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Distribution and Polymorphism of the Flagellin Genes from Isolates of Campylobacter coli and Campylobacter jejuni

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The complex flagellar filaments of the LIO8 serogroup member Campylobacter coli VC167 are composed of two highly related subunits encoded by the flaA and flaB genes which share 92% identity. Using oligonucleotides based on the known DNA sequence of both the flaA and flaB genes from C. coli VC167 in the polymerase chain reaction, we have shown conservation of both fla genes among isolates within the LIO8 heat-labile serogroup by digestion of the amplified product with PstI and EcoRI restriction endonucleases. Amplification and subsequent restriction analysis of the flaA flagellin gene from Campylobacter isolates belonging to 13 different LIO serogroups further identified 10 unique polymorphic groups. Within most of the serogroups examined, isolates appeared to contain flaA genes with conserved primary structures. Only in serogroups LIO11 and LIO29 did independent isolates possess flagellin genes with different primary structures. Furthermore, by employing primers specific for helicobacter pylori (25), the flagellar filament of C. pylori (14), and Rhizobium meliloti (35). The periplasmic flagella of Spirochaeta aurantia (32) and Treponema pallidum (31) are also composed of multiple species of flagellin subunits, as are the flagellar filaments of archaea such as Helobacterium halobium, Methanococcus spp., and Methanospirillum spp. (13, 22, 23, 41). For several of these organisms, the flagellin genes coding for the two or more flagellin species have been sequenced. However, as a group, the complex flagella remain poorly described. For example, in most cases the information available is restricted to a single strain of the species, and little is known regarding the antigenic and structural diversity of flagellins which form complex flagella.

Regardless of whether the flagellar filament is simple or complex, the structures of most bacterial flagellin subunits are strikingly similar. For a large range of organisms, the amino- and carboxy-terminal domains of flagellin molecules which have roles in flagellin export, polymerization of the flagellar filament, and filament stability (11, 46, 47) do not display significant amino acid sequence homology (21, 45, 49). However, the central region of the flagellin molecule often displays large differences in both size and amino acid content and appears to be responsible for the extensive antigenic diversity seen among flagellins from members of the family Enterobacteriaceae (18–20, 26). Preliminary immunological evidence suggests that such a model may also be valid for Campylobacter spp. (28). Moreover, genetic data by Thor.ton et al. (43) demonstrated that serotype-specific DNA probes can be constructed by using the central region of the Campylobacter flagellin gene.

The flagellar filament of C. coli VC167 can undergo antigenic variation between two antigenic forms, T1 and T2 (15, 17), each of which contains two flagellin subunits, FlaA and FlaB. These flagellins are coded for by two tandemly oriented, highly homologous genes which show 92.0% identity in VC167-T1 and 91.6% identity in VC167-T2 (14, 16). C. jejuni 81-116 also possesses two tandem flagellin genes which display 92.8% identity with each other (27), and the total identity between the flagellin genes of strains VC167 and 81-116 is 82%. The flaA genes of two other C. jejuni strains, IN1 and TGH9011, have also been sequenced (12, 24) and show 74.9 and 73.6% identity to the flaA gene of VC167, respectively. This suggests that there may be considerable genetic diversity among the flaA genes in Campylobacter spp. Although a number of studies have suggested the presence of a second flaA gene in other strains of Campylobacter (12, 43), it is not known how widespread flaB is throughout Campylobacter spp., as tandem gene duplications are often rapidly eliminated by recombination (7, 44), or indeed how related flaB is to flaA.

One of the major serotyping schemes of Campylobacter spp. is the heat-labile scheme of Lior (26a), which recognizes more than 100 serogroups. It had previously been suggested that flagella were the serodeterminant of this scheme, but recent genetic data have indicated that in most serogroups flagella are not the Lior serodeterminant (3). Nonetheless, surface-exposed serospecific determinants on flagella have been demonstrated and flagellin genes are highly conserved.

