Comparison of lipooligosaccharide biosynthesis genes of *Campylobacter jejuni* strains with varying abilities to colonize the chicken gut and to invade Caco-2 cells

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Campylobacter jejuni strains develop a high variability of lipooligosaccharide (LOS) structures on the cell surface based on variations in the genetic content of the LOS biosynthesis locus. While the importance of these variations for ganglioside mimicry as a critical factor in the triggering of Guillain–Barré syndrome has already been shown, little work has been done on the investigation of LOS structures and their function in the pathogenesis of gastrointestinal disease. In this study, the presence of several LOS genes in 40 *C. jejuni* strains with different abilities to colonize the chicken gut and to invade Caco-2 cells was investigated by PCR. Two genes, *cgtB* and *wlaN*, encoding putative β -1,3-galactosyltransferases were detected in most strongly invasive strains and rarely in non-invasive strains. A homopolymeric tract within the *wlaN* gene resulted in an intact gene product only in strongly invasive strains. The specific function of these genes during LOS biosynthesis is still unknown. *cgtB* and *wlaN* gene products are suggested to be involved in development of the colonization and invasion ability of *C. jejuni*. After a classification of the complete LOS loci, an association between a particular LOS class and colonization and invasion ability of the *C. jejuni* strain could not be detected. Lack of the *pglB* gene involved in protein glycosylation in one strain could be responsible for the weak colonization and invasion ability of this strain. There is some evidence that different genetic characteristics were responsible for strong or weak colonization and the invasion ability of *C. jejuni* strains.

Received 22 March 2007

Accepted 24 July 2007

INTRODUCTION

Campylobacter jejuni is the leading cause of bacterial food-borne diarrhoeal disease throughout the world (Blaser, 1997). The most common source of human infections is poultry products. The clinical spectrum of the disease ranges from asymptomatic infections to severe inflammatory bloody diarrhoea (Ketley, 1997). Furthermore, campylobacteriosis is also the most frequent antecedent infection before the onset of the post-infectious peripheral neuropathy Guillain–Barré syndrome (GBS; Nachamkin *et al.*, 1998). Structural similarity between human gangliosides and *C. jejuni* lipooligosaccharides (LOSs), the so-called ganglioside mimicry, is thought to be involved in the elicitation of GBS and the related Miller–Fisher syndrome.

The completion of the sequencing of the genomes of *C. jejuni* NCTC 11168 and RM1221 has enabled the rapid identification of a large cluster of genes involved in LOS biosynthesis (Parkhill *et al.*, 2000; Fouts *et al.*, 2005). The LOS biosynthesis locus is one of the highly variable regions in the *C. jejuni* genome as indicated by the results of both

DNA sequencing of these loci from different *C. jejuni* strains (Gilbert *et al.*, 2002; Guerry *et al.*, 2002) and microarray analysis (Dorrell *et al.*, 2001; Leonard *et al.*, 2004). The comparison of LOS biosynthesis loci from a variety of strains resulted in a grouping into eight LOS classes (A–H; Parker *et al.*, 2005), based on the organization of about 40 distinct genes. The authors were able to classify a majority (80 %) of the LOS biosynthesis loci from 123 *C. jejuni* strains utilizing PCR amplifications of genes from the cluster. The differences in the gene content of the LOS loci provide a basis for differences in LOS outer cores among *C. jejuni* strains. However, the same set of homologous genes is able to result in different LOS core structures. This is the result of multiple mechanisms that are used by *C. jejuni* to turn on or off a gene or to modulate the substrate specificities of the glycosyltransferases. Mechanisms responsible for the generation of variability in the LOS outer core structure include phase-variation because of homopolymeric tracts of DNA and gene inactivation by deletion or insertion of a single base.

While current research is focused on the role of LOS in the development of GBS, little attention has been placed on the

Abbreviations: GBS, Guillain–Barré syndrome; LOS, lipooligosaccharide.

function of LOS structures in the pathogenesis of gastrointestinal disease. There is some evidence that LOS structures are important for the invasion of *Campylobacter* into epithelial cells. A site-specific insertional mutant in the *cgtA* gene of *C. jejuni* strain 81-176, which encodes an *N*-acetylgalactosaminyltransferase, showed a significant more than twofold increase in invasion of INT407 cells, compared to wild-type (Guerry *et al.*, 2002). Variations in a homopolymeric tract of G-residues in the *cgtA* gene of *C. jejuni* strain 81-176 affected the length of the open reading frame, and these changes in *cgtA* expression were associated with changes of the LOS structure (Guerry *et al.*, 2002). Similar phase variations and corresponding changes of LOS structure have been found in another LOS biosynthesis gene, *wlaN* of *C. jejuni* strain NCTC 11186 (Linton *et al.*, 2000). We recently reported strain-specific differences in the occurrence of *cgtB* and *wlaN* genes, which both encode a β -1,3-galactosyltransferase, connected with different abilities to colonize the chick gut and to invade Caco-2 cells (Müller *et al.*, 2006). However, *cgtB* was mostly detectable only in strains with strong colonization and invasion abilities but was absent in some colonizing strains.

