The lipopolysaccharide biosynthesis locus of *Campylobacter jejuni* 81116

Ben N. Fry,^{1†} Victoria Korolik,² Janna A. ten Brinke,¹
Maarten T. T. Pennings,² Robert Zalm,¹ Bart J. J. Teunis,¹
Peter J. Coloe² and Bernard A. M. van der Zeijst^{1‡}

Author for correspondence: Bernard A. M. van der Zeijst. Tel: +31 30 274 41 64. Fax: +31 30 274 44 39.
e-mail: ben.van.der.zeijst@rivm.nl

¹ Department of Bacteriology, Institute of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands

² Department of Applied Biology, RMIT GPO Box 2476V, Melbourne 3001, Australia

Most *Campylobacter jejuni* strains express lipo-oligosaccharides. Some strains also express lipopolysaccharides (LPS), with O-antigen-like carbohydrate repeats. *C. jejuni* 81116 expresses an LPS containing both lipo-oligosaccharides and O-antigen-like repeats, but nothing is known about the structure or sugar composition of these LPS species. A cosmid library of the genome of *C. jejuni* 81116 was constructed and probed with *Campylobacter hyoilei* genes involved in LPS synthesis. Five cosmids hybridized with the probe and two of these expressed *C. jejuni* 81116 LPS in *Escherichia coli*. By subcloning, a 16 kb DNA region was identified which contains the genetic information required to express *C. jejuni* LPS. DNA sequence analysis revealed 11 ORFs homologous to genes involved in LPS synthesis of other bacteria. They consisted of three homologues of sugar biosynthesis genes, two homologues of transport genes and six homologues of sugar transferases.

Keywords: *Campylobacter jejuni*, *rfb* gene cluster, lipo-oligosaccharide (LOS), lipopolysaccharide (LPS), O-antigen

INTRODUCTION

Campylobacter jejuni is an agent causing human enterocolitis and is the most common cause of bacterial diarrhoea in many countries (Tauxe, 1992). Symptoms most frequently seen are acute abdominal pain and inflammatory diarrhoea, often with fever (Butzler & Skirrow, 1979). *C. jejuni* is also a commensal in the intestine of birds (Blaser & Reller, 1981; Stern *et al.*, 1988), which probably explains why it grows well at 42 °C, the body temperature of birds.

Lipopolysaccharides (LPS) are an abundant surface component of the outer membrane of Gram-negative bacteria. The LPS molecule consists of three distinct regions. Anchored in the outer membrane is the lipid A moiety, which is the endotoxic part of the LPS molecule.

Attached to the lipid A is the core, a branched-chain oligosaccharide linked to a ketodeoxy octulosonic acid (KDO) molecule, which has an inner and an outer part. Specific for the inner core is the presence of KDO and heptose residues, which are not found in the outer core. Extending from the cell surface is the O-antigen, a repeat of 10–30 oligosaccharides composed of 1–5 sugar residues (Schnaitman & Klena, 1993). Bacterial O-antigen protects the organism against complement and other serum components (Hackett *et al.*, 1987; Joiner *et al.*, 1986) and is involved in resistance to microbicidal human polymorphonuclear leukocytes (Stinavage *et al.*, 1989).

A variant of the LPS molecule, seen in bacteria like *Neisseria* spp., *Haemophilus* spp. and *Bordetella* spp., is the lipo-oligosaccharide (LOS) molecule (Inzana *et al.*, 1985; Jennings *et al.*, 1980; Ray *et al.*, 1991), which lacks the O-antigen polymer. *C. jejuni* strains synthesize LOS molecules. In addition, some strains also possess polysaccharide repeats (Preston & Penner, 1987). In this paper, the term LPS will be used to indicate both saccharide structures of *Campylobacter* (LOS or LPS containing additional polysaccharide repeats). The LPS molecules of *Campylobacter* have been shown to have endotoxic properties (Branquinho *et al.*, 1983; Naess &

[†] **Present address:** Department of Applied Biology and Biotechnology, Royal Melbourne Institute of Technology, 124 LaTrobe St, Melbourne, VIC 3000, Australia.

[‡] **Present address:** National Institute of Public Health and the Environment, Vaccine Division, PO Box 1, 3720 Bilthoven, The Netherlands.

Abbreviations: GalE, UDPgalactose 4-epimerase; KDO, ketodeoxy octulosonic acid; LOS, lipo-oligosaccharide; LPS, lipopolysaccharide.

The EMBL accession number for the complete 16 kb sequence reported in this paper is Y11648.

 

“

 

Table 1. Bacterial strains and plasmids used in this work

Strain or plasmid	Description	Reference
<i>C. jejuni</i> strains		
81116	Penner 6 serotype	Palmer <i>et al.</i> (1983)
<i>E. coli</i> strains		
HB101	$\Delta(gpt-proA)62 leuB6 thi-1 lacY1 hsdS_{B20} recA rpsL20 (Str^R) ara-14 galK2 xyl-5 mtl-1 subE44 mcrB_B$	Boyer & Roulland-Dussoix (1969)
DH5 α	F'/endA1 hsdR17($r_{K^+} m_{K^+}^+$) subE44 thi-1 recA1 gyrA (Nal ^R) relA1 $\Delta(lacZYA-argF)U169 (\phi 80 lacZ \Delta M15)$	Hanahan (1983)
S ϕ 874	K-12 $\Delta(sbcB-rfb)86 lacZ4053 trp-355 upp-12 relA1 rpsL150$	Neuhard & Thomassen (1976)
O111		Bray (1945)
Plasmids		
pBR322	Cloning vector	Bolivar <i>et al.</i> (1977)
pBl	Sequencing vector pBluescript KS M13 +	Short <i>et al.</i> (1988)
pUC18	Sequencing vector	Yanisch-Perron <i>et al.</i> (1985)
pLA2917	Cosmid vector	Allen & Hanson (1985)
pBT9502	pLA2917 containing a 25 kb fragment from <i>C. jejuni</i> 81116	This study
pBT9503	pLA2917 containing a 30 kb fragment from <i>C. jejuni</i> 81116	This study
pBT9504	pLA2917 containing a 29 kb fragment from <i>C. jejuni</i> 81116	This study
pBT9505	pLA2917 containing a 27 kb fragment from <i>C. jejuni</i> 81116	This study
pBT9506	pLA2917 containing a 25 kb fragment from <i>C. jejuni</i> 81116	This study
pBTLPS	Subclone (<i>Bgl</i> II) of pBT9502 containing a 16 kb fragment in pBR322	This study
pBF81	7 kb <i>Clal</i> fragment from pBT9502 in pBl	This study
pBF84	3.2 kb <i>Bgl</i> II fragment from pBT9502 in pBl	This study
pBF92	4.5 kb <i>Bgl</i> II fragment from pBT9502 in pBl	This study
pBF71	3 kb <i>Sau</i> 3A fragment from pBT9502 in pUC18	This study
pBF77	1.4 kb <i>Hind</i> III fragment from pBT9502 in pBl	This study

transduced into the host *E. coli* HB101 as recommended by the manufacturers. Of the resulting transformants, 600 were taken to assemble the genomic library, ensuring that the complete *C. jejuni* genome of 1600 kb was present.

Library screening by Southern blot analysis. The library was plated onto LB plates and grown overnight at 37 °C. The bacteria were transferred to nylon membranes (Hybond-N; Amersham) and lysed on Whatman 3 MM paper saturated with 2 × SSPE, 5 % SDS. The DNA was fixed by microwaving the filters for 2.5 min at 650 W. The filters were prehybridized for 2 h in hybridization mix (6 × SSPE, 5 × Denhardt's, 0.5 % SDS) with 0.1 mg herring sperm DNA ml⁻¹ at 60 °C. The insert from pBT9105 (pBR322 with an 11.8 kb *Eco*RV fragment from *Campylobacter hyoilei* RMIT-32A containing several genes showing homology with genes involved in LPS synthesis, including a *rfbP*-like gene; Korolik *et al.*, 1997) was labelled with ³²P using a random-priming labelling kit (Amersham). After boiling the probe for 3 min, it was added to the filters in the hybridization mix and hybridized overnight at 60 °C. The filters were washed twice with 2 × SSPE, 0.1 % SDS for 15 min at 60 °C and exposed to Fuji RX film overnight.

Subcloning of the *C. jejuni* LPS coding region. Recombinant cosmid pBT9502 DNA was partially cleaved with *Bgl*III. The resulting fragments were subcloned into *Bam*HI-cleaved, alkaline-phosphatase-treated plasmid vector pBR322 (Bolivar *et al.*, 1977) and transformed into *E. coli* HB101.

DNA sequence analysis. The sequence of the cloned DNA was determined by the dideoxy chain-termination method (Sanger *et al.*, 1977) with an Automated laser fluorescent DNA sequencer (Pharmacia), the autoread sequencing kit using T7

DNA polymerase (Pharmacia) and fluorescein-labelled nucleotide primers (Pharmacia). PC/GENE 6.70 (Korn & Queen, 1984) was used to analyse nucleotide and amino acid sequences, which were compared to databases available at GenomeNet using the BLAST program (Altschul *et al.*, 1990). The Macaw program (Lawrence *et al.*, 1993) was used for multiple sequence alignment.