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TABLE 1. Strains used in this study

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Strain(s)</th>
<th>Polymorphism group</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIO8</td>
<td>C. coli VC20, VC143, VC144, VC167, VC189; C. jejuni VC156, VC159</td>
<td>flaA</td>
</tr>
<tr>
<td>LIO8(29)</td>
<td>C. jejuni VC152, VC157</td>
<td>flaB</td>
</tr>
<tr>
<td>LIO6</td>
<td>C. jejuni VC34, VC209, VC223, 81-116</td>
<td></td>
</tr>
<tr>
<td>LIO4</td>
<td>C. jejuni VC33, VC207, VC226, VC331, VC237</td>
<td></td>
</tr>
<tr>
<td>LIO5</td>
<td>C. jejuni VC29, 81-176</td>
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</tr>
<tr>
<td>LIO20</td>
<td>C. coli VC97, VC225</td>
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</tr>
<tr>
<td>LIO29</td>
<td>C. coli VC168</td>
<td></td>
</tr>
<tr>
<td>LIO42</td>
<td>C. coli VC236</td>
<td></td>
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<tr>
<td>LIO1</td>
<td>C. jejuni VC228, VC232</td>
<td></td>
</tr>
<tr>
<td>LIO7</td>
<td>C. jejuni VC95</td>
<td></td>
</tr>
<tr>
<td>LIO55</td>
<td>C. coli VC235</td>
<td></td>
</tr>
</tbody>
</table>

within Lior serogroups (4, 17). In this study, we undertook to determine the conservation of the primary flagellin gene structure within various LIO serogroups and the polymorphism of the flagellin genes among serogroups. Further, we could determine the presence or absence of flaB in a range of Campylobacter strains belonging to a variety of LIO serogroups, and, by restriction endonuclease digestion of polymerase chain reaction (PCR)-amplified products that represent ~85% of the flagellin genes, we could investigate the similarity of the primary structure of the flaB gene, if present, to that of flaA.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The C. coli and C. jejuni strains listed in Table 1 were obtained from H. Lior, National Enteric Reference Centre, Ottawa, Canada. Campylobacter spp. were grown on Mueller-Hinton agar (Difco) at 37°C in an atmosphere containing 5% oxygen and 10% CO2.

Preparation of chromosomal DNA. A plate of fresh over-night Campylobacter growth was harvested in 2.5 ml of ice-cold 25% sucrose–50 mM Tris-HCl, pH 8.0. One milliliter of lysozyme (10 mg/ml in 0.25 M EDTA, pH 8.0) solution was added, and incubation was continued on ice for 20 min. This was followed by the addition of 0.75 ml of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), 0.25 ml of lysis solution (5% N-lauroylsarcosine; 50 mM Tris-HCl, pH 8.0; 62.5 mM EDTA), and 10 mg of pronase (Boehringer-Mannheim Canada Ltd., Laval, Québec, Canada), and incubation was continued at 58°C for 90 min. The viscous DNA solution was then extracted twice with Tris-saturated phenol and once with diethyl ether before being placed in dialysis bags and dialyzed against 2 liters of TE at 4°C for 16 to 24 h with one change.

PCR amplification. Oligonucleotides used for amplification were constructed on an Applied Biosystems 392 DNA synthesizer and were based on the known sequences of the flagellin genes of C. coli VC167. The flaA-specific 18-bp primer pg50 (5'-ATGGGATTGGTTAGAATAAAC-3') (30) used for PCR amplification is located on the plus strand beginning at the N terminus (Fig. 1A). The flaB-specific 32-bp primer RAA9 (5'-AAGGATTAAAAATGGGTTTATAAACC-3') is also located on the plus strand and begins 11 bp upstream of the translational start site of flaB (Fig. 1A). The 26-bp primer RAA19 [5'-GCCC(GC)T]TTAG(AG)TTACAC(TG)-3'], which binds to both flagellin genes 274 bp from the 3' end, was used as the reverse primer in both cases (Fig. 1A), and PCR amplification generated the fragments depicted in Fig. 1B. Other primers used were RAA10 (5'-TCTTGCITTTAATCITTTTCGATGCA-3'), which binds between 10 and 33 bp upstream of RAA9 on the same strand, and RAAS (5'-TTGCAACACGGTTACGTGGC-3'), which binds to the flaB coding region 25 bp from the translational stop codon on the minus, or noncoding, strand. AmpliTaq polymerase (Perkin-Elmer Cetus, Rexdale, Ontario, Canada) (0.4 U/10 μl of reaction mixture) was used in a hot-air thermocycler (Idaho Technology, Idaho Falls, Idaho) in the presence of 1× standard PCR buffer, 2.5 mM MgCl2 (GeneAmp; Perkin-Elmer Cetus), a final concentration of 250 μM each primer, and 50 ng of genomic template. Thirty to 40 cycles of amplification were performed in thin-walled capillary tubes, each cycle consisting of 1 s at 94°C, 1 s at 50°C, and 20 s at 72°C.