In this study, we compared the presence of LOS biosynthesis genes from a panel of *C. jejuni* strains with different abilities to colonize the chick gut and to invade Caco-2 cells. We used PCR probing with specific primers and DNA sequencing to determine differences in LOS gene content. The aim of the study was to determine whether specific classes of genes are associated with the chicken colonization potential, invasive properties for human epithelial cells or animal reservoirs.

METHODS

Bacterial isolates. Forty *C. jejuni* isolates used in this study were of human, bovine or turkey origin. The strains were cultivated on Müller–Hinton blood agar at 37 °C under microaerobic conditions (5% O₂, 10% CO₂, 85% N₂) for 24 h. All isolates were characterized by their invasion ability determined in a Caco-2 cell culture model and their colonization ability determined in a chick model as described in previous studies (Hänel *et al.*, 2004, 2007).

DNA preparation and PCR. Chromosomal DNA of the bacteria was extracted using the High Pure PCR Template Preparation kit (Roche Diagnostics). The target genes, primers and annealing temperatures are listed in Table 1. For the generation of PCR primers, the Primer Express software (Applied Biosystems) was used. PCR was performed in a DNA Thermal Cycler (Eppendorf) using standardized cycling parameters: 95 °C for 1 min for initial denaturation followed by 35 cycles of denaturation at 95 °C for 30 s, variable annealing temperature (Table 1) for 1 min, primer extension step at 72 °C for 1 min, and final extension step at 72 °C for 5 min. PCR products were analysed by electrophoresis on 1 or 2% agarose gels. DNA bands were stained with ethidium bromide and visualized under UV illumination using a Bio Imaging System (Syngene). The expected lengths of PCR products are shown in Table 1.

Sequencing of the *wlaN* gene. For sequencing of the *wlaN* gene homopolymeric tract, an approximately 435 bp intragenic fragment of the gene was amplified using genomic DNA and primer pair

Cj1139cF and DL39 (Table 1). After purification of the expected bands with a QIAquick Gel Extraction kit (Qiagen), cycle sequencing reactions were carried out using the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) according to the manufacturer's instructions. Sequencing primers were Cj1139cF and DL39 (see above), and analysis was performed on an ABI 310 genetic analyser (Applied Biosystems).

Analysis of the transcription of *cgtB* in strain 972/96. Bacterial cells that had adhered to and invaded Caco-2 cells were recovered from an invasion assay (Hänel *et al.*, 2004) after cell lysis by centrifugation and processed for RNA isolation using a RNeasy Protect Bacteria Mini kit (Qiagen) according to the manufacturer's instructions. The concentration of RNA was determined by measuring the A₂₆₀. One hundred and fifty nanograms of RNA was reverse-transcribed with random hexamer primers using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen). A real-time PCR was carried out using qPCR MasterMix Plus for SYBR Green I (Eurogentec) and a MX 3000P real-time PCR system (Stratagene Europe). Primers for real-time PCR were *cgtB*-q-F (5'-GTT TGT GAA AAA AAT TAT TGC AAA GAA-3') and *cgtB*-q-R (5'-TCG AGT CTT AAA CTC GCA AAA GC-3').