LPS isolation, PAGE, silver staining and immunoblotting. Cell envelopes were isolated following the procedure of Lugtenberg *et al.* (1975) and treated with proteinase K for 1 h at 56 °C. The isolated LPS was resolved by Tricine-SDS-PAGE (Lesse *et al.*, 1990; Schagger & von Jagow, 1987) and analysed by silver staining (Tsai & Frasch, 1982) and Western blotting (Sambrook *et al.*, 1982). Penner 6 antiserum was used in immunoblotting diluted 1:1000 in PBS containing 5 % skimmed milk and 0.3 % Tween 20. Goat anti-rabbit alkaline-phosphatase-conjugated immunoglobulins (Promega) were used as the second antibody diluted 1:5000 in PBS containing 5 % skimmed milk and 0.3 % Tween 20. The bound phosphatase was visualized with NBT/BCIP (nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate). The Multi-mark multi-coloured standard (Novel Experimental Technology) was used as a molecular-mass marker.

RESULTS

Isolation of the DNA region involved in LPS synthesis

A genomic cosmid library of *C. jejuni* 81116 (Newell *et al.*, 1985; Palmer *et al.*, 1983) was constructed and screened by Southern hybridization with the *rfbP* gene

Fig. 2. Restriction map of the genomic region of *C. jejuni* 81116 encoding the synthesis of O-antigen. The inserts of cosmids pBT9502, pBT9505 and pBT9506 are shown as bars. The insert of plasmid pBTLPS is also shown. Black bars indicate clones that express O-antigen in *E. coli*. C and B are abbreviations for *ClaI* and *BglII* respectively.

from *C. hyoilei* (Korolik *et al.*, 1997). This gene is homologous to genes involved in transferring the first sugar of the O-antigen subunit to the lipid precursor undecaprenol phosphate. Five hybridizing clones were isolated. The cosmids contained in these strains were designated pBT9502–pBT9506 (Table 1). Restriction analysis showed that pBT9502, pBT9505 and pBT9506 contained overlapping inserts (Fig. 2). The three clones carrying these overlapping cosmids were tested for expression of *C. jejuni* LPS antigens by colony blotting. Both *E. coli*/pBT9502 and *E. coli*/pBT9506 reacted with Penner 6 antiserum in a Western blot.

LPS analysis

The silver-stained *C. jejuni* 81116 LPS showed one band of 6 kDa after separation by Tricine-SDS-PAGE. This size is in accordance with LPS isolated from other *Campylobacter* strains. Using the Tricine-SDS-PAGE system allows the use of protein markers to estimate the molecular mass of LPS components (Lesse *et al.*, 1990), as opposed to the standard glycine-buffer system in which the LPS components do not migrate as a function of their molecular masses. Moreover, aggregates were observed for *C. jejuni* 81116 LPS when the standard glycine-SDS-PAGE method of Laemmli (1970) was used (data not shown); this was never seen using the Tricine-SDS-PAGE method.

The *E. coli* strains carrying recombinant cosmids showed different LPS profiles when stained with silver. In the control lane, *E. coli*/pLA2917, three bands around 5.5 kDa and two bands around 4 kDa were visible. *E. coli*/pBT9505 LPS demonstrated the same pattern (data not shown). *E. coli*/pBT9502 and *E. coli*/pBT9506 LPS showed identical patterns with additional bands of 4.5, 6.5, 7.5 and 8 kDa compared to *E. coli*/pLA2917. The LPS from *E. coli*/pBT9502 is shown in Fig. 3. The changed LPS pattern shows that the cloned DNA region is involved in LPS synthesis.

Western blotting analysis using Penner 6 antiserum demonstrated that *C. jejuni* 81116 LPS is of the smooth type, with a high-molecular-mass O-antigen-specific ladder pattern between 6 and 40 kDa. LPS isolated from *E. coli*/pBT9502 and *E. coli*/pBT9506 (not shown) gave

Fig. 3. LPS profiles in Tricine-SDS-PAGE. (a) Silver-stained samples. (b) Western blots reacted with Penner 6 antiserum. Lanes: 1, *E. coli* O111; 2, *C. jejuni* 81116; 3, *E. coli* HB101(pBT9502); 4, *E. coli* HB101(pLA2917); 5, *E. coli* HB101(pBTLPS).

identical profiles, i.e. an O-antigen ladder pattern with immunoreactive bands between 10 and 45 kDa. This complex appeared to have a higher molecular mass than that of *C. jejuni* 81116 LPS. This may be explained by the fact that the lipid A-core complex of *E. coli* is used as a carrier for the *C. jejuni* O-antigen subunits. As expected, the *C. jejuni* O-antigen expressed in *E. coli* does not stain with silver. LPS prepared from *E. coli*/pBT9505 did not react with Penner 6 antiserum (data not shown).

Further definition of the LPS-expressing DNA region

The smallest insert still able to encode the complete ladder structure reacting with Penner 6 antiserum was 16 kb; it was subcloned into the pBR322 plasmid vector. The plasmid containing this insert was named pBTLPS (Figs 2 and 3). To exclude possible complementation of the *E. coli* HB101 LPS genes by the *C. jejuni* genes, pBT9502 and pBTLPS were also expressed in *E. coli* Sϕ874, a strain lacking all genes involved in O-antigen synthesis. LPS isolated from these Sϕ874 strains showed the same O-antigen ladder pattern reacting with Penner 6 antiserum (data not shown).

DNA sequence analysis

Fragments of the insert of plasmid pBTLPS, generated by the restriction endonucleases *BglII*, *ClaI*, *HindIII* and *Sau3AI*, were subcloned into sequencing vectors. Nested deletions and subcloning of these fragments allowed the

Fig. 4. The 16 kb region containing the *wla* cluster (open arrows) and flanking genes of *C. jejuni* 81116. The *waaC* and *orf1* ORFs are incomplete on this fragment.

Table 2. Characteristics of the found ORFs

ORF	Translation start*	Spacing†	Position (nt)	Size (aa)	Molecular mass (kDa)	pI	G + C (mol %)
<i>waaC</i> ‡	aatattAGGcttttaATGaaa	5	254–1	> 84	> 9.7	9.8	27.4
<i>galE</i>	gaaaAAGGAatgatgATGaaa	6	1169–2155	328	36.8	5.9	34.4
<i>wlaB</i>	tgattGGGAaaaacaGTGcta	6	2149–3843	564	64.5	9.2	28.4
<i>wlaC</i>	taAAGAGGAgaaatgATGaaa	6	3843–4919	358	41.1	8.4	30.5
<i>wlaD</i>	tagtcGAGGTtaaaaATGcct	5	4912–5841	309	35.5	9.1	29.7
<i>wlaE</i>	aattcgGGTGgctttATGcaa	5	5829–6926	365	41.3	8.7	29.2
<i>wlaF</i>	ttattAGGAtaaaagATGttg	6	6930–9071	713	82.2	8.3	29.7
<i>wlaG</i>	attTAAGGGttgaaaATGaga	6	9081–10211	376	42.9	9.2	30.0
<i>wlaH</i>	atAGGGtaattaagaATGtat	9	10204–10806	200	23.1	9.7	32.5
<i>wlaI</i>	taagcaAAGAaggccATGtta	5	10772–11383	203	22.1	9.0	31.7
<i>wlaK</i>	tacaAAGGAtaaatcATGaga	6	11481–12641	386	43.8	6.2	30.7
<i>wlaL</i>	gcataAAGGcttaaaATGatt	6	12643–14415	590	66.7	7.8	30.4
<i>wlaM</i>	taaagAAGGcatataATGtat	5	14415–15308	297	33.9	6.9	29.9
<i>cheY</i>	tgaaAGGAcgaaattGTGaaa	7	15421–15813	130	14.4	5.0	34.4
<i>orf1</i> ‡	tttttAAAAtcttaaATGcaa	6	15836–16293	> 152	> 18.0	6.5	28.2

* Potential Shine–Dalgarno sequences and start codons (bold) are capitalized.

† Spacing between Shine–Dalgarno sequence and start codon.

‡ Incompletely characterized ORF.

sequencing of the complete 16 kb region. To demonstrate continuity of adjacent but non-overlapping fragments, specifically primed sequences were generated.

Sequence analysis of the insert from pBTLPS showed that it contains 13 complete and two incomplete ORFs (Fig. 4; Table 2). Twelve ORFs showed homology to genes involved in LPS synthesis (Table 2) and were named *galE* and *wlaB*–*wlaM* (*wla* genes), according to the newly proposed bacterial polysaccharide gene nomenclature (Reeves *et al.*, 1996). The remaining complete ORF was that of the chemotaxis gene *cheY* from *C. jejuni*, which is not thought to be involved in LPS synthesis. The incomplete ORF at the 5' terminus was similar to *waaC* genes and was therefore named *waaC*. At the 3' end, an incomplete ORF without homology was found. *waaC* is transcribed in the opposite direction to the other genes (Fig. 4). Not all the genes start with ATG codons preceded by typical Shine–Dalgarno sequences. *wlaB* and *cheY* have the putative start codon GUG (Table 2); to our knowledge, this is the first time this start codon has been suggested for a *Campylobacter* gene. As only a few *Campylobacter* genes have been identified, not much is known about the promoter consensus sequences. Using the *E. coli* σ^{70} promoter consensus sequence, weak putative promoter sites were recognized along the sequence, which were located

upstream of the *waaC*, the *galE* and the *cheY* genes. *galE* and *wlaB*–*wlaM* are very closely spaced, with most of the designated ORFs showing overlap (Table 2). The largest spacing between two ORFs (97 bp) was found between genes *wlaI* and *wlaK*. No putative promoter sequences were found within this spacing. All these genes have the same transcriptional direction and therefore it is likely they are transcribed as a single operon. No putative *rho*-independent terminator-like hairpin loops were found. All ORFs possessed a G + C content between 28 and 34 mol % (Table 2), which is consistent with the low G + C content of *Campylobacter* spp. of 30 mol %.

The characteristics of the genes within the analysed region will be discussed in the order in which they are present, starting with *waaC*. The BLAST algorithm (Altschul *et al.*, 1990) was used to find proteins homologous to the deduced gene products (Table 3).

***waaC*.** The deduced partial protein sequence is homologous to the N-terminal regions of LPS heptosyl-transferases -I, which transfer a heptose residue to the KDO molecule, initiating the synthesis of the inner core (Ray & Raetz, 1987). At least four other genes adjacent to *waaC* are similar to genes involved in inner core synthesis (data not shown). Therefore, *waaC* may be the first of a gene cluster involved in inner core synthesis.

Table 3. Deduced proteins present in the *C. jejuni* *wla* region and their homologues

<i>C. jejuni</i> protein	Similar protein	Organism	Function	Identity/similarity (%)*	Accession no.
WaaC†	RfaC	<i>Escherichia coli</i>	Heptosyltransferase I	28.6/51.2	P24173
	RfaC	<i>Neisseria gonorrhoeae</i>	Heptosyltransferase I	28.6/51.2	S60783
	RfaC	<i>Bordetella pertussis</i>	Heptosyltransferase I	28.6/48.8	X90711
	RfaC	<i>Salmonella typhimurium</i>	Heptosyltransferase I	26.2/52.4	P26469
GalE	GalE	<i>Haemophilus influenzae</i>	UDPglucose 4-epimerase	37.5/52.4	A64063
	GalE	<i>Neisseria meningitidis</i>	UDPglucose 4-epimerase	36.3/53.1	S39638
	GalE	<i>Escherichia coli</i>	UDPglucose 4-epimerase	36.0/52.2	P09147
WlaB	HetA	<i>Anabaena</i> sp.	Polysaccharide involvement	30.1/48.7	P22638
	HlyB	<i>Escherichia coli</i>	Export haemolysin	30.1/46.2	P08716
	HlyB	<i>Actinobacillus pleuropneumoniae</i>	Export haemolysin	27.5/44.0	P26760
	MsbA	<i>Escherichia coli</i>	Export†	24.3/43.3	P27299
	ValA	<i>Francisella novicida</i>	Secretion LPS	23.4/41.5	L17003
WlaC	TrsD	<i>Yersinia enterocolitica</i>	Glycosyltransferase‡	28.8/47.0	S51263
	AmsD	<i>Erwinia amylovora</i>	Glycosyltransferase‡	27.4/44.8	S52144
	EpsG	<i>Streptococcus thermophilus</i>	Glycosyltransferase‡	26.8/40.8	U40830
WlaD	Orf8.7	<i>Yersinia pseudotuberculosis</i>	Abequosyltransferase‡	27.2/38.9	L01777
	TrsB	<i>Yersinia enterocolitica</i>	Glycosyltransferase‡	23.3/42.4	S51261
	EpsI	<i>Streptococcus thermophilus</i>	Glycosyltransferase‡	23.0/38.8	U40830
	SpsA	<i>Bacillus subtilis</i>	Polysaccharide synthesis	22.2/41.0	P39621
WlaE	AmsD	<i>Erwinia amylovora</i>	Glycosyltransferase‡	25.1/41.3	S52144
	TrsD	<i>Yersinia enterocolitica</i>	Glycosyltransferase‡	24.9/40.5	S51263
	TrsE	<i>Yersinia enterocolitica</i>	Glycosyltransferase‡	25.6/40.1	S51264
	CapH	<i>Staphylococcus aureus</i>	Glycosyltransferase‡	23.1/37.8	U10927
WlaF	STT-3	<i>Caenorhabditis elegans</i>	Oligosaccharyltransferase	19.5/35.9	P46975
	Wzy (Rfc)	<i>Escherichia coli</i>	O-antigen polymerase	18.8/39.7	P37748
	STT3	<i>Saccharomyces cerevisiae</i>	Oligosaccharyltransferase	16.3/34.5	JC4355
WlaG	CpsF	<i>Proteus mirabilis</i>	Glycosyltransferase‡	30.8/48.1	L36873
	RfbF	<i>Serratia marescens</i>	Galactantransferase‡	27.1/41.2	L34167
	RfpB	<i>Shigella dysenteriae</i>	Galactosyltransferase	26.6/44.7	S27671
	RfbF	<i>Klebsiella pneumoniae</i>	Galactantransferase	26.1/41.5	L31762
WlaH	WbaP (RfbP)	<i>Salmonella enterica</i>	Galactosyltransferase first step	39.5/55.5	P26406
	WbaP (RfbP)	<i>Haemophilus influenzae</i>	Galactosyltransferase‡	38.5/53.0	B64099
	EpsE	<i>Streptococcus thermophilus</i>	Galactosyltransferase first step	36.0/53.0	U40830
	ExoY	<i>Rhizobium</i> sp.	Galactosyltransferase first step‡	36.0/52.0	X16704
WlaI	NeuD	<i>Escherichia coli</i>	Polysialic acid capsule synthesis	28.1/45.8	U05248
	LpxD	<i>Salmonella enterica</i>	Acyltransferase	26.6/44.3	P18482
	LpxD	<i>Yersinia enterocolitica</i>	Acyltransferase	26.1/42.0	P32203
	LpxD	<i>Escherichia coli</i>	Acyltransferase	25.1/43.8	P21645
WlaK	RfbE	<i>Vibrio cholerae</i>	Perosamine synthetase‡	32.2/51.2	S28471
	DegT	<i>Bacillus stearothermophilus</i>	Transamination	29.0/44.4	P15263
	FlaA2	<i>Caulobacter crescentus</i>	Motility/polysaccharide	25.9/44.0	U27301
	SpsC	<i>Bacillus subtilis</i>	Polysaccharide synthesis	25.4/43.0	P39623
WlaL	CapD	<i>Staphylococcus aureus</i>	Capsule synthesis	38.5/55.6	P39853
	TrsG	<i>Yersinia enterocolitica</i>	Acetylglactosamine synthesis‡	37.5/51.7	S51266
	WpbM	<i>Pseudomonas aeruginosa</i>	LPS B-band synthesis	34.4/53.2	U44089
WlaM	AcfB	<i>Vibrio cholerae</i>	Accessory colonization factor	26.9/48.2	L25660
CheY	CheY	<i>Campylobacter jejuni</i>	Chemotaxis regulator	100	U75208
	CheY	<i>Pseudomonas aeruginosa</i>	Chemotaxis regulator	48.4/65.3	S20545
	CheY	<i>Escherichia coli</i>	Chemotaxis regulator	45.3/57.8	P06143
	CheY	<i>Salmonella typhimurium</i>	Chemotaxis regulator	45.3/57.8	P06657
Orf1†	—	—	—	—	—

* The program PALIGN from PCGene (Korn & Queen, 1984) was used to compare the deduced gene products. Open gap cost: 6. Unit gap cost: 2. Percentage similarities represent the sum of identical amino acids (%) plus conserved substitutions (%).

† Incomplete.

‡ Putative function.

galE. The protein encoded by *galE* is homologous to UDPgalactose 4-epimerase (GalE), an enzyme catalysing the interconversion between UDPGlc and UDPGal. Gal

is a component of the LPS core structure of *E. coli* and *Salmonella typhimurium*. Defects in *galE* result in a rough-LPS phenotype (Rick, 1987). In *Neisseria mening-*

WlaB-Cj	KYLKLNLNLIKKEKIAFIESSGCSHVLVDLIGLLKPKESQILIDKQELNASNAKNYFQKIGYIPQNIYLFNDSIAKN	442
HetA-An	NLVNLTITLTIKERTTALVSGAGHITLADLIPRFYDPTESQILVQGLDVQYFEINSLRRKMVVSQDTFIFNTSIRDN	457
ValA-Fn	HKVLSGVSVDIKAGQTVAFVTKGCSHITLTSISRFRYDQHKESILLDQVDTRELTLENLRSHLSIVSQNVHFLDDTVYNN	422
MsbA-Ec	VPALRNINLKIPASKTVALVRECSHESIASLITRFYDIDESEILMDGHDLREYTLASLRNQVALVSONVHFLNDTVANN	436
HlyB-Ec	PVILDNINLSIKQSEVIGIVRECSHESLTKLQRFYIPENESQVILDGHDALADPNWLRQVGVVLDQNVLLNRSIIDN	562
HlyB-Ac	PVILNDVNLSIQQSEVIGIVRECSHESLTKLQRFYIPENESQVILDGHDALADPNWLRQVGVVLDQNVLLNRSIRDN	562
	G----GKS A-----T	
WlaB-Cj	ITFGD--AVDEEKLNVKVIQANLEHFIKNLPQVQIKVSDGSSNLSSGGKQRIATARALYLEPEILVLDEATSALDTSSEA	521
HetA-An	IAYGT-SGASEABIREVARLANALQIEMPEPDIKLDPRVRLSSGGGRRIATARALLRDPEILILDEATSALDSVSESR	537
ValA-Fn	IAPGLSREVSSEVIDALKRANAYEVQELSDSIHININNNSSKLSGGGRRIATARALLKNAPVILFDEATSALDNESER	503
MsbA-Ec	IAYARTEQYSREQIEBAARMAYAMDFINKMDNLDIVISENVLSGGGRQRIATARALLRDSPIILFDEATSALDTESESR	517
HlyB-Ec	ISLAN-PGMSVEKVIYAAGLAGHDSISELRECYNIIVSEQAGLSGGGRQRIATARALLVNNPKILFDEATSALDYESEH	642
HlyB-Ac	IALAD-PGMPMEKIVHAAGLAGHDSISELRECYNIIVSEQAGLSGGGRQRIATARALLVNNPKILFDEATSALDYESEH	642
	182Gxxx34x56	
WlaB-Cj	KIMDEIYKISKDKIMIIIAHRLSTITQCKVYRLEHAKLKEEK-----	564
HetA-An	LIQESIEKLSVGRIVIAIAHRLSTIAKADKVVVMEQSRIVEQGNYSQELIQRGKLWkyhqmheagqtms	607
ValA-Fn	VVQQALESILTESCITIVIAHRLSTIVENASKIVVMDSKVVESGKHQELIEGGGLYtgseinrdfnetyar-	572
MsbA-Ec	AIQAALDELQKNRISLVIAHRLSTIEKADSIIVVEDSVIVERGTHNDLIEHRGVYaqihkmqfgq----	582
HlyB-Ec	VIMRNMHKICKGRIVIIIAHRLSTIVKNADRIIVMEKQKIVEQGHKELISEPESLYsylyqlqsd----	707
HlyB-Ac	IIMRNMHQICKGRIVIIIAHRLSTIVKNADRIIVMEKQKIVEQGHKELIADPNGLYhyhlqlqse----	707

Fig. 5. Alignment of the C-terminal part of WlaB with homologous proteins. Identical amino acids within all six sequences are highlighted. The ATP-binding site motif and the ABC-transporter family signature (underlined) are presented below the alignment. When multiple amino acids are possible in one of the positions of the ABC-transporter family signature, numbers are inserted: 1 (L, I, V, M, F or Y); 2 (S, A or G); 3 (R, K or A); 4 (L, I, V, M, Y or F); 5 (L, I, V, M or F); 6 (S, A or G). Cj, *C. jejuni*; An, *Anabaena* sp.; Fn, *Francisella novicida*; Ec, *E. coli*; Ac, *Actinobacillus pleuropneumoniae*.

itidis, a mutated *galE* gene resulted in truncation of the LOS chain by deletion of the outer core (Hammer-schmidt *et al.*, 1994). The N-terminal region of the predicted GalE protein possesses the consensus NAD-binding domain (GxxGxxG; Macpherson *et al.*, 1994) commonly present in epimerases. All known *Campylobacter* LPS structures contain both Gal and Glc; therefore it is likely that GalE functions as a UDP-galactose 4-epimerase.

wlaB. The protein product of *wlaB* contains an ATP-binding site and the signature for ABC transporters (Higgins *et al.*, 1990; Walker *et al.*, 1982), suggesting that this protein is involved in transport across the cytoplasmic membrane. The protein contains six potential transmembrane segments, which are also seen in other transmembrane transport molecules (Blight & Holland, 1990). Homology to other transport proteins is mainly located in the C-terminal part, where the ATP-binding site and the ABC transporters family motif are located (Fig. 5). WlaB is also homologous to mammalian multidrug resistance proteins (P-proteins), which bind to and export a variety of drugs, thereby conferring resistance (Endicott & Ling, 1989). Comparison of the hydrophobicity plots of these proteins indicated that the six transmembrane segments were located at identical positions (data not shown). WlaB probably has a signal peptide which is cleaved between amino acids 45 and 46. The similarities described above suggest that the WlaB

protein from *C. jejuni* is involved in the transport of LPS molecules across the cytoplasmic membrane.

wlaC, wlaD and wlaE. The protein products of these three genes all possess one or two putative transmembrane segments. All three deduced proteins are similar to putative glycosyltransferases. TrsD and TrsE from *Y. enterocolitica*, showing homology to WlaC and WlaE, are putative galactosyl- or N-acetylgalactosaminyl-transferases involved in outer core synthesis (Skurnik *et al.*, 1995). WlaC and WlaE are closely related, with 30.2% identical amino acids and 43.6% similar amino acids. WlaC and WlaE may link Gal or GalNAc to the growing polysaccharide chain.

wlaF. *wlaF* is the largest gene found in the cluster, encoding a predicted protein of 82.2 kDa (Table 2). This protein is similar to the transmembrane oligosaccharyl-transferases (STT-3) from eukaryotes and the O-antigen polymerase from *E. coli*. The STT3 protein from yeast is possibly involved in the transfer of an oligosaccharide from a lipid precursor to a protein (Yoshida *et al.*, 1995). The amino acid sequence of WlaF revealed a putative signal peptide, which is most probably cleaved between amino acids 36 and 37, as also seen for the STT-3 protein. The N-terminal part of WlaF contains 12 putative transmembrane segments. The C-terminal part is hydrophilic. These latter two characteristics are also seen in the STT-3 protein. The Rfc polymerase is often also highly hydrophobic, containing 12 putative trans-

Fig. 6. Hydrophobicity plots of WlaH and homologous proteins (Table 2). The plots were made using SOAP from PCGENE (Korn and Queen, 1984) using an interval of 15 amino acids. Se, *S. enterica*; Hi, *Haemophilus influenzae*.

membrane segments. We speculate that the *C. jejuni* WlaF protein is involved in the transfer of oligosaccharides, possibly from a lipid carrier onto the lipid A-core complex. However, it may also have flippase activity. Wzx proteins are also hydrophobic and their genes are often found in LPS gene clusters (Reeves, 1994).

wlaG. Throughout the whole gene, *wlaG* is homologous to genes encoding galactosyltransferases. The *rfbF* gene products of *Klebsiella pneumoniae* and *Serratia marcescens*, transfer the disaccharide Gal(1 → 3)Gal, representing a single repeating unit of D-galactan I, to the GlcNAc-containing lipid intermediate (Clarke *et al.*, 1995; Szabo *et al.*, 1995). The RfpB protein of *Shigella dysenteriae* transfers a single Gal residue to a GlcNAc-containing lipid (Gohmann *et al.*, 1994). Unlike the RfpB protein of *S. dysenteriae* and the CpsF protein of *Proteus mirabilis* (Gygi *et al.*, 1995), the predicted WlaG protein of *C. jejuni* is probably not membrane bound, as it has no significant hydrophobic regions. However, the RfbF protein of *Klebsiella pneumoniae* is also not membrane bound. As at least two galactose residues are present in the LPS molecules of all *C. jejuni* strains analysed, we suggest that the WlaG protein functions as a galactosyltransferase.

wlaH. The *wlaH* gene is homologous to genes involved in transferring the first sugar of the subunits of O-antigen to the lipid precursor undecaprenol phosphate. The present nomenclature for this type of gene is *wbaP*. This first sugar differs between bacterial species and strains, but in most cases Gal is the first sugar with which O-antigen synthesis is initiated. The proteins showing highest similarity with WlaH transfer a Gal residue. Like other WbaP proteins, WlaH contains a hydrophobic domain required for the interaction with the undecaprenol phosphate lipid carrier (Fig. 6). WbaP-like