FIG. 1. Flagellin genes from C. coli VC152. (A) Schematic representation indicating the localization of the primary sites for the oligonucleotides used for PCR amplification and the restriction endonuclease sites used. (B) PCR products generated with primer pairs pg50-RAA19 and RAA9-RAA19 that represent the majority of the flaA and flaB genes, respectively. (C) Restriction fragments generated upon digestion of the amplified product with PstI and EcoRI.
n distinguishable in size from the 1,448-bp product from UJ and are indicated schematically in Fig. 1. Known sequences of the flavellin genes from VC167-T2 (lanes 2 and 6), the flaA flaB mutant KX15 (lanes 3 and 7), and the flaA flaB mutant KX5 (lanes 3 and 7). No DNA controls are seen in lanes 1 and 5. Fragment sizes are 1,448 bp (lanes 2 and 4), 2,848 bp (lane 3), 1,459 bp (lanes 6 and 7), and 2,859 bp (lane 6).

Genomic DNA from the seven strains listed in Table 1 were subjected to PCR amplification with specific flaA and flaB primer pairs. Certain strains, the ability of each primer pair to specifically amplify either flagellin gene had first to be determined. To accomplish this, we employed the previously described flagellin typing sera and are classed as serogroup L108(29). The expected larger fragment due to the Km' cassette was 202 bp.

Presence and conservation of fla genes among L108 isolates. Genomic DNA from the seven L108 and two L108(29) strains listed in Table 1 were subjected to PCR amplification with specific flaA and flaB primer pairs. Certain Campylobacter isolates agglutinate with both the L108 and L109 typing sera and are classed as serogroup L108(29). The flaA and flaB genes amplified from the L108 and L108(29) strains possessed the same size as that seen with VC167 (Fig. 3A and B). To determine whether the primary structure of the flagellin genes was conserved within the L108 serogroup, the PCR products were digested with PstI and analyzed on polyacrylamide gels.

Restriction analysis of PCR products. Following PCR amplification, the 10-μl reaction mixtures were either analyzed directly on 1% agarose gels and visualized by staining with ethidium bromide or subjected to restriction enzyme digestion. PCR products (10 μl) were digested in a total volume of 12 μl with 3 U of both PstI and EcoRI for 90 min at 37°C. After digestion, the fragments were separated on 12% polyacrylamide gels in a minislab gel apparatus (Hoefer Scientific Instruments, San Francisco, Calif.) in 1x TBE buffer by the method of Sambrook et al. (36) and visualized by staining with ethidium bromide.

RESULTS

Specificity of PCR primers in L108 strains. Because the flaA and flaB genes of C. coli VC167 are so highly homologous, the ability of each primer pair to specifically amplify either flagellin gene had first to be determined. To accomplish this, we employed the previously described flagellin typing sera and are classed as serogroup L108(29). The flaA flaB primer pair (pg50-RAA19) amplified a 1,448-bp product from wild-type VC167-T2 (Fig. 2, lane 2) and the flaA flaB mutant KX5 (Fig. 2, lane 4). A larger product was amplified from the KX15 mutant because of its flaA gene being inactivated by a Km' cassette (Fig. 2, lane 3). Similarly, the flaB primer pair (RAA9-RAA19) amplified a 1,459-bp product from VC167-T2 (Fig. 2, lane 6) and KX15 (Fig. 2, lane 7). The expected larger fragment due to the Km' cassette was amplified from mutant KX5 (Fig. 2, lane 8). These results indicated that in this strain, these primer pairs could specifically amplify the two flagellin genes.

Distinctive and conservation of fla genes among L108 isolates. Genomic DNA from the seven L108 and two L108(29) strains listed in Table 1 were subjected to PCR amplification with specific flaA and flaB primer pairs. Certain Campylobacter isolates agglutinate with both the L108 and L109 typing sera and are classed as serogroup L108(29). The flaA and flaB genes amplified from the L108 and L108(29) strains possessed the same size as that seen with VC167 (Fig. 3A and B). To determine whether the primary structure of the flagellin genes was conserved within the L108 serogroup, the PCR products were digested with PstI and analyzed on polyacrylamide gels.

Polyorphism of the flaA gene of other LIO serogroups. By employing the pg50-RAA19 flaA primer pair, we examined the 34 Campylobacter strains from 13 other LIO serogroups listed in Table 1. Under the amplification conditions employed, all strains were shown to produce a product indistinguishable in size from the 1,448-bp product from VC167-T2 detected by agarose gel electrophoresis (data not shown). To determine the polymorphic groupings of these strains, the PCR products generated with the pg50-RAA19 primer pair were digested with PstI and EcoRI and analyzed on polyacrylamide gels.

Presence and conservation of fla genes among L108 isolates. Genomic DNA from the seven L108 and two L108(29) strains listed in Table 1 were subjected to PCR amplification with specific flaA and flaB primer pairs. Certain Campylobacter isolates agglutinate with both the L108 and L109 typing sera and are classed as serogroup L108(29). The flaA and flaB genes amplified from the L108 and L108(29) strains possessed the same size as that seen with VC167 (Fig. 3A and B). To determine whether the primary structure of the flagellin genes was conserved within the L108 serogroup, the PCR products were digested with PstI and EcoRI, which cut within the central region of the VC167 flagellin genes (Fig. 1A). The sizes of the fragments based on the known sequences of the flagellin genes from VC167 (14, 16) are indicated schematically in Fig. 1C. Indeed, when the PCR products that represented the L108 and L108(29) flaA and flaB genes were digested, they displayed perfect conservation with the VC167-T2 control (Fig. 4A and B).

Polyorphism of the flaA gene of other LIO serogroups. By employing the pg50-RAA19 flaA primer pair, we examined the 34 Campylobacter strains from 13 other LIO serogroups listed in Table 1. Under the amplification conditions employed, all strains were shown to produce a product indistinguishable in size from the 1,448-bp product from VC167-T2 detected by agarose gel electrophoresis (data not shown). To determine the polymorphic groupings of these strains, the PCR products generated with the pg50-RAA19 primer pair were digested with PstI and EcoRI and analyzed on polyacrylamide gels.
The restriction profile of a representative strain from each \( L.\) \( I.\) serogroup is shown in Fig. 5. It can be seen that there is a wide range of primary structure polymorphism within the \( flaB\) genes from the various \( L.\) \( I.\) serotypes on the basis of digestion with these two enzymes, and 10 distinct group- phylic patterns generated by \( Psl\) and \( EcoR\) digestion of the PCR fragments generated with the pg50-RAA19 primer pair from the following strains: VC235 \( (L.055,\) lane 1), VC88 \( (L.05,\) lane 2), 81-116 \( (L.006,\) lane 3), VC83 \( (L.004,\) lane 4), VC94 \( (L.015,\) lane 5), VC87 \( (L.01,\) lane 6), VC104 \( (L.019,\) lane 7), VC92 \( (L.012,\) lane 8), VC97 \( (L.020,\) lane 9), VC167 \( (L.008,\) lane 10), VC74 \( (L.011,\) lane 12), VC230 \( (L.011,\) lane 13), VC322 \( (L.011,\) lane 14), VC91 \( (L.011,\) lane 15), VC168 \( (L.029,\) lane 16), VC236 \( (L.029,\) lane 17). For reference, the fragment sizes in lane 11 are (top to bottom) 739, 250, 232, and 202 bp.