RESULTS AND DISCUSSION

An overview of the selected LOS genes and the PCR results in relation to colonization and invasion ability is shown in Table 2. In only 20% (3 of 15) of strains with no or weak colonization and invasion ability could the gene *cgtB* be detected (strains 972/96, 292/94 and 315/94), whereas it was found in 52% (13 of 25) of strongly invasive and colonizing strains. If one assumes that the β -1,3-galactosyltransferase encoded by *cgtB* (Gilbert *et al.*, 2000) is a characteristic enzyme of strongly invasive strains, then the role of another β -1,3-galactosyltransferase encoded by *wlaN* (Cj1139c) should also be investigated. The overall level of amino acid identity between the two sequences is 58.4% (Linton *et al.*, 2000). We found *wlaN* in 46.7% (7 of 15) of strains with no or weak colonization and invasion ability and in 60% (15 of 25) of strains with strong colonization and invasion ability. Linton *et al.* (2000) reported that the presence of an intragenic homopolymeric tract renders the expression of a functional *wlaN* gene product phase-variable, resulting in distinct cell populations with alternative LOS structures. Homopolymeric nucleotide tracts have been previously identified in the genome sequences of *C. jejuni* NCTC 11168 (Parkhill *et al.*, 2000) and RM 1221 (Fouts *et al.*, 2005). Investigations of several homopolymeric tracts in *C. jejuni* resulted in the hypothesis that the formation and/or selection of these tracts is not a random process, but is driven by as yet unknown mechanisms (Wassenaar *et al.*, 2002). The polymorphism of these genes may be a mechanism by which *C. jejuni* survives selection bottlenecks between opportunities for growth within a host. In this study, an intragenic homopolymeric tract was found with six, eight or nine G-residues within *wlaN* by DNA sequencing (Table 2, Fig. 1). Only eight G-residues allow the transcription of a functional galactosyltransferase, whereas six or nine G-residues lead to two open reading frames

Table 1. List of target genes, PCR primers and annealing temperatures

Target gene/ORF	Primers	Sequence	Annealing temp. (°C)	Amplicon length (bp)	Reference
<i>cgtB</i>	DL39	5'-TTA AGA GCA AGA TAT GAA GGT G-3'	56	561	Linton <i>et al.</i> (2000)
	<i>cgtB</i> Brev	5'-GCA CAT AGA GAA CGC TAC AA-3'			
<i>wlaN</i>	Cj1139cF	5'-TGC TGG GTA TAC AAA GGT TGT G-3'	60	330	Wassenaar <i>et al.</i> (2002)
	Cj1139cR or	5'-AAT TTT GGA TAT GGG TGG GG-3'			
	DL39	5'-TTA AGA GCA AGA TAT GAA GGT G-3'	60	435	See above
<i>galE</i>	<i>galE</i> 1	5'-CTT ATG GCG AAC CAC AAA CTC C-3'	60	555	This study
	<i>galE</i> 2	5'-TAG GCT GCC AAG AAG TAA GA-3'			
<i>neuB</i> -a	<i>neuB</i> -a-F	5'-ATT ATA GCC ATT TGC TCA CTT TG-3'	52	755	Parker <i>et al.</i> (2005)
	<i>neuB</i> -a-R	5'-AAA GCA CCC TTA GTC GTA CCT G-3'			
<i>cgtA</i> 2	<i>cgtA</i> 2 F	5'-CTG TGA TGA TGG GAG TGA AGA GC-3'	52	539	Parker <i>et al.</i> (2005)
	<i>cgtA</i> 2-R	5'-GGT AAT CGT TTC GGC GGT ATT-3'			
<i>orf17d</i>	<i>orf17d</i> -F	5'-TTG AAC AAC CTG CTT ATG AGC TTT AT-3'	52	438	Parker <i>et al.</i> (2005)
	<i>orf17d</i> -R	5'-TTT CTT TAG TAG ATC TTC CCA CGC-3'			
<i>orf19df</i>	<i>orf19df</i> -F	5'-AAA ATT TCC GTC ATA ATC CCA ATC T-3'	52	442	Parker <i>et al.</i> (2005)
	<i>orf19df</i> -R	5'-TAT CAG GTA AAT CTT GAA TGA TAA AGT CA-3'			
<i>wlaH</i>	<i>wlaH</i> -q-F	5'-GGG ATA TGA GTT TTG TTG GAC CTA G-3'	60	114	This study
	<i>wlaH</i> -q-R	5'-CCT GCG CCC ATC CTG TTA TAC-3'			
<i>wlaG</i>	<i>wlaG</i> -q-F	5'-CTC TGG CGC GGT GCA TTA T-3'	60	170	This study
	<i>wlaG</i> -q-R	5'-CCT CCA CAC AAC CTT CAC AAT CA-3'			
<i>pglB</i>	<i>pglB</i> f	5'-ACT TGG TGG GAT TAT GGT TAT CCT G-3'	60	312	This study
	<i>pglB</i> r	5'-TCA CGA GTT TTT GGT GTA TCG ATT TT-3'			
CJE1278	CJE1278-q-F	5'-CCG AAG ATA TGC TTG CGG TT-3'	60	118	This study
	CJE1278-q-R	5'-CGT CGC TTA GTA ATC GAA AAA GAA T-3'			
CJE1280	CJE1280-q-F	5'-AGA GCT AGA TAT GAA GGA GTT AAG GCA-3'	60	101	This study
	CJE1280-q-R	5'-CTC ACA CAC TCT TCA CAA GCA TTA AG-3'			
Cj1136	Cj1136-q-F	5'-GGG TCA AAA AAC AAG GAT GAA TGT A-3'	60	121	This study
	Cj1136-q-R	5'-AAC ACC CCA GCT AAA CGA GAT A-3'			
Cj1138	Cj1138-q-F	5'-CAT AGA AGA ATG TGT ACC TAG AAT GGA TG-3'	60	103	This study
	Cj1138-q-R	5'-ACG ACT ATG ATG CTT GAA ATA ACT TTT T-3'			
Cj1434c	Cj1434-q-F	5'-TAA TGT TTT GAG AAA TTC TTG TGA AGA T-3'	60	169	This study
	Cj1434-q-R	5'-ATA GGA AGC TCC TGG AGT TTC GT-3'			
Cj1438c	Cj1438-q-F	5'-TCG GGG ATG GTG ATA TGA AAA TA-3'	60	123	This study
	Cj1438-q-R	5'-TCC TTT CAG AGG CAC CAA AA-3'			
Cj1440c	Cj1440-q-F	5'-GGG AAT TAA ATT GCA TAG AAG AAT GTG T-3'	60	104	This study
	Cj1440-q-R	5'-CCA TTG TTT CTT AAA TCC TTC TTC AAA T-3'			