WlaI_Cj	115	KAK	IE KGVI	NT SSV	IE HECV	IE EFSH	IS VGAK	CAGNVK	153
NeuD_Ec	116	DTR	IE HDAVV	IN TRSL	IE HGNE	IE CCSN	IS TNVV	LNGDVS	154
LpxD_Se	221	DTV	IE NGVI	IN QCO	IE HNVV	IE DNTA	IS GGVI	MAGSLK	259
LpxD_Ec	221	DTI	IE NGVI	IN QCO	IE HNVV	IE DNTA	IS GGVI	MAGSLK	259
LpxD_Ye	221	NTI	IE NGVI	IN QCO	IE HNVV	IE DNTA	IS GGVI	MAGSLK	259
			..*	..*	..*	..*	..*	..*	
WlaI_Cj	154	IE KNCF	IE GINSC	IE PNLS	IE ADDSI	IE GGAT	IS VKSQNEKG		192
NeuD_Ec	155	IE GETF	IE GSVTV	IE GNQLK	IE GSKSI	IE SGSV	IS IRNIPSNV		193
LpxD_Se	260	IE GRYCM	IE GGASV	IE NGHME	IE CDKVT	IE TGMGM	IS MRPITEPG		298
LpxD_Ec	260	IE GRYCM	IE GGASV	IE NGHME	IE CDKVT	IE TGMGM	IS MRPITEPG		298
LpxD_Ye	260	IE GRYCM	IE GGASV	IE NGHME	IE CDKVT	IE TGMGM	IS MRPITEPG		298
			..*	..*	..*	..*	..*		

Fig. 7. Protein sequences of WlaI and homologous proteins (Table 2). The first residues of the isoleucine patch are boxed and in bold type. Glycine residues in the second position are also in bold face. Asterisks indicate identical amino acids and dots conserved substitutions. Cj, *C. jejuni*; Ec, *E. coli*; Se, *S. enterica*; Ye, *Y. enterocolitica*.

proteins have been shown to be bifunctional, with the above-mentioned transferase activity assigned to the C-terminal domain. Recently it has been proposed that the N-terminal domain of the WbaP protein of *Salmonella enterica* is involved in the release of undecaprenol-pyrophosphate-linked galactose from WbaP (Wang *et al.*, 1996) and does not have a flippase function as earlier reported (Wang & Reeves, 1994). From the protein alignment it can be concluded that WlaH from *C. jejuni* probably only possesses the transferase activity, as the N-terminal domain is missing, compared to the WbaP proteins of *S. enterica* and *Haemophilus influenzae* (Fig. 6).

wlaI. This ORF, encoding a protein of 203 amino acids, is most similar to the 207 amino acid NeuD protein from *E. coli*, an enzyme involved in the synthesis of the K1 polysaccharide (Annunziato *et al.*, 1995), an α -2,8-linked linear polymer of about 200 NeuNAc residues. The NeuD protein is suggested to be involved in NeuNAc transfer (Annunziato *et al.*, 1995). The C-terminal part of the WlaI protein is also similar, although to a lesser extent, to the 340 amino acid UDP-N-acetylglucosamine acyltransferases, LpxD, of *E. coli*, *S. enterica* and *Y. enterocolitica*, which play a role in lipid A synthesis (Dicker & Seetharam, 1991; Hirvas *et al.*, 1990; Vuorio *et al.*, 1994). *lpxD*-like genes have been found in several gene clusters involved in LPS O-antigen synthesis. The BplB protein from *Bordetella pertussis* (Allen & Maskell, 1996) and the WbpD protein from *Pseudomonas aeruginosa* (Burrows *et al.*, 1996) are both acetyl transferases. The WlaI deduced protein sequence also shows homology to this group of transferases.

A hexapeptide motif, also termed the isoleucine patch (Dicker & Seetharam, 1992), is present in some transferases (Vaara, 1992). Each motif starts with an isoleucine, leucine or valine residue and often contains a glycine in the second position. In the WlaI protein of *C. jejuni*, as well as in all the homologous proteins described above, this repeating motif is present (Fig. 7). We propose a function for the WlaI protein as a NeuNAc transferase, since the NeuD protein from *E. coli* shows the highest similarity. The NeuD protein, like the WlaI protein, is small, containing only 207 amino acids,

whereas all known LpxD proteins contain around 340 amino acids. The presence of NeuNAc residues in seven of the eight analysed *C. jejuni* LPS molecules indicates the need of such an enzyme activity.

wlaK. The predicted protein designated WlaK shows the highest similarity with the RfbE protein from *Vibrio cholerae*, which encodes a putative perosamine synthetase (Manning *et al.*, 1995). Homology with DegT from *Bacillus stearothermophilus* is also found. This protein is required in the pathway to synthesize 2,6-, 3,6- and 4,6-dideoxyhexoses and is thought to be an enzyme for transaminations leading to amino sugars (Thorson *et al.*, 1993). The SpsC protein from *Bacillus subtilis* (Glaser *et al.*, 1993), also belonging to the DegT family, and involved in spore coat polysaccharide biosynthesis, is also homologous to the *C. jejuni* WlaK protein. The *flaA2* gene product from *Caulobacter crescentus*, involved in flagellin synthesis (B. Ely, personal communication) is also similar to WlaK. It is thought that polysaccharide may be involved in *C. crescentus* flagellum biogenesis (B. Ely, personal communication). The WlaK protein of *C. jejuni* is probably also involved in the synthesis of an amino sugar, possibly GalNAc or NeuNAc, which are both common components of the *Campylobacter* LPS molecule. *N. gonorrhoeae*, however, uses exogenous NeuNAc to sialylate its LOS by an outer-membrane-bound NeuNAc transferase which has been detected in the outer membrane (Mandrell *et al.*, 1993). If exogenous NeuNAc is also used by *C. jejuni*, no enzymes for the synthesis of NeuNAc would be needed.

wlaL. The WlaL protein is homologous throughout its entire length to the CapD, WbpM and TrsG proteins from *Staphylococcus aureus*, *P. aeruginosa* and *Y. enterocolitica*, respectively (Burrows *et al.*, 1996; Lin *et al.*, 1994; Skurnik *et al.*, 1995). All proteins contain five hydrophobic domains in the N-terminal part that are predicted to be transmembrane segments. The central part contains an NAD-binding site and is homologous to UDPglucose 4-epimerases. The function of the CapD protein is not known. The TrsG protein is thought to be involved in the biosynthesis of GalNAc or FucNAc (acetylfucosamine), the WbpM protein is possibly a dehydrogenase or epimerase needed for the biosynthesis of a 2-acetamido-2,6-dideoxy-D-galactose residue. A putative signal peptide has been found in WlaL, which is probably cleaved at position 29. We suggest the WlaL protein is involved in the synthesis of an amino sugar, probably GalNAc or NeuNAc.

wlaM. The *wlaM* gene product shows only low similarity to an accessory colonization factor, AcfB (Everiss *et al.*, 1994). This protein contains the methyl-accepting chemotaxis motif as found in chemotaxis genes (Alley *et al.*, 1992; Dahl *et al.*, 1989). However the similarity with WlaM was confined to the N-terminal part not containing this motif. Like WlaF, WlaM is a hydrophobic protein containing six putative transmembrane regions. Therefore WlaM could also function as a flippase or a polymerase.

cheY. This gene is identical to the *cheY* gene of *C. jejuni*

(Ketley, 1997; Yao *et al.*, 1997), a gene involved in chemotaxis. It is unlikely that this gene is an LPS synthesis gene. Furthermore, unlike *galE* and *wla*, the *cheY* gene has its own promoter consensus sequence.

orf1. No homology was found for this incomplete ORF or its putative protein product.

DISCUSSION

We have shown that a 16 kb chromosomal DNA region of *Campylobacter jejuni* strain 81116 is able to express LPS in *E. coli*. These heat-stable antigens are known to react with Penner 6 antiserum. The sequence analysis of this region is presented.

The *wla* cluster

The sequence data show that the 16 kb region contains 11 genes, of which the deduced protein sequences show homology to proteins involved in LPS synthesis in other bacteria. On the basis of the observed similarity of the deduced Wla proteins to proteins involved in LOS synthesis, and especially the outer-core synthesis of *Y. enterocolitica* and *Bordetella pertussis* (Allen & Maskell, 1996; Skurnik *et al.*, 1995), it is likely that the *wla* gene cluster of *C. jejuni* is involved in the synthesis of the O-antigen-like polysaccharide, as well as the outer core, but not of the inner core. This is in agreement with the finding of the *waaC* gene, situated at the beginning of a separate gene cluster of at least five genes involved in inner core synthesis (data not shown).

The presence of the *wlaH* gene, which probably encodes a galactosyltransferase, linking a Gal residue to the lipid carrier, and the observation that almost all analysed outer core molecules from *C. jejuni* start with a Gal residue, suggests that the outer core molecule is synthesized on a lipid carrier. This has also been suggested for the outer core of *Y. enterocolitica*, where also a Gal-lipid carrier transferase gene was found to be present in the outer-core-synthesis gene cluster (Skurnik *et al.*, 1995). Both the O-antigen-like polysaccharide and the outer core may be synthesized on lipid carriers, in which case the WlaH protein would catalyse the first step.

