziprin et al., 1999).

Epidemiological studies on large numbers of C. jejuni isolates have shown a high degree of phenotypic and genotypic diversity (Owen et al., 1997; Lindstedt et al., 2000; Dingle et al., 2001; Wassenaar & Blaser, 1999). It seems likely that such diversity is reflected in phenotypic variation in the colonization and disease-causing potentials of strains. Certainly strain variation in virulence properties has been observed in both in vitro (Gorelov et al., 1990) and in vivo models, though such models may poorly reflect the spectrum of disease seen in humans (Pang et al., 1987; Black et al., 1988; Wassenaar & Blaser, 1999). Understanding the molecular basis and biological consequences of genetic diversity among strains is important for disease control. To this end the recent genome sequencing of C. jejuni NCTC 11168 (Parkhill et al., 2000) now enables, by DNA array technology (Lan & Reeves, 2000), the absence of genes in other C. jejuni strains to be detected. However, the identification of the presence of additional genes in other strains is not feasible using this approach.

C. jejuni NCTC 11168 was derived from a case of human campylobacteriosis in 1977 (M.B. Skirrow, personal communication). Preliminary studies indicated that this strain displayed a relatively low colonization potential in chickens compared to that previously reported for the well-characterized strain 81116 (Cawthraw et al., 1996). The inability of chicken passage of strain 11168 to fully restore its colonization potential indicated that this phenotype had a genetic basis. Therefore, the method of subtractive hybridization was used to identify genetic material, potentially associated with colonization, present in C. jejuni strain 8116 but not present in the genome sequence of strain 11168.

**METHODS**

**Bacterial strains.** C. jejuni NCTC 11168 and strains 8116 and 81-176, were all derived from human cases of campylobacteriosis (Parkhill et al., 2000; Palmer et al., 1983; Kroll et al., 1985, respectively) and are all routinely used in laboratory investigations. Other isolates included in this investigation were randomly selected from the collection of C. jejuni strains held at the Veterinary Laboratories Agency: strains 99/189, 99/322 and 99/388 were from the faeces of patients with diarrhoea; strains 94/194, 99/346 and 99/385 were from the caeca of broilers in unrelated poultry flocks; strain EX114 was an environmental strain from a poultry farm; and strain 99/312 was from the faeces of a cow.

**Fig. 1.** The strategy adopted for subtractive hybridization. Digested tester DNA (8116) is subdivided into two portions and the 5’ ends are ligated to an adapter. Each aliquot is denatured and hybridized to excess denatured driver DNA (11168). In the second hybridization, the aliquots are combined and excess denatured driver is again added. Amplification with PCR primer 1 exponentially concentrates the type f molecules, which represent DNA present in the tester but which is absent from the driver. A second PCR with nested primers 1 and 2R further enriches tester-specific DNA.
Table 1. Summary of the bacterial strains, plasmids and primers used in subtractive hybridization, cloning and sequencing

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
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<tbody>
<tr>
<td>Adapter 1</td>
<td>5′-CTAATAGCTACTATAGGTCGAGCGGCGCCGCCGGCAGGT-3′ 3′-GGCCGCTCAG-5′</td>
</tr>
<tr>
<td>Adapter 2R</td>
<td>5′-CTAATAGCTACTATAGGTCGAGCGGCGCCGCCGGCAGGT-3′ 3′-GGCCGCTCAG-5′</td>
</tr>
<tr>
<td>PCR primer 1</td>
<td>5′-CTAATAAGCTCTAGTTTACACAACTAGAG-3′</td>
</tr>
<tr>
<td>Nested primer 1</td>
<td>5′-TCGAGGCGGGTCCGGGCGGAGGT-3′</td>
</tr>
<tr>
<td>Nested primer 2</td>
<td>5′-AGGGGCGGGTCCGGGCGGAGGT-3′</td>
</tr>
<tr>
<td>M13 (forward)</td>
<td>5′-GGCCAGGTGGTCCGGTCAAGTG-3′</td>
</tr>
<tr>
<td>M13 (reverse)</td>
<td>5′-TCACAGGGAAACAGCTATGAC-3′</td>
</tr>
<tr>
<td>Tester C. jejuni</td>
<td>81116; colonizes the chicken gut</td>
</tr>
<tr>
<td>Driver C. jejuni</td>
<td>NCTC 11168; poorly colonizes the chicken gut</td>
</tr>
<tr>
<td>E. coli TOP10F*</td>
<td>F′ [lacI Tn10 (TetR) mcrA Δ(mrr-lbsdIRMS–mcrBC) g80lacZAM15 ΔlacX7 recA1 deoR araD(ara–leu)7697 galU galK rpsL (StrR) endA1 mapG</td>
</tr>
<tr>
<td>pCR2.1-TOPO</td>
<td>Plasmid map shown at <a href="http://www.invitrogen.com">http://www.invitrogen.com</a></td>
</tr>
</tbody>
</table>