encoding two truncated hypothetical peptides. From the seven non- or weakly invasive strains with *wlaN* we found no strain with eight G-residues, six strains with six G-residues and one with nine G-residues. These results indicate that 80 % of strains with no or weak colonization and invasion ability have no functional β -1,3-galactosyltransferase encoded by *cgtB* or *wlaN*. However, we detected eight G-residues in the *wlaN* gene of four strongly invasive strains lacking *cgtB*. Our findings show that a functional β -1,3-galactosyltransferase encoded by *cgtB* or *wlaN* was detected in 68 % of strains with strong colonization and invasion ability.

Furthermore, in an analysis of transcriptional activity of *cgtB* in the non-invasive strain 972/96, we could not detect any mRNA of this gene by real-time PCR (data not shown). This indicates that unknown transcriptional

regulation processes or defects in expression of *cgtB* could be a putative cause for the lack of colonization and invasion ability in this strain. After sequencing the *cgtB* gene of non- and weakly invasive strains 972/96, 292/94 and 315/94, we detected a single nucleotide exchange from G to A at position 528 in strain 972/96 leading to an amino acid change from arginine to lysine at position 176. In strain 292/94, we detected two single nucleotide changes which do not result in amino acid changes, and in strain 315/94, nucleotide changes were not detectable in the *cgtB* locus.

In eight strains with strong colonization and invasion ability we could not detect intact β -1,3-galactosyltransferase genes of *cgtB* and *wlaN* by PCR.

After complete sequencing of *C. jejuni* NCTC 11168, besides *wlaN* (Cj1139c) seven other genes encoding

Table 2. PCR amplification results, LOS classification according to Parker *et al.* (2005), and sequencing results of the *wlaN* gene compared with invasion and colonization ability

ND, Not determined; NA, not applicable; unk, unknown; G-res, guanosine residues; +, positive PCR result/weak invasion/colonization; –, negative PCR result/no invasion/colonization; + + +, strong invasion/colonization.