Sugar biosynthesis

Three genes involved in the biosynthesis of sugars are present in the *wla* cluster. The presence of *galE*, converting Glc to Gal, is not surprising, as all known outer core molecules of *C. jejuni* contain Gal residues. The *wlaK* and *wlaL* genes are probably involved in the synthesis of amino sugars. GalNAc and NeuNAc are amino sugars found in core molecules of several *C. jejuni* strains.

Transport of sugar structures

Three transport steps have been observed during LPS synthesis of *E. coli* and *Salmonella typhimurium*: flipping of an oligosaccharide subunit attached to a lipid carrier across the inner membrane; transport of saccharide structures from a lipid carrier to the

lipid A-core complex; and transport of the complete LPS molecule from the inner membrane to the outer membrane.

We assume that the outer-core molecule of *C. jejuni* is synthesized at the cytoplasmic side of the inner membrane, like an LPS O-antigen subunit. After completion it has to be 'flipped' to the periplasmic side of the inner membrane. This could happen before or after it has been ligated to the lipid A-inner core complex. Possibly WlaM is involved in this flippase process and WlaF could be used to link the outer core to the lipid A-inner core complex, or vice versa. The complete LOS molecule is then translocated across the inner membrane to the outer membrane by WlaB.

Sugar transferases

The proposed sugar transferases within the *wla* gene cluster consist of Gal or GalNAc transferases WlaC and WlaE, an unknown transferase WlaD, a Gal transferase WlaG, a Gal-to-lipid-carrier transferase WlaH and a NeuNAc transferase WlaI. If, as suggested above, the outer core is synthesized on a lipid carrier, WlaH probably initiates the synthesis of the outer core by transferring a Gal residue onto the lipid carrier.

All but one of the known core molecules of *C. jejuni* contain a GalNAc residue bound to a backbone Gal residue. The proposed function of WlaC and WlaE as GalNAc transferases would predict the presence of a GalNAc residue in the outer core of strain 81116. Most Gal residues in the outer core molecules of *C. jejuni* are branched with a NeuNAc residue, for which WlaI is the proposed transferase.

O-antigen-like polysaccharide structure and synthesis

Three observations suggest that the O-antigen-like repeat of *C. jejuni* is not linked to the lipid A-inner core complex and therefore is not a proper O-antigen. (1) Significant amounts of lipid A-inner core residues were not found in any of the analysed O-antigen-like repeats (Aspinall *et al.*, 1993b, c, 1994b, 1995a; Salloway *et al.*, 1996). (2) The O-antigen-like repeat unit is not stained by the silver-staining method, whereas the lipid A-core complex is. Thus, if the repeat unit were linked to the lipid A-core complex it would surely be seen using this technique. (3) Expression of the *wla* cluster in *E. coli* shows a lipid A-core molecule larger than that of *Campylobacter*, which reacts with the serospecific antiserum of *C. jejuni* 81116. This suggests that the lipid A-core complex of *E. coli* is used, to which a specific *C. jejuni* sugar structure is linked. The molecular mass of the expressed O-antigen-like repeat, however, is not increased. The O-antigen-like repeat therefore resembles capsular polysaccharide or enterobacterial common antigen as found in *E. coli* and not LPS O-antigen.

Three variable O-antigen-like polysaccharides from different *Campylobacter* strains have been examined. Two consist of a disaccharide and one of a trisaccharide repeat. In two of these repeat units, a heptose derivative

is present, as also found in *Campylobacter coli* O:30 (Aspinall *et al.*, 1993a) and in strains of *Campylobacter lari* (Aspinall *et al.*, 1995b, 1995c). In all three analysed repeat units, an amino sugar is present; in two of these it is a GlcNAc residue.

We predict that the polysaccharide synthesis follows the same route as the start of the outer-core assembly, namely on a lipid carrier. The first sugar, a Gal residue, will be linked by WlaH. After this, the synthesis of the outer core and the O-antigen-like polysaccharide would diverge.

When comparing the known O-antigen-like polysaccharide structures with the core structures of *C. jejuni*, a remarkable difference is seen. The Gal residues in the repeat units are not sialylated, whereas all Gal residues found in the backbone of the outer core molecule are. It could therefore be reasoned that the outer core oligosaccharide is sialylated in a compartment where the O-antigen-like polysaccharide is not present. Sialylation may occur on the periplasmic side of the inner membrane or in the outer membrane, where the O-antigen-like polysaccharide never goes.

The characterization of this first *wla* gene cluster of *C. jejuni* marks a starting point for further studies and applications, possibly in serotyping, diagnostics and vaccine development. Also the study of LPS-induced diseases can be advanced.

Insertional-inactivation experiments in *C. jejuni* and in the cloned *wla* region in *E. coli* are needed to confirm the functions assigned to the various proteins. In addition, attempts to complement defined *S. typhimurium* LPS mutants are in progress. The LOS structure of *C. jejuni* 81116 is currently being analysed by Dr A. P. Moran and coworkers in Galway, Ireland.

ACKNOWLEDGEMENTS

We are grateful to Dr D. G. Newell for her gift of the Penner 6 antiserum and to Dr J. M. Ketley for his help with designing Fig. 1. We thank Dr P. R. Reeves for his help with the gene nomenclature. We thank A. J. A. M. van Asten, L. Heijmen-van Dijk and K. A. Zwaagstra for technical assistance with the automated sequencing.

REFERENCES

- Allen, A. & Maskell, D. (1996). The identification, cloning and mutagenesis of a genetic locus required for lipopolysaccharide biosynthesis in *Bordetella pertussis*. *Mol Microbiol* **19**, 37–52.
- Allen, L. N. & Hanson, R. S. (1985). Construction of broad-host-range cosmid cloning vectors: identification of genes necessary for growth of *Methylobacterium organophilum* on methanol. *J Bacteriol* **161**, 955–962.
- Alley, M. R., Maddock, J. R. & Shapiro, L. (1992). Polar localization of a bacterial chemoreceptor. *Genes Dev* **6**, 825–836.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Annunziato, P. W., Wright, L. F., Vann, W. F. & Silver, R. P. (1995). Nucleotide sequence and genetic analysis of the *neuD* and *neuB* genes in region 2 of the polysialic acid gene cluster of *Escherichia coli* K1. *J Bacteriol* **177**, 312–319.