*Adapter 1 and 2R homology to PCR primer 1 (shown in bold). Adapter 1 homology to nested primer 1 (shown in italic) and adapter 2R homology to nested primer 2 (shown in bold italic).

Media and growth conditions. C. jejuni strains were routinely grown for 24 h at 42 °C under microaerobic conditions (85% N₂, 7.5% CO₂, 7.5% O₂) on blood agar plates supplemented with 10% sheep blood, 50 µg Actidione ml⁻¹ and selective antibiotics (Oxoid) (Skirrow, 1977). Escherichia coli was maintained on Luria–Bertani (LB) agar or in Luria–Bertani broth (Difco) at 37°C in atmospheric conditions. When required the media were supplemented with 100 µg ampicillin ml⁻¹ or 50 µg kanamycin ml⁻¹. C. jejuni and E. coli were stored as frozen cultures at -80°C as frozen cultures at -80°C.

Chicken-colonization model. A quantitative oral chicken-colonization assay was performed as previously described (Wassenaar et al., 1993). Briefly, groups of 10 specific pathogen-free chickens (SPAFAS, Charles River), housed in isolators, were dosed at day 1 of age by oral gavage. Doses ranged from 10⁹ to 10¹⁰ c.f.u. (100 µl 0.1 M PBS, pH 7.2)⁻¹ of C. jejuni NCTC 11168, administered in 100 µl 0.1 M PBS (0.0234 M disodium hydrogen phosphate; 0.0066 M potassium dihydrogen orthophosphate; 0.07 M NaCl; pH 7.2). Doses were prepared by harvesting bacteria, grown overnight on blood agar plates at 42°C, into sterile PBS. At 3 days post-challenge, the levels of colonization were determined by plating out dilutions of caecal contents. Chicken-colonization levels were given as c.f.u. (g caecal contents)⁻¹ for individual birds. The limit of detection was 100 c.f.u. (g caecal contents)⁻¹. The dose response for the colonization potential of C. jejuni 81116 has been previously described (Wassenaar et al., 1993) and has been consistent in over 20 experiments.

Subtractive hybridization. A schematic diagram of the subtractive-hybridization technique is shown in Fig. 1. The DNA sequences of adapters and primers used in this study are shown in Table 1. Initially, genomic DNA preparations from C. jejuni 81116 (tester) and strain 11168 (driver) were isolated using the CTAB method for DNA isolation (Ausubel et al., 1994). Each preparation was digested with Alul and DraI, according to the methods described in the PCR-Select Bacterial Subtraction kit (Clonetech), to give a range of blunt-ended fragments of between 0·1 and 2·0 kbp, as determined by gel electrophoresis. The blunt-ended DNA fragments from C. jejuni 81116 were subdivided into two aliquots and each aliquot was ligated to a different adapter (1 or 2R). This resulted in double-stranded DNA fragments with an extended, single-stranded adapter sequence on the 5’ end of each complementary strand. Both aliquots were denatured and combined with an excess of denatured driver DNA and the mixture was incubated at 63°C for 1·5 h (Fig. 1, hybridization one). In the second hybridization (Fig. 1, hybridization two) the two aliquots were combined and incubated at 63°C overnight with more fresh denatured driver DNA. During this stage tester-specific DNA (ligated to adapter 1) annealed to tester-specific DNA (ligated to adapter 2R). The recessed ends of the adapters were filled and the primary PCR was carried out to exponentially amplify tester-specific DNA (type f molecules). In the secondary PCR, nested primers to adapters 1 and 2R were used to further enrich the tester-specific DNA (type f molecules). The first genomic subtraction was carried out with a hybridization temperature of 63°C, as recommended by the manufacturer. A second round of subtractive hybridization was performed with 10 µl of the secondary PCR products, but this time a hybridization temperature of 61°C was used.

Preparation of the subtractive-hybridization library. The secondary PCR products were ligated into pCR2.1-TOPO vector using the TOPO TA Cloning kit (Invitrogen). The ligated products were transformed into TOP10F⁺ competent E. coli cells, as recommended, and transformants were selected for kanamycin and ampicillin resistance. PCR amplification was performed directly on the transformants using 50 pmol µl⁻¹ of M13 forward and reverse primers together with HotStar Taq master mix (Qiagen). PCR parameters were as follows: 95°C for 15 min followed by 25 cycles of 94°C for 45 s, 60°C for 45 s and 72°C for 1 min 45 s, with a final incubation at 72°C for 10 min. Agarose-gel electrophoresis confirmed the presence of DNA inserts (data not shown).

Dot-blot and Southern-blot hybridizations. For the dot blots, 50 ng of each PCR product was denatured by incubating in a boiling water bath for 5 min and spotted onto a Hybond-N + membrane (Amersham). DNA was cross-linked to the membrane by UV light. For probe labelling, 200 ng of genomic DNA digested with Alul and DraI was labelled using alkaline

1205
phosphatase (AP) according to AlkPhosDirect labelling kit guidelines (Amersham). The blots were prehybridized in AlkPhos Direct Hybe buffer for 1 h at 60 °C and then hybridized overnight at 60 °C with 200 ng of the AP probe. For Southern-blot analysis, 10 µg of each C. jejuni genomic DNA was digested with either HindIII or HinfI, electrophoresed through a 1% (w/v) agarose gel and transferred onto a Hybond-N+ membrane. Each DNA insert (50 ng) of interest was labelled with AP; hybridizations and stringency washes were carried out at 60 °C as before.

Sequencing and analysis. Strain-81116-specific clones were cultured in 1 ml LB broth with kanamycin selection, purified using a QIAprep spin miniprep kit (Qiagen) and sequenced by using the CEQ DTCS sequencing system (with 3-2 pmol M13 primer µl−1) on a CEQ 2000 DNA sequencer (Beckman Coulter). The DNA sequences were edited using the EDITSEQ program (DNAstar, Lasergene) and homology searches were performed using the BLAST suite of programs (Altschul et al., 1997).

RESULTS

C. jejuni 81116 colonizes the chick model in a dose-dependent manner, with a dose of 10⁸ c.f.u. maximally colonizing 100% of the birds (Wassenaar et al., 1993). This differs from other freshly isolated strains tested, which display all-or-nothing-type colonization patterns and maximally colonize all the birds with doses of 10⁶–10⁸ c.f.u. (Cawthraw et al., 1996). However, this apparent lower colonization potential of strain 81116 can be overcome by a single passage through chicks, so that the passaged strain (81116P) maximally colonizes birds with a dose as low as 35 c.f.u. (Cawthraw et al., 1996). In comparison with strain 81116, strain 11168 was a poor colonizer (Fig. 2). A dose of 10⁸ c.f.u. gave a detectable colonization level in only two out of 10 birds. A dose of 10⁹ c.f.u. was required to colonize 100% of birds challenged, and even then colonization was at a mean level below the maximum level seen with freshly isolated campylobacter strains. Chick-passaged strain 11168 (11168P) did show some increased ability to colonize, but this was still lower than that of the unpassaged strain 81116 (Fig. 2).