Presence and polymorphism of \( flaB\) among \( L.\) \( I.\) serogroups. The \( flaB\) gene has previously been identified with certainty only in \( C.\) \( jejuni\) VC167 \( (L.008)\) and \( C.\) \( jejuni\) 81-116 \( (L.006)\) \( (27),\) although its presence in \( C.\) \( jejuni\) INI \( (L.007),\) VC208 \( (L.003),\) VC212 \( (L.006),\) and CL99 \( (L.010)\) has been suggested \( (2,\) 43). With the \( flaB\) primer pair \( (RAA9-RAA19)\) that selectively amplified the \( flaB\) gene in \( L.\) \( I.08\) isolates, the presence of PCR products of identical size was detected for all serotypes tested, confirming the presence of a second flagellin gene (Fig. 6A). The PCR product generated by amplification with RAA9-RAA19 was also digested with \( Psl\) and \( EcoR\) during the 41 \( C.\) \( jejuni\) strains for which a PCR product was generated with this primer pair, the restriction patterns obtained for 35 isolates belonging to all but two of the serotypes examined were identical to that seen for the corresponding \( flaA\) gene (summarized in Table 1). The restriction pattern of the \( flaB\) gene from the four \( L.\) \( I.06\) strains was the same as that of the \( flaB\) gene of the two \( L.\) \( I.05\) isolates examined and was different from the \( flaA\) patterns of both \( L.\) \( I.05\) and \( L.\) \( I.06\) strains (Fig. 6B).

The possibility existed that although the RAA9-RAA19 primer pair can specifically amplify the \( flaB\) gene in \( L.\) \( I.08,\) \( L.\) \( I.05,\) and \( L.\) \( I.06\) strains as determined directly by restriction fragment length polymorphism, these primers could be nonspecifically priming from the \( flaA\) gene in the other \( L.\) \( I.0\) serotypes for which the polymorphic pattern generated by these primers is identical to the pattern generated with the pg50-RAA19 primer pair. To eliminate this possibility, PCR amplification with both primer pairs was performed with the isogenic flagellin mutants from \( L.\) \( I.01,\) \( L.\) \( I.04,\) \( L.\) \( I.011,\) \( L.\) \( I.012,\) \( L.\) \( I.015,\) \( L.\) \( I.019,\) and \( L.\) \( I.20\) described previously \( (3).\) Amplification with the pg50-RAA19 primer pair generated fragments of 2.85 kb from each mutant (data not shown), which corresponded to an increase of 1.4 kb in size due to the presence of the Km' cassette in the \( flaA\) gene. Further, amplification with the RAA9-RAA19 primer pair generated wild-type-size fragments of approximately 1.45
The one exception in the case of \textit{C. jejuni} Z genes sequenced to date is in region V of the 0: restricting profile is conserved by isolates within a given Natural transformation may also be responsible for the *Z* c groups of thermophilic campylobacters. However, in all but part to continuous natural transformation and recombination among strains during epidemiologic evaluations (33). However, the application of individual leucine and serine nucleic acid polymorphism (33) demonstrated reasonably close relationships among separate epidemiologic-associated isolates of this frequently occurring serogroup.

This intragroup conservation of two flagellin genes is the first evidence for the existence of a class of discrete, stable clonal groupings within \textit{Campylobacter} spp., which appear to parallel the L10 serogroups. Although clonality exists in other pathogenic bacteria, with well-studied examples being salmonellae (8, 37), \textit{Neisseria meningitidis} (9, 29), \textit{Escherichia coli} (1, 38, 51, 52), and \textit{Listeria monocytogenes} (34), previous attempts to identify clonal groupings within \textit{Campylobacter} spp. by a variety of immunologic and genetic techniques have been generally unsuccessful. For example, restriction endonuclease analysis, multilocus enzyme electrophoresis, pulsed-field gel electrophoresis, and restriction fragment length polymorphism (RFLP) analysis with RNA or genes coding for RNA (ribotyping) have all been employed to examine chromosomal DNA and to attempt to detect minor alterations at the nucleotide level. Indeed, the combination of these sensitive techniques has proven to be useful when attempting to discriminate among strains during epidemiologic evaluations (33). However, the application of individual leucine and serine nucleic acid polymorphism (33) demonstrated reasonably close relationships among separate epidemiologic-associated isolates of this frequently occurring serogroup.