- Aspinall, G. O., McDonald, A. G. & Pang, H. (1992a). Structures of the O chains from lipopolysaccharides of *Campylobacter jejuni* serotypes O:23 and O:36. *Carbohydr Res* 231, 13–30.
- Aspinall, G. O., McDonald, A. G., Raju, T. S., Pang, H., Mills, S. D., Kurjanczyk, L. A. & Penner, J. L. (1992b). Serological diversity and chemical structures of *Campylobacter jejuni* low-molecular-weight lipopolysaccharides. *J Bacteriol* 174, 1324–1332.
- Aspinall, G. O., McDonald, A. G., Pang, H., Kurjanczyk, L. A. & Penner, J. L. (1993a). An antigenic polysaccharide from *Campylobacter coli* serotype O:30: structure of a teichoic acid-like antigenic polysaccharide associated with the lipopolysaccharide. *J Biol Chem* 268, 18321–18329.
- Aspinall, G. O., McDonald, A. G., Raju, T. S., Pang, H., Kurjanczyk, L. A., Penner, J. L. & Moran, A. P. (1993b). Chemical structure of the core region of *Campylobacter jejuni* serotype O:2 lipopolysaccharide. *Eur J Biochem* 213, 1029–1037.
- Aspinall, G. O., McDonald, A. G., Raju, T. S., Pang, H., Moran, A. P. & Penner, J. L. (1993c). Chemical structures of the core regions of *Campylobacter jejuni* serotypes O:1, O:4, O:23, and O:36 lipopolysaccharides. *Eur J Biochem* 213, 1017–1027 (erratum published in *Eur J Biochem* 216, 880).
- Aspinall, G. O., McDonald, A. G. & Pang, H. (1994a). Lipopolysaccharides of *Campylobacter jejuni* serotype O:19: structures of O antigen chains from the serostrain and two bacterial isolates from patients with the Guillain-Barré syndrome. *Biochemistry* 33, 250–255.
- Aspinall, G. O., McDonald, A. G., Pang, H., Kurjanczyk, L. A. & Penner, J. L. (1994b). Lipopolysaccharides of *Campylobacter jejuni* serotype O:19: structures of core oligosaccharide regions from the serostrain and two bacterial isolates from patients with the Guillain-Barré syndrome. *Biochemistry* 33, 241–249.
- Aspinall, G. O., Lynch, C. M., Pang, H., Shaver, R. T. & Moran, A. P. (1995a). Chemical structures of the core region of *Campylobacter jejuni* O:3 lipopolysaccharide and an associated polysaccharide. *Eur J Biochem* 231, 570–578.
- Aspinall, G. O., Monteiro, M. A. & Pang, H. (1995b). Lipooligosaccharide of the *Campylobacter lari* type strain ATCC 35221. Structure of the liberated oligosaccharide and an associated extracellular polysaccharide. *Carbohydr Res* 279, 245–264.
- Aspinall, G. O., Monteiro, M. A., Pang, H., Kurjanczyk, L. A. & Penner, J. L. (1995c). Lipo-oligosaccharide of *Campylobacter lari* strain PC 637. Structure of the liberated oligosaccharide and an associated extracellular polysaccharide. *Carbohydr Res* 279, 227–244.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1995). *Current Protocols in Molecular Biology*. New York: John Wiley.
- Blaser, M. J. & Reller, L. B. (1981). *Campylobacter* enteritis. *N Engl J Med* 305, 1444–1452.
- Blight, M. A. & Holland, I. B. (1990). Structure and function of haemolysin B, P-glycoprotein and other members of a novel family of membrane translocators. *Mol Microbiol* 4, 873–880.
- Bolivar, F., Rodriguez, R. L., Greene, P. J., Betlach, M. C., Heyneker, H. L. & Boyer, H. W. (1977). Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. *Gene* 2, 95–113.
- Boyer, H. W. & Roulland-Dussoix, D. (1969). A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *J Mol Biol* 41, 459–472.
- Branquinho, M. R., Alviano, C. S. & Ricciardi, I. D. (1983). Chemical composition and biological action of lipopolysaccharide (LPS) of *Campylobacter fetus* ss. *jejuni*. *Rev Microbiol* 14, 90–96.
- Bray, J. (1945). Isolation of antigenically homogeneous strains of *Bact. coli neapolitanum* from summer diarrhoea in infants. *J Pathol Bacteriol* 57, 239–247.
- Burrows, L. L., Charter, D. F. & Lam, J. S. (1996). Molecular characterization of the *Pseudomonas aeruginosa* serotype O5 (PAO1) B-band lipopolysaccharide gene cluster. *Mol Microbiol* 22, 481–495.
- Butzler, J. P. & Skirrow, M. B. (1979). *Campylobacter* enteritis. *Clin Gastroenterol* 8, 737–765.
- Clarke, B. R., Bronner, D., Keenleyside, W. J., Severn, W. B., Richards, J. C. & Whitfield, C. (1995). Role of Rfe and RfbF in the initiation of biosynthesis of D-galactan I, the lipopolysaccharide O antigen from *Klebsiella pneumoniae* serotype O1. *J Bacteriol* 177, 5411–5418.
- Dahl, M. K., Boos, W. & Manson, M. D. (1989). Evolution of chemotactic-signal transducers in enteric bacteria. *J Bacteriol* 171, 2361–2371.
- Demarco de Hormaeche, R., Macpherson, A., Bowe, F. & Hormaeche, C. E. (1991). Alterations of the LPS determine virulence of *Neisseria gonorrhoeae* in guinea-pig subcutaneous chambers. *Microb Pathog* 11, 159–170.
- Dicker, I. B. & Seetharam, S. (1991). Cloning and nucleotide sequence of the *firA* gene and the *firA200(Ts)* allele from *Escherichia coli*. *J Bacteriol* 173, 334–344.
- Dicker, I. B. & Seetharam, S. (1992). What is known about the structure and function of the *Escherichia coli* protein FirA? *Mol Microbiol* 6, 817–823.
- Endicott, J. A. & Ling, V. (1989). The biochemistry of P-glycoprotein-mediated multidrug resistance. *Annu Rev Biochem* 58, 137–171.
- Everiss, K. D., Hughes, K. J., Kovach, M. E. & Peterson, K. M. (1994). The *Vibrio cholerae* *acfB* colonization determinant encodes an inner membrane protein that is related to a family of signal-transducing proteins. *Infect Immun* 62, 3289–3298.
- Glaser, P., Kunst, F., Arnaud, M. & 17 other authors (1993). *Bacillus subtilis* genome project: cloning and sequencing of the 97 kb region from 325 degrees to 333 degrees. *Mol Microbiol* 10, 371–384.
- Gohmann, S., Manning, P. A., Alpert, C. A., Walker, M. J. & Timmis, K. N. (1994). Lipopolysaccharide O-antigen biosynthesis in *Shigella dysenteriae* serotype 1: analysis of the plasmid-carried *rpf* determinant. *Microb Pathog* 16, 53–64.
- Gygi, D., Rahman, M. M., Lai, H. C., Carlson, R., Guard-Petter, J. & Hughes, C. (1995). A cell-surface polysaccharide that facilitates rapid population migration by differentiated swarm cells of *Proteus mirabilis*. *Mol Microbiol* 17, 1167–1175.
- Hackett, J., Wyk, P., Reeves, P. & Mathan, V. (1987). Mediation of serum resistance in *Salmonella typhimurium* by an 11-kilodalton polypeptide encoded by the cryptic plasmid. *J Infect Dis* 155, 540–549.
- Hammerschmidt, S., Birkholz, C., Zahringer, U., Robertson, B. D., van Putten, J., Ebeling, O. & Frosch, M. (1994). Contribution of genes from the capsule gene complex (*cps*) to lipooligosaccharide biosynthesis and serum resistance in *Neisseria meningitidis*. *Mol Microbiol* 11, 885–896.
- Hanahan, D. (1983). Studies on transformation of *Escherichia coli* with plasmids. *J Mol Biol* 166, 557–580.
- Higgins, C. F., Hyde, S. C., Mimmack, M. M., Gileadi, U., Gill, D. R. & Gallagher, M. P. (1990). Binding protein-dependent transport systems. *J Bioenerg Biomembr* 22, 571–592.