Genomic subtractive hybridization was applied to identify DNA sequences present in strain 81116 and absent in strain 11168 (Fig. 1). The initial round of subtractive hybridization at 63 °C indicated that subtraction had occurred at only a low efficiency, given that relatively few transformants were recovered and that many of the clones contained DNA inserts with sequences present in 11168. Consequently, the second round of subtraction was performed at a lower hybridization temperature (61 °C), which greatly enhanced the annealing of homologous driver and tester DNA, and the subsequent amplification of tester-specific DNA. Cloning of these PCR products into pCR2.1-TOPO resulted in 255 colonies, of which 167 contained inserts. Dot-blot hybridization was performed to test the specificity of the DNA fragments using the genomic DNA (labelled with AP) of strains 81116 and 11168 as probes (Fig. 3). Forty-two of the 167 inserts were chosen for sequencing. An example of a strain-81116-specific insert has been circled.
to strongly hybridize to strain 81116 and either weakly or not at all to strain 11168. DNA sequencing revealed that 24 of these 42 clones contained inserts that were absent in strain 11168 (Table 2), of which one DNA sequence was represented twice (inserts 126 and 136, absent in strain 11168 (Table 2), of which one DNA insert was unique to strain 81116 (Fig. 4a). The remaining 18 DNA inserts (43%) were false positives, i.e. analysis using the BLAST suite of programs revealed high sequence similarities in strain 11168 and also predicted similarities of 75–100% at the amino acid level when each DNA sequence was translated in all six reading frames. Most of these false-positive inserts were present in the cloning plasmid as chimeras (13 inserts), in which two different inserts had ligated into one vector joined by adapter sequences. Five inserts (46, 141, 149, 209 and 212) had weaker, but significant, similarities (40–61% identity at the amino acid level) with genes present in strain 11168. Twelve sequences had no similarity to any gene in the strain 11168 genome sequence, but they did have significant similarity to genes of other organisms. The remaining six sequences (inserts 30, 50, 65, 117, 121 and 243) had no significant similarity with any sequence present in the NCBI databases searched. The 23 strain-81116-unique DNA fragments (mean size of 272 bp; range 124–644 bp) were further analysed for the presence of potential surface structures and conserved motifs in protein families by Predict Protein (http://dodo.cpmc.columbia.edu/predictprotein/submit.def.html). The prevalence of these unique sequences, among a diverse range of other C. jejuni strains, was determined by Southern-blot analysis (Table 3). The chromosomal DNA of each strain, digested with either HinIII or HinfII, was probed with each of the 23 strain-81116-specific DNA inserts. The results confirmed the presence of all 23 sequences in strain 81116 and their absence from strain 11168 DNA (Table 3). Only one of the 23 inserts (insert 121) was unique to strain 81116 (Fig. 4a). In contrast, only insert 50 hybridized to all other strains tested, with the exception of strain 11168 (Fig. 4b). The remaining 21 sequences gave variable distributions and banding patterns, of which two examples are shown (Fig. 4c, d, insert 246 and 135, respectively). The results are summarized in Table 3. Insert 46 is an example of a chimeric insert, in which two DNA fragments (excisional nuclease ABC subunit A and putative oxidoreductase)
Table 3. Southern-blot analysis of each insert against randomly chosen C. jejuni strains

<table>
<thead>
<tr>
<th>Insert</th>
<th>Hybridization with C. jejuni strain*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>183</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>68</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>135</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>65</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>236</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>141</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>205</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>246</td>
<td>+</td>
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<td>136</td>
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</tr>
<tr>
<td>31</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>186</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>212</td>
<td>+</td>
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<td>149</td>
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<td>209</td>
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<td>−</td>
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<tr>
<td>117</td>
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<td>−</td>
</tr>
<tr>
<td>121</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>17</td>
</tr>
</tbody>
</table>

*+., Indicates a positive hybridization; −., indicates a negative reaction (total absence of bands).

were ligated together (Fig. 5a). Removal of the exci-
nuclease sequence and generation of a new AP-labelled
probe, containing only the putative oxidoreductase,
removed those bands that were common to all the
strains (~2 kbp band for HindIII profile, Fig. 5b),
leaving a 1–1 kbp band in strains 99/385, 81116, 99/388
and 99/346, and a 2–4 kbp band for strain EX114 (data
not shown). For the other chimeric inserts, the individual
strain-81116-specific sequences were also amplified by
PCR from strain 81116 genomic DNA and then used to
probe the Southern blots.