\textbf{TABLE 2. Sequence homologies of Campylobacter flagellin genes}

<table>
<thead>
<tr>
<th>Gene</th>
<th>% Homology* in region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>C. coli VC167-T2 flaA</td>
<td>100</td>
</tr>
<tr>
<td>C. coli VC167-T2 flaB</td>
<td>87.2</td>
</tr>
<tr>
<td>C. coli VC167-T1 flaA</td>
<td>100</td>
</tr>
<tr>
<td>C. coli VC167-T1 flaB</td>
<td>87.2</td>
</tr>
<tr>
<td>C. jejuni IN1 flaA</td>
<td>83.3</td>
</tr>
<tr>
<td>C. jejuni TGH9011 flaA</td>
<td>87.4</td>
</tr>
<tr>
<td>C. jejuni 81-116 flaA</td>
<td>86.7</td>
</tr>
<tr>
<td>C. jejuni 81-116 flaB</td>
<td>82.8</td>
</tr>
</tbody>
</table>

* Compared with the fla genes from \textit{C. jejuni} VC167-T2 (14).
* Regions I to V are as previously defined by Guerry et al. (15).

DISCUSSION

This study has provided further information on the primary structure of \textit{Campylobacter} flagellin genes, especially within the coding areas for the N-terminal and central domains of flagellin. Previous sequencing studies of \textit{Campylobacter} flagellin genes have shown that regardless of the LIO serogroup, the N-terminal region of the flagellin protein are relatively conserved, as indeed they are in the better-described enterobacterial flagellins compared with the central variable domain (Table 2). The one exception in the case of \textit{Campylobacter} flagellin genes sequenced to date is in region V of the flaA gene of \textit{C. jejuni} TGH9011, which displays only 71.5% identity to region V of the flaA gene of \textit{C. coli} VC167-T2 (14) (Table 2). However, the flaI gene encodes a flagellin with a deduced M, of 56,000, compared with the protein with an M, of 60,000 produced by \textit{C. coli} VC167. In this study, restriction analysis of specific PCR-generated fragments has demonstrated that the central region of the flaA gene is highly polymorphic among strains belonging to various heat-labile LIO serogroups of thermophilic campylobacters. However, in all but two serogroups examined, L101 and L102, the flaA restriction profile is conserved by isolates within a given serogroup.

Using a PCR with oligonucleotide primers shown with L108 strains to specifically amplify the flaB gene, we have also confirmed the presence of a second flagellin gene among \textit{C. jejuni} and \textit{C. coli} isolates representing 11 serogroups. This is the first study to examine either the distribution or the diversity of flaB. For all strains examined except those isolates belonging to the L105 and L106 serotypes, the polymorphic pattern of the secondary fla gene was identical to that of flaA, supporting previous data that demonstrated the high homology of the two flagellin genes (15), and leads us to believe that \textit{C. coli} and \textit{C. jejuni} strains uniformly possess two highly homologous flagellin genes. The reasons for the maintenance of two flagellin genes may be related to regulation by alternate promoters (5) and/or to ensure that motility is maintained (6).

This intragroup conservation of two flagellin genes is the first evidence for the existence of a class of discrete, stable clonal groupings within \textit{Campylobacter} spp., which appear to parallel the L10 serogroups. Although clonality exists in other pathogenic bacteria, with well-studied examples being salmonellae (8, 37), \textit{Neisseria meningitidis} (9, 29), \textit{Escherichia coli} (1, 38, 51, 52), and \textit{Listeria monocytogenes} (34), previous attempts to identify clonal groupings within \textit{Campylobacter} spp. by a variety of immunologic and genetic techniques have been generally unsuccessful. For example, restriction endonuclease analysis, multilocus enzyme electrophoresis, pulsed-field gel electrophoresis, and restriction fragment length polymorphism (RFLP) analysis with RNA or genes coding for RNA (ribotyping) have all been employed to examine chromosomal DNA and to attempt to detect minor alterations at the nucleotide level. Indeed, the combination of these sensitive techniques has proven to be useful when attempting to discriminate among strains during epidemiologic evaluations (33). However, the application of individual leucine and serine nucleic acid polymorphism (33) demonstrated reasonably close relationships among separate epidemiologic-associated isolates of this frequently occurring serogroup.