- Hirvas, L., Koski, P. & Vaara, M. (1990). Primary structure and expression of the Ssc-protein of *Salmonella typhimurium*. *Biochem Biophys Res Commun* 173, 53–59 (erratum published in *Biochem Biophys Res Commun* 174, 1054).
- Inzana, T. J., Seifert, W. E., Jr & Williams, R. P. (1985). Composition and antigenic activity of the oligosaccharide moiety of *Haemophilus influenzae* type b lipooligosaccharide. *Infect Immun* 48, 324–330.
- Jansson, P. E., Lindberg, A. A., Lindberg, B. & Wollin, R. (1981). Structural studies on the hexose region of the core in lipopolysaccharides from Enterobacteriaceae. *Eur J Biochem* 115, 571–577.
- Jennings, H. J., Bhattacharjee, A. K., Kenne, L., Kenny, C. P. & Calver, G. (1980). The R-type lipopolysaccharides of *Neisseria meningitidis*. *Can J Biochem* 58, 128–136.
- Joiner, K. A., Grossman, N., Schmetz, M. & Leive, L. (1986). C3 binds preferentially to long-chain lipopolysaccharide during alternative pathway activation by *Salmonella montevideo*. *J Immunol* 136, 710–715.
- Ketley, J. M. (1997). Pathogenesis of enteric infection by *Campylobacter*. *Microbiology* 143, 5–21.
- Korn, L. J. & Queen, C. (1984). Analysis of biological sequences on small computers. *DNA* 3, 421–436.
- Korolik, V., Fry, B. N., Alderton, M. R., van der Zeijst, B. A. M. & Coloe, P. J. (1997). Expression of *Campylobacter hyoilei* lipooligosaccharide (LOS) antigens in *Escherichia coli*. *Microbiology* 143, 3481–3489.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680–685.
- Lawrence, C. E., Altschul, S. F., Boguski, M. S., Liu, J. S., Neuwald, A. F. & Wootton, J. C. (1993). Detecting subtle sequence signals: a Gibbs sampling strategy for multiple alignment. *Science* 262, 208–214.
- Lesse, A. J., Campagnari, A. A., Bittner, W. E. & Apicella, M. A. (1990). Increased resolution of lipopolysaccharides and lipooligosaccharides utilizing tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J Immunol Methods* 126, 109–117.
- Lin, W. S., Cunneen, T. & Lee, C. Y. (1994). Sequence analysis and molecular characterization of genes required for the biosynthesis of type 1 capsular polysaccharide in *Staphylococcus aureus*. *J Bacteriol* 176, 7005–7016.
- Lugtenberg, B., Meijers, J., Peters, R., van der Hoek, P. & van Alphen, L. (1975). Electrophoretic resolution of the 'major outer membrane protein' of *Escherichia coli* K12 into four bands. *FEBS Lett* 58, 254–258.
- Macpherson, D. F., Manning, P. A. & Morona, R. (1994). Characterization of the dTDP-rhamnose biosynthetic genes encoded in the rfb locus of *Shigella flexneri*. *Mol Microbiol* 11, 281–292.
- McSweeney, E. & Walker, R. I. (1986). Identification and characterization of two *Campylobacter jejuni* adhesins for cellular and mucous substrates. *Infect Immun* 53, 141–148.
- Mandrell, R. E. & Apicella, M. A. (1993). Lipo-oligosaccharides (LOS) of mucosal pathogens: molecular mimicry and host-modification of LOS. *Immunobiology* 187, 382–402.
- Mandrell, R. E., Smith, H., Jarvis, G. A., Griffiss, J. M. & Cole, J. A. (1993). Detection and some properties of the sialyltransferase implicated in the sialylation of lipopolysaccharide of *Neisseria gonorrhoeae*. *Microb Pathog* 14, 307–313.
- Manning, P. A., Stroehrer, U. H., Karageorgos, L. E. & Morona, R. (1995). Putative O-antigen transport genes within the rfb region of *Vibrio cholerae* O1 are homologous to those for capsule transport. *Gene* 158, 1–7.
- Mills, S. D., Kuzniar, B., Shames, B., Kurjanczyk, L. A. & Penner, J. L. (1992). Variation of the O antigen of *Campylobacter jejuni* in vivo. *J Med Microbiol* 36, 215–219.
- Moxon, R. E. & Maskell, D. (1992). *Haemophilus influenzae* lipopolysaccharide: the biochemistry and biology of a virulence factor. In *Molecular Biology of Bacterial Infection: Current Status and Future Perspectives*, pp. 75–96. Edited by C. E. Hormaeche, C. W. Penn & C. J. Smyth. Cambridge: Cambridge University Press.
- Naess, V. & Hofstad, T. (1984). Chemical composition and biological activity of lipopolysaccharides prepared from type strains of *Campylobacter jejuni* and *Campylobacter coli*. *Acta Pathol Microbiol Immunol Scand Sect B Microbiol* 92, 217–222.
- Neuhard, J. & Thomassen, E. (1976). Altered deoxyribonucleotide pools in P2 eductants of *Escherichia coli* K-12 due to deletion of the *dcd* gene. *J Bacteriol* 126, 999–1001.
- Newell, D. G., McBride, H. & Dolby, J. M. (1985). Investigations on the role of flagella in the colonization of infant mice with *Campylobacter jejuni* and attachment of *Campylobacter jejuni* to human epithelial cell lines. *J Hyg* 95, 217–227.
- Palmer, S. R., Gully, P. R., White, J. M., Pearson, A. D., Suckling, W. G., Jones, D. M., Rawes, J. C. & Penner, J. L. (1983). Water-borne outbreak of campylobacter gastroenteritis. *Lancet* 1, 287–290.
- Pavliak, V., Brisson, J. R., Michon, F., Uhrin, D. & Jennings, H. J. (1993). Structure of the sialylated L3 lipopolysaccharide of *Neisseria meningitidis*. *J Biol Chem* 268, 14146–14152.
- Penner, J. L. (1988). The genus *Campylobacter*: a decade of progress. *Clin Microbiol Rev* 1, 157–172.
- Penner, J. L. & Hennessy, J. N. (1980). Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. *J Clin Microbiol* 12, 732–737.
- Preston, M. A. & Penner, J. L. (1987). Structural and antigenic properties of lipopolysaccharides from serotype reference strains of *Campylobacter jejuni*. *Infect Immun* 55, 1806–1812.
- Ray, A., Redhead, K., Selkirk, S. & Poole, S. (1991). Variability in LPS composition, antigenicity and reactogenicity of phase variants of *Bordetella pertussis*. *FEMS Microbiol Lett* 63, 211–217.
- Ray, B. L. & Raetz, C. R. (1987). The biosynthesis of gram-negative endotoxin. A novel kinase in *Escherichia coli* membranes that incorporates the 4'-phosphate of lipid A. *J Biol Chem* 262, 1122–1128.
- Reeves, P. R. (1994). Biosynthesis and assembly of lipopolysaccharide. In *Bacterial Cell Wall*, pp. 281–317. Edited by J.-M. Ghuyssen & R. Hakenbeck. Amsterdam: Elsevier.
- Reeves, P. R., Hobbs, M., Valvano, M. A. & 8 other authors (1996). Bacterial polysaccharide synthesis and gene nomenclature. *Trends Microbiol* 4, 495–503.
- Rick, P. D. (1987). Lipopolysaccharide biosynthesis. In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, pp. 648–662. Edited by F. C. Neidhart and others. Washington, DC: American Society for Microbiology.
- Salloway, S., Mermel, L. A., Seamans, M., Aspinall, G. O., Nam Shin, J. E., Kurjanczyk, L. A. & Penner, J. L. (1996). Miller-Fisher syndrome associated with *Campylobacter jejuni* bearing lipopolysaccharide molecules that mimic human ganglioside GD3. *Infect Immun* 64, 2945–2949.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1982). *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

- Sanger, F., Nicklen, S. & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* **74**, 5463–5467.
- Schagger, H. & von Jagow, G. (1987). Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem* **166**, 368–379.
- Schnaitman, C. A. & Klena, J. D. (1993). Genetics of lipopolysaccharide biosynthesis in enteric bacteria. *Microbiol Rev* **57**, 655–682.
- Schwerer, B., Neisser, A., Polt, R. J., Bernheimer, H. & Moran, A. P. (1995). Antibody cross-reactivities between gangliosides and lipopolysaccharides of *Campylobacter jejuni* serotypes associated with Guillain-Barré syndrome. *J Endotoxin Res* **2**, 395–403.
- Short, J. M., Fernandez, J. M., Sorge, J. A. & Huse, W. D. (1988). Lambda ZAP: a bacteriophage lambda expression vector with in vivo excision properties. *Nucleic Acids Res* **16**, 7583–7600.
- Skirrow, M. B. (1977). *Campylobacter* enteritis: a 'new' disease. *Br Med J* **2**, 9–11.
- Skurnik, M., Venho, R., Toivanen, P. & al-Hendy, A. (1995). A novel locus of *Yersinia enterocolitica* serotype O:3 involved in lipopolysaccharide outer core biosynthesis. *Mol Microbiol* **17**, 575–594.
- Stern, N. J., Bailey, J. S., Blankenship, L. C., Cox, N. A. & McHan, F. (1988). Colonization characteristics of *Campylobacter jejuni* in chick ceca. *Avian Dis* **32**, 330–334.
- Stinavage, P., Martin, L. E. & Spitznagel, J. K. (1989). O antigen and lipid A phosphoryl groups in resistance of *Salmonella typhimurium* LT-2 to nonoxidative killing in human polymorphonuclear neutrophils. *Infect Immun* **57**, 3894–3900.
- Szabo, M., Bronner, D. & Whitfield, C. (1995). Relationships between *rfb* gene clusters required for biosynthesis of identical D-galactose-containing O antigens in *Klebsiella pneumoniae* serotype O1 and *Serratia marcescens* serotype O16. *J Bacteriol* **177**, 1544–1553.
- Tauxe, R. V. (1992). Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In *Campylobacter jejuni: Current Status and Future Trends*, pp. 9–19. Edited by I. Nachamkin, M. J. Blaser & L. S. Tompkins. Washington, DC: American Society for Microbiology.
- Thorson, J. S., Lo, S. F. & Liu, H.-W. (1993). Biosynthesis of 3, 6-dideoxyhexoses: new mechanistic reflections upon 2,6-dideoxy, 4,6-dideoxy, and amino sugar construction. *J Am Chem Soc* **115**, 6993–6994.
- Tsai, C. M. & Frasch, C. E. (1982). A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. *Anal Biochem* **119**, 115–119.
- Vaara, M. (1992). Eight bacterial proteins, including UDP-N-acetylglucosamine acyltransferase (LpxA) and three other transferases of *Escherichia coli*, consist of a six-residue periodicity theme. *FEMS Microbiol Lett* **76**, 249–254.
- Vuorio, R., Harkonen, T., Tolvanen, M. & Vaara, M. (1994). The novel hexapeptide motif found in the acyltransferases LpxA and LpxD of lipid A biosynthesis is conserved in various bacteria. *FEBS Lett* **337**, 289–292.
- Walker, J. E., Saraste, M., Runswick, M. J. & Gay, N. J. (1982). Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide-binding fold. *EMBO J* **1**, 945–951.
- Wang, L. & Reeves, P. R. (1994). Involvement of the galactosyl-1-phosphate transferase encoded by the *Salmonella enterica* *rfbP* gene in O-antigen subunit processing. *J Bacteriol* **176**, 4348–4356.
- Wang, L., Liu, D. & Reeves, P. R. (1996). C-terminal half of *Salmonella enterica* WbaP (RfbP) is the galactosyl-1-phosphate transferase domain catalyzing the first step of O-antigen synthesis. *J Bacteriol* **178**, 2598–2604.
- Yanisch-Perron, C., Vieira, J. & Messing, J. (1985). Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**, 103–119.
- Yao, R., Burr, D. H. & Guerry, P. (1997). CheY-mediated modulation of *Campylobacter jejuni* virulence. *Mol Microbiol* **23**, 1021–1031.
- Yoshida, S., Ohya, Y., Nakano, A. & Anraku, Y. (1995). *STT3*, a novel essential gene related to the PKC1/STT1 protein kinase pathway, is involved in protein glycosylation in yeast. *Gene* **164**, 167–172.

Received 10 October 1997; revised 13 March 1998; accepted 2 April 1998.