Strain	Source	Invasion	Colonization	LOS classification									
				<i>cgtB</i>	<i>wlaN</i>	G-res (<i>wlaN</i>)	<i>neuB-a</i>	<i>cgtA2</i>	<i>orf17d</i>	<i>orf19df</i>	LOS class	<i>galE</i>	<i>pglB</i>
51/89	Human	–	–	–	–	NA	–	–	–	–	unk	+	+
81116	Human	–	–	–	+	6	+	–	–	–	E	+	+
C130	Bovine	–	–	–	–	NA	–	–	–	+	F	+	+
128/94	Human	–	–	–	+	6	–	–	–	–	C	+	+
129/94	Human	–	–	–	+	6	–	–	–	–	C	+	+
972/96	Bovine	–	–	+	+	6	+	–	–	+	unk	+	+
P1	Ref. str.	+	+	–	+	9	–	–	–	–	C	+	+
Z2	Unknown	+	+	–	–	NA	+	+	–	–	B2	+	+
292/94	Bovine	+	+	+	+	6	+	–	–	–	unk	+	–
315/94	Bovine	+	+	+	+	6	+	+	–	–	unk	+	+
av67/3	Turkey	+	ND	–	–	NA	–	–	–	–	unk	+	+
av356	Turkey	+	ND	–	–	NA	+	–	–	–	A2	+	+
av347	Turkey	+	ND	–	–	NA	+	–	–	–	A2	+	+
av355B	Turkey	+	ND	–	–	NA	+	–	–	–	A2	+	+
av64/3	Turkey	+	ND	–	–	NA	–	–	–	–	unk	+	+
81-176	Human	+++	+++	+	–	NA	+	+	–	–	B1	+	+
T313W	Bovine	+++	+++	+	–	NA	+	–	–	–	A1	+	+
340W	Bovine	+++	+++	–	+	6	–	–	–	+	unk	+	+
44/96	Human	+++	+++	–	–	NA	+	–	–	–	E	+	+
73/96	Bovine	+++	+++	–	+	6	+	–	–	+	unk	+	+
157/96	Human	+++	+++	+	–	NA	+	–	–	–	A1	+	+
158/96	Human	+++	+++	+	–	NA	+	–	–	–	A1	+	+
163/96	Human	+++	+++	+	–	NA	+	–	–	–	A1	+	+
164/96	Human	+++	+++	+	+	ND	+	–	–	–	unk	–	+
165/96	Human	+++	+++	+	–	NA	+	–	–	–	A1	+	+
167/96	Human	+++	+++	–	+	8	–	–	–	–	C	+	+
170/96	Human	+++	+++	–	+	8	–	–	–	–	C	–	+
216/04	Human	+++	+++	+	+	9	+	–	–	–	unk	+	+
277/94	Bovine	+++	+++	+	+	6	–	–	–	–	unk	+	+
288/94	Bovine	+++	+++	–	+	6	–	–	–	–	C	+	+
305/94	Bovine	+++	+++	–	+	6	–	–	–	–	C	+	+
320/94	Bovine	+++	+++	–	+	8	+	–	–	–	unk	+	+
av245	Turkey	+++	ND	–	+	8	–	–	–	–	C	+	+
av322B	Turkey	+++	ND	–	+	9	–	–	–	–	C	+	+
av324B	Turkey	+++	ND	–	+	9	–	–	–	–	C	+	+
av503	Turkey	+++	ND	+	–	NA	–	–	–	–	unk	+	+
av518	Turkey	+++	ND	+	+	9	–	–	–	–	unk	+	+
av618	Turkey	+++	ND	+	–	NA	+	+	–	–	B1	+	+
av519/1	Turkey	+++	ND	–	+	9	–	–	–	–	C	+	+
B/IV/509	Turkey	+++	ND	+	–	NA	+	–	–	–	A1	+	+

putative galactosyltransferases (*wlaH*, *wlaG*, Cj1136, Cj1138, Cj1434c, Cj1438c and Cj1440c) were annotated (Parkhill *et al.*, 2000). Sequencing of *C. jejuni* RM1221 resulted in the annotation of two putative galactosyltransferases (CJE1278 and CJE1280) with no similarity to genes of NCTC 11168. PCR detection of all these gene loci in 40 *C. jejuni* isolates used in this study indicated no correlation between the occurrence of the genes and the colonization and invasion

ability of the strains (data not shown). These putative galactosyltransferases were not able to compensate for the lack of *cgtB* and *wlaN* gene products in non-invasive strains.

Finally, the results suggest a correlation between the occurrence of a β -1,3-galactosyltransferase encoded by *cgtB* or *wlaN* in *C. jejuni* strains and their strong colonization and invasion ability *in vivo* and *in vitro*.

Fig. 1. Sequencing of the homopolymeric tract of *wlaN* (strain 129/94, six G-residues; strain 167/96, eight G-residues; strain 216/04, nine G-residues).