**DISCUSSION**

All freshly isolated C. jejuni strains, i.e. those with
minimal laboratory passage, tested to date maximally
colonize 10–100 c.f.u. (g caecal contents −1) one-day-
old chicks within 5 days with oral doses of 10–106 c.f.u.
(Cawthraw et al., 1996). Although the laboratory strain
81116 has a comparatively low colonization potential,
this can be upregulated by in vivo passage (81116P) to a
level similar to that of freshly isolated strains. In
contrast, strain 11168 appears to be a very poor
colonizer, and even in vivo passage will not upregulate
its colonization potential to that seen in freshly isolated
strains. It therefore seems likely that the observed lower
colonization potential of C. jejuni 11168, compared with
strain 81116, is a reflection of genetic difference(s). In
this study subtractive hybridization has been success-
fully applied to identify genetic material present in C.
jejuni 81116 but absent in strain 11168. This approach
was rendered easier because strain 11168 has recently
had its genome sequenced.

The low percentage of genuine strain-81116-specific
sequences, obtained from the initial subtractive-
hybridization experiment, may have been due to the
inadequate annealing of homologous DNA sequences
from strain 11168 to 81116. Decreasing the temperature
from 63 °C (optimal for bacteria with a GC ratio of
40–51 mol%) to 61 °C improved this efficiency by
allowing the preferential amplification of 81116-specific
sequences. Improved optimization might be achieved by
decreasing the hybridization temperature further. The
presence of a number of chimeric clones also confounded
the overall efficiency, as these were detected as positive
clones. Nonetheless, a total of 23 DNA sequences
identified six sequences had no significant similarities to
any entry in the databases searched. In several cases, two
or more of the strain-81116-specific inserts appeared to
have been derived from the same genomic location, as indicated by Southern blotting, suggesting that such strain-specific genes may be clustered; for example, the *Hinfl* profiles of inserts 60, 243 and 246 were identical.

The appearance of identical banding profiles on Southern blots for the two *C. jejuni* strains 99/312 and 99/322, when probed with inserts 50, 68, 135 and 202, suggests that the genetic arrangement of these strains is similar. Indeed, PFGE profiles of these two strains are also identical, indicating that they are clonally related (A.M. Ridley, personal communication).

Although subtractive hybridization was developed to screen for the presence or absence of genes, it can also be used to detect polymorphisms between two strains. This was apparent for inserts 205 and 141. These inserts have similarities to dTDP-glucose 4,6-dehydratase (*rmlB*) from *Legionella pneumophila*, an enzyme involved in the glycosylation of the cell wall of pathogenic bacteria (Allard et al., 2000), and a putative glycosyltransferase from *C. jejuni* 11168, respectively. Both inserts had identical *Hinfl* Southern-blot profiles and both were either present or absent in the *C. jejuni* strains tested. This suggests that the functions of these two genes, involved in polysaccharide biosynthesis, are coordinated. That inserts were identified that do not hybridize to strain 11168, despite significant amino acid similarity as in the case of insert 141, suggests that (at least some) polymorphic genes can be detected by subtractive hybridization. These results confirm DNA microarray observations indicating that strain 81116 differs from strain 11168 within this genetic region (Dorrell et al., 2001). Thus, subtractive hybridization not only complements DNA microarray technology but provides the additional benefit of detecting novel sequences in test strains.

It has been reported that sequences within the flagellar modification region and the capsule locus vary between strains. In particular, it has been reported that a region between Cj1318 and Cj1336 of the flagella modification locus has been lost from strain 81116 (Dorrell et al., 2001). However, subtractive hybridization identified insert 209 to have similarity to Cj1333. One explanation of this anomaly would be a genetic rearrangement in strain 81116, so that Cj1333 was no longer located where expected relative to strain 11168. However, the identification of a second insert (149) with similarity to Cj1337, and the identical Southern-blot profiles using inserts 149 and 209 as probes suggests that these two DNA fragments are in close proximity on the 81116 genome.
Fig. 5. An example of a chimeric clone, insert 46. (a) The DNA sequence between 426 and 16 has an amino acid identity of 61% to a putative oxidoreductase in C. jejuni 11168; the sequence between 431 and 595 has 98% amino acid identity to excinuclease ABC subunit A from C. jejuni 11168. The amino acid consensus is shown in between the amino acid sequence for insert 46 and the putative oxidoreductase or excinuclease ABC subunit A; conservative substitutions are indicated by +. (b) Southern-blot hybridization using insert 46 as a probe. The oxidoreductase part of chimeric insert 46 was shown to hybridize to strains 99/385, 99/388 and 99/346 (1–1 kbp band) and to EX114 (2 kbp band) in a separate experiment (data not shown). The excinuclease part of insert 46 was shown to hybridize to all strains, including strain 11168, as shown by the band in all lanes at 2 kbp.
revealed a substantial amount of overlapping DNA sequence, suggesting that they might be part of the same R–M gene. There is increasing evidence for the existence of multiple R–M systems in C. jejuni (Miller, 2001). The functions of those R–M genes found in strain 81116 are currently under investigation. Interestingly, the chromosomal DNA of this strain does not cut with SmaI (G. Manning, personal communication), even though SmaI sites have been shown to be present (I.H. Ahmed, unpublished data). It is therefore interesting to speculate that the R–M genes detected have a role in this enzyme restriction.

Two more inserts (31 and 186), which were absent from the strain 11168 genome, have similarities with genes involved in arsenic resistance in several bacteria. Insert 31 has 64% amino acid identity to ArsB from Sinorhizobium sp. As4, an integral membrane protein needed for the extrusion of arsenite. Insert 186 has 35% amino acid identity to ArsC from Aquifex aeolicus. This gene encodes arsenate reductase, which reduces intracellular arsenite to arsenate. The Southern-blot profiles of inserts 31 and 186 were different (data not shown), suggesting that these two genes do not form part of an operon. However, insert 186 had identical HindIII and HinfI profiles (data not shown) to those of insert 212, which has similarity to Cj1560 (a putative membrane protein). This suggests that ArsB and Cj1560 are co-located in the genome of all strains tested. Generally, in Gram-negative bacteria the arsenic-efflux pump comprises ArsA, the ATPase subunit, ArsB, which anchors the former to the membrane, and ArsC in the cytosol (Cervantes et al., 1994). The observation of arsenite-metabolizing genes in strain 81116 and not in strain 11168 is interesting especially since phenylarsionic compounds have been used, in the past, in poultry feed for controlling caecal coccidiosis and to promote growth (Pergantis et al., 1997). The use of such compounds may have led to the contamination of some agricultural lands on which poultry litter has been used as manure (Gupta & Charles, 1999). Thears operon is widespread among the Enterobacteriaceae and arsenic has also been shown to induce the synthesis of heat-shock proteins, indicative of the stress response triggered due to exposure to this toxic chemical (Chang et al., 1989). Thus, the biological role of these genes in the colonization of chickens by campylobacters requires further investigation.

Insert 236 has 72% amino acid identity to the H. pylori γ-glutamyl transpeptidase (ggt) gene. Southern-blot analysis of insert 236 revealed an identical HinfI pattern, to that of insert 65, for which no significant protein similarities could be identified. This suggests that these inserts are located in close proximity in the genome of strain 81116 and that they may be linked. As well as functions in the antioxidant glutathione pathway (Carmel-Harel & Storz, 2000), GGT appears to have a role in the colonization of the gastric mucosa by H. pylori (Chevalier et al., 1999; McGovern et al., 2001). Whether this gene product has a similar role in colonization of the chicken intestinal tract by C. jejuni will require further investigation.

The successful application of subtractive hybridization, to genetically compare two strains of C. jejuni with differing colonization potentials, has revealed a number of strain-specific differences, some of which may be potential colonization factors. It should, however, be noted that the sequences identified in this study are only fragments of much larger genes and that expression of the corresponding genes has not yet been proven. Moreover, future work involving the mutagenesis of the 81116-specific sequences in tandem with further chick-colonization experiments will be necessary to determine the importance of such genes in colonization. Insert 50 is particularly interesting since it was universally present in the other C. jejuni strains tested (apart from strain 11168) and for one strain (94/194) was the only insert hybridizing. As this strain colonizes the chick model to the same extent as freshly isolated strains (K.J. Clow, personal communication), insert 50 may be especially important in chicken colonization.

In conclusion, the 23 novel DNA segments identified in this study represent a selection of genes present in strain 81116 and absent in strain 11168, and they indicate extensive genetic variation even among a small number of C. jejuni strains. These results imply that we have, to date, found only a portion of the total gene pool existing within the C. jejuni species. While C. jejuni genome sequence data are limited techniques like subtractive hybridization can be used to identify novel genes, which can then be used to construct more comprehensive DNA arrays to accurately determine genomic diversity.

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REFERENCES