The conservation of the primary flagellin gene structure within heat-labile serogroups and the polymorphism among serogroups demonstrated by this study illustrate the N-terminal domain of the flagellin gene is highly conserved within serogroups. This finding is somewhat unexpected because the previously observed genetic diversity and apparent lack of clonal groupings suggested that the central domain would be one of the last regions of the flagellin genes to demonstrate conservation. The reason why the flagellin gene structure is so highly conserved within serogroups while the flagellin antigens are serospecific is not yet elucidated. However, flagellar antigens do appear to be serospecific and antigenically conserved within a given LIO serogroup (4, 17), a finding which is consistent with the conservation of primary flagellin gene structure within a serogroup. The high level of identity of the fla genes within a given strain may be due at least in part to continuous natural transformation and recombination of flagellin information released from neighboring cells. Natural transformation may also be responsible for the duplication of flagellin genes.

Not all of the serogroups examined displayed singular discrete lineage of their flagellin genes. For example, the two L1029 strains, both of which were distinct from the L108/ (29) isolates which agglutinate with both the L108 and the L1029 typing antisera, were classified into separate polymorphic groups. The presence of four distinct polymorphic patterns within isolates of the L1011 serogroup is intriguing. In a previous study, we were unable to isolate an isogenic nonflagellated mutant of VC91 after transformation with VC16783 genomic DNA in which both the flaA and flaB genes had been disrupted (3). These data, in conjunction with the nonconservation of the primary structure of L1011 flagellin genes demonstrated here, indicate that there may be an unusual arrangement of flagellin information in this serogroup and that this serogroup fails to demonstrate clonality. Importantly, however, in all the L1029 and L1011 isolates examined, the restriction profile of the flaB gene was identical to that of the corresponding flaA gene. This configuration of homologous fla genes appears to be preferred in \textit{Campylobacter} spp., because the only examples we observed which diverged from this pattern were isolates be-
ing to serogroups L105 and L106, whose flaA and flaB genes were in polymorphism groups 2 and 3, respectively (Table 1).

The subunit flagellin size of Campylobacter strains is generally large, ranging between 58 and 62 kDa (17), which indicates that the central domain, although shown here to be highly variable at the sequence level and thought to vary antigenically, may be under certain structural constraints. This is further reflected by the conservation in size of the cellular flagellin genes in highly variable at the sequence level and thought to vary in publication. It indicates that the central domain, although shown here to be promoter is subject to environmental regulation. Submitted for generally large, ranging between (Table 1). Variation in antigenicity and molecular weight of genes were in polymorphism groups 2 and 3, respectively. Mim, R. A., P. Guerry, M. E. Power, and T. F. Fischer, 1988. The flagellar filament protein. Can. Medical Research Council of Canada and by U.S. Navy Research 15:2-1-228.

This work was supported in part by a grant to T. J. T. from the National Science and Engineering Research Council of Canada. filament protein of Campylobacter jejuni. Annu. Rev. Genet. 11:161-182.

ACKNOWLEDGMENTS

We thank Corinne Gustafson and Paul O'Toole for valuable discussions.

This work was supported in part by a grant to T. J. T. from the Medical Research Council of Canada and by U.S. Navy Research and Development Command Research Work Unit no. 20. Joys, T. M. 1985. The covalent structure of the phase-1 flagellar protein. Can. J. Microbiol. 31:1115-1158.

We thank Dr. Corinne Gustafson and Paul O'Toole for valuable discussions. This work was supported in part by a grant to T. J. T. from the Medical Research Council of Canada and by U.S. Navy Research and Development Command Research Work Unit no. 20. Joys, T. M. 1985. The covalent structure of the phase-1 flagellar protein. Can. J. Microbiol. 31:1115-1158.