A UDP-glucose 4-epimerase of *C. jejuni* encoded by *galE* was shown to be involved in LOS synthesis and virulence (Fry *et al.*, 2000). A *galE* deletion mutant of *C. jejuni* 81116 showed a reduction in its ability to adhere to and invade INT407 cells, whereas it was still able to colonize chickens to the same level as the wild-type strain. This is in contrast to our findings of a good correlation between colonization of the chick gut and ability of the strain to invade Caco-2 cells (Hänel *et al.* 2004). Nevertheless, we checked the 40 *C. jejuni* strains used in this study with respect to the occurrence of the *galE* gene by PCR. We detected this gene in all non- or weakly invasive strains as well as in nearly all strongly invasive strains (Table 2). Additionally, transcriptional analysis of *galE* in three invasive and three non-invasive strains showed no differences in transcription activity between the strains (data not shown). From these results, it can be concluded that *galE* is not responsible for the different colonization and invasion abilities of the investigated *C. jejuni* strains.

The *C. jejuni* LOS loci have been grouped into eight classes (A–H) based on gene content and organization utilizing PCR of genes from these loci (Parker *et al.*, 2005). The detected LOS classes were not associated with the Penner serotypes but 14 of 16 GBS-associated isolates shared the same LOS class (A1). As *cgtB* and *wlaN* were among the genes used for the classification, a possible association of invasive and non-invasive strains with specific LOS classes was investigated. With additional primers targeting *neuB-a*, *cgtA2*, *orf17d* and *orf19df* (Table 1), a classification of the

majority (65 %) of LOS biosynthesis loci of 40 investigated *C. jejuni* strains became possible. In contrast to Parker *et al.* (2005) we found seven strains with concurrent detection of *cgtB* and *wlaN*. Furthermore, an association of invasive or non-invasive strains with a specific LOS class could not be detected.

An interesting correlation between LOS classes and source of the *C. jejuni* strains is shown in Table 3. Most of the human isolates possessed a class A1 or C locus whereas turkey isolates were positive for classes A2, C and unknown LOS classes. Most bovine isolates belonged to unknown LOS classes and a minority of them were positive for class C. These results are similar to the findings of Parker *et al.* (2005). LOS class C was detected in *C. jejuni* from all sources, suggesting a particular importance for the transmission of the bacteria from animals to humans. Moreover, classes A1 and B1 detected in turkey and human isolates are probably important for the transmission from poultry to humans. Further characterization of unknown LOS classes is necessary to determine the potential of bovine *C. jejuni* isolates to infect humans.

Another factor probably involved in colonization and invasion processes is *Campylobacter* protein glycosylation. *C. jejuni* 81-176 has been shown to contain a general protein glycosylation (*pgl*) system affecting many soluble and membrane-associated proteins (Szymanski *et al.*, 1999). Glycosylated flagellins of *Campylobacter* spp. have been characterized previously (Doig *et al.*, 1996; Thibault *et al.*, 2001). A *pglB* mutant of strain 81-176 encoding a putative oligosaccharyl transferase had reduced adhesion (38 %) and invasion (4.4 %) ability compared to the wild-type strain whereas growth rate and motility had not changed (Szymanski *et al.*, 2002). Furthermore, the glycoprotein VirB10 involved in a plasmid-encoded type IV secretion system could not be detected by Western blot analysis in the *pglB* mutant (Larsen *et al.*, 2004). In our study, we detected the *pglB* gene in all strains tested with one exception. The lack of *pglB* in strain 292/94 could be a reason for its weak colonization and invasion ability.

Table 3. Relationship between LOS classes and sources of the *C. jejuni* strains

Source	A1	A2	B1	C	E	F	Unknown
Turkey	1	3	1	4	0	0	4
Human	4	0	1	4	2	0	3
Bovine	0	0	0	2	0	1	7

However, in this strain we could not detect the Vir plasmid by PCR (data not shown). These results indicate additional functions of *pglB* beside glycosylation of VirB10.

This study describes the putative involvement of glycosyltransferases in the pathogenicity of *C. jejuni*. Glycosyltransferases participate in different cellular processes with high variability such as LOS biosynthesis and protein glycosylation. LOS and glycosylated flagellins are structures of the outer surface of *C. jejuni* and are involved in interactions with the host cells. The variability of these outer structures seems to be an important virulence factor enabling the bacteria to overcome several bottlenecks of their surroundings. Homopolymeric tracts in single genes have been suggested as a specific mechanism to generate such variability. Nevertheless, the complete network from a single gene to structures determining the pathogenicity still remains unknown. Further work with specific mutants and gene expression studies has to be done.

ACKNOWLEDGEMENTS

We thank Byrgit Hofmann, Kerstin Kluge, Peggy Methner and Karola Zmuda for excellent technical assistance.

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