Influence of growth conditions on diverse polysaccharide production by Campylobacter jejuni

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Abstract

Campylobacter jejuni is the leading bacterial cause of gastroenteritis worldwide. The present study was undertaken to determine the forms of polysaccharide-related compounds (PRCs) produced by C. jejuni and the culture conditions influencing their production. Expression of polysaccharides by C. jejuni was influenced by culture medium composition and growth phase. In addition to the production of lipooligosaccharide and capsular polysaccharide, a previously undescribed polysaccharide, not related to capsular polysaccharide, was shown to occur in C. jejuni in batch liquid and chemostat cultures. Thus, a variety of PRCs are produced by C. jejuni, and this should be considered when growing the bacterium in vitro for pathogenesis studies.

Introduction

The Gram-negative bacterium, Campylobacter jejuni, is recognized as the most common cause of bacterial gastroenteritis in humans in developed and developing countries (Prendergast & Moran, 2000). Complications arising after C. jejuni infection include reactive arthritis (Kosunen et al., 1980; Hannu et al., 2004) and the neuropathy Guillain–Barré syndrome (GBS) (Moran & Prendergast, 2001), and there is evidence that molecular mimicry of human gangliosides by C. jejuni contributes to the latter disorder (Prendergast & Moran, 2001), and there is evidence that molecular mimicry of human gangliosides by C. jejuni low-molecular-weight (low-Mr) lipooligosaccharide contributes to the latter disorder (Prendergast & Moran, 2000). In spite of this, the pathogenic role played by other polysaccharides produced by C. jejuni is unclear and their molecular characterization is incomplete.

In the past, molecular serospecificity in the heat-stable (HS) serotyping scheme of C. jejuni was attributed to lipopolysaccharide (Mills et al., 1985). However, Aspinall et al. (1995) isolated and described the structure of a polysaccharide independent of lipopolysaccharide in C. jejuni HS:3, although its contribution to serospecificity was unclear. More recent studies have shown that lipopolysaccharide-independent polysaccharides are produced by C. jejuni and contribute to serospecificity. Based on mutational analysis of the kpsM, kpsS and kpsC genes of C. jejuni, Karlyshev et al. (2000) suggested that high-Mr lipopolysaccharide O-chains were capsular polysaccharides in nature, and that C. jejuni produced lipopolysaccharide and capsular polysaccharide only. Nevertheless, Bacon et al. (2001) showed that C. jejuni 81–176 (HS:23/36) contains two high-Mr antigenic polysaccharides, both of which appeared independent of lipid A and to have a phospholipid anchor as determined by phospholipase treatment. Mutation of the kpsM capsular biosynthesis gene resulted in loss of expression of one of these polysaccharides, and concurrently the strain became nontypeable with specific antisera. Confirming the independence of C. jejuni polysaccharide from lipopolysaccharide, studies by Oldfield et al. (2002) with NCTC 11828 (strain 81116, HS:6) showed that, when the waaF gene, a heptosyltransferase II enzyme involved in lipopolysaccharide core biosynthesis, was deleted, the electrophoretic mobility of high-Mr antigenic polysaccharide was unaffected. In addition to the chemically characterized polysaccharide in the C. jejuni HS:3 serostrain (Aspinall et al., 1995), a high-Mr hyaluronic acid-like polysaccharide has been structurally characterized in the HS:19 serostrain (Aspinall et al., 1994). The expression of this polysaccharide has been implicated in serospecificity but also in virulence (Moran & O’Malley, 1995). Although it has been deduced that capsular polysaccharides are expressed in a number of C. jejuni strains (St Michael et al., 2002; Karlyshev et al., 2005a, b; McNally et al., 2005; Papp-Szabo et al., 2005),...
more than one polysaccharide can be produced in a given C. jejuni strain (Bacon et al., 2001). In particular, in C. jejuni strain 81116, the structures of two high-Mr polysaccharides have been elucidated (Muldoon et al., 2002), one of which is neutral, and the other acidic. Importantly, the acidic polysaccharide reacts with anti-HS:6 antiserum in Western blotting, whereas the neutral polysaccharide, which produces a ladder-like series of high-Mr bands upon silver staining, reacts with HS:7 antiserum (Kilcoyne et al., 2006), thereby explaining the serological cross-reactions of this strain (Karlyshev et al., 2000).

More recent analysis of the HS serotyping scheme of C. jejuni has shown that, although capsular polysaccharide may be a dominant contributor, low-Mr lipooligosaccharide also contributes to the typing reactions (Moran et al., 2001). Also, there is substantial evidence from studies investigating the contribution of ganglioside-mimicking lipooligosaccharide in the generation of autoreactive antibodies in GBS that C. jejuni lipooligosaccharide is antigenic (Moran & Prendergast, 2001). Furthermore, C. jejuni can form biofilms of matrix-enclosed cell populations adherent to surfaces or interfaces (Joshua et al., 2006), and it is generally accepted that production of lipooligosaccharide and polysaccharide can play a key role in biofilm formation (Moran & Ljungh, 2003). The characterization and nature of the various polysaccharide-related compounds (PRCs), including lipooligosaccharide, produced by C. jejuni is, therefore, of paramount importance in understanding the pathogenesis of C. jejuni.

This study was undertaken to determine the forms of PRCs produced by C. jejuni and the influence of growth conditions in vitro on their production. To achieve this, C. jejuni was grown in liquid media, both in a chemostat and in batch liquid culture, and also on a variety of solid media. To allow a more relevant investigation of the PRCs produced by C. jejuni under routine laboratory conditions, commonly used growth media that would be applied in laboratories investigating the pathogenesis of C. jejuni were chosen. Moreover, extracted PRCs were analysed using a range of techniques including electrophoretic and thin-layer chromatographic methods, serological analyses, and lectin typing assays.

Materials and methods

Bacterial strains and growth conditions

Campylobacter jejuni strains used in this study were NCTC 11168 (HS : 2) from the National Collection of Type Cultures (Colindale, London, UK), 81–176 (Korlath et al., 1985; HS : 23/36), 81116 (NCTC 11828; HS : 6), the HS : 19 serostain (American Type Culture Collection, Manassas, VA; ATCC 43446), and a HS : 41 clinical isolate, 16971.94 (donated by Dr Albert J. Lastovica, Cape Town, South Africa). Campylobacter jejuni strains were routinely grown on blood agar [Columbia agar (Oxoid Ltd., Hampshire, UK) containing 7% (v/v) horse blood]. NCTC 11168 was also grown on Brucella agar or Brucella broth (both from Becton, Dickinson and Co., Sparks, MD). Bacteria were routinely incubated in a microaerobic atmosphere at 37 °C for 48 h.

For the growth study, batch cultivation of C. jejuni was performed in 10 mL of Brucella broth in universal bottles incubated at 37 °C or 42 °C. For the construction of growth curves, three universals were removed each hour from the group of inoculated universal bottles, checked for contamination by Gram staining, and the OD_{600 nm} of cultures recorded. Exponential phase growth of a 5-h culture (see Fig. 1) at 37 °C was used as an inoculum for all the test media (agar-based, batch liquid culture and chemostat culture). The controlled transfer of cells with a common ancestry facilitated comparison of PRC production when bacteria were grown under differing culture conditions. For cultivation of C. jejuni and comparison of PRC production, media were chosen that are commonly used when cultivating C. jejuni for pathogenesis studies. Columbia blood agar and Brucella agar were used as solid culture media, and Brucella broth was used as a liquid medium both in batch culture (to provide stationary phase PRCs) and in the chemostat (to provide exponential phase PRCs).

Growth in the chemostat

Campylobacter jejuni was grown in a chemostat in Brucella broth (Becton, Dickinson and Co.) in a 5-L glass vessel with a working volume of 1 L to which fresh medium was supplied and waste broth removed using a peristaltic pump.

![Fig. 1. Growth curve of Campylobacter jejuni NCTC 11168 grown in batch culture in Brucella broth at 37 °C over a 24-h period. Data are the mean of three determinations, and bars represent the SD. The doubling time was calculated at 97.5 min, while the cells entered stationary phase after 18 h of growth.](https://academic.oup.com/femspd/article-abstract/49/1/124/582825/fig1)
(model ISM834A, Ismatec SA, Glattbrug-Zürich, Switzerland). In order to create a microaerobic atmosphere, the chemostat was sealed immediately after autoclaving, and no additional O2 was added to the system. Working volumes were maintained by removing waste and adding fresh medium at the same rate using the peristaltic pump. Using a syringe, appropriate quantities of C. jejuni Skirrow selective supplement (Oxoid) were added to the fresh medium. For inoculation, 100 mL of an overnight C. jejuni culture was added to 900 mL of fresh medium in the reaction chamber, and the chemostat was operated at a dilution rate of 21.5 mL h\(^{-1}\) with constant agitation at 100 r.p.m. at 37 °C.

**Staining of biofilm structures using Ruthenium Red and Coomassie Blue**

In order to grow the bacteria as a biofilm, glass cover slips were submerged in the liquid medium (batch or chemostat cultures) or glass slides were suspended half-submerged at the air/medium interface. After 4 days, the cover slips/slides were removed and the biofilms were stained using the method of Borucki et al. (2003). Briefly, slides were subjected to stepwise staining first with 0.1% (w/v) Ruthenium Red (Sigma-Aldrich, St Louis, MO) and then with 0.1% (w/v) Coomassie Blue (Bio-Rad Laboratories, Hercules, CA), both of which were dissolved in 100-mM sodium cacodylate (Sigma) containing 0.5% (v/v) glutaraldehyde (Fluka Chemical GmbH, Buchs, Germany), for 1-h periods each at room temperature, and then washed three times for 5-min periods in 100-mM sodium cacodylate. The slides were subsequently viewed and photographed using a Nikon Optiphot-2 Microscope connected to a Dell desktop computer with IRFANVIEWER IMAGING software.

**Lectin typing**

Samples for lectin typing were prepared according to a previously described method (Hynes et al., 1999). In brief, cells were washed three times in 0.1-M phosphate-buffered saline pH 7.3 (PBS), and then incubated in PBS (adjusted to pH 4 with 1-M HCl) for 30 min at 20 °C. The resulting cellular debris was washed twice in PBS, then resuspended in PBS containing 2% (w/v) proteinase K (Sigma) and incubated for 1 h at 60 °C. After centrifugation, the resultant pellet was resuspended in PBS to an OD\(_{550nm}\) of 0.9.

Samples were lectin-typed as previously described (Hynes et al., 1999). In summary, bacterial samples (40 μL) were mixed with 10 μL of lectin solution in the round-bottomed wells of 96-well microtitre plates (Orange Scientific, Braine-l’Alleud, Belgium) for 5 s or, alternatively, were mixed with 10 μL of PBS (negative control) and allowed to settle overnight, undisturbed, at 20 °C. A suspension of human type O erythrocytes (0.75%) was used as a positive control. Assays were performed in duplicate.

The following freeze-dried native lectins (Sigma) with defined specificities [in parentheses] were used: Solanum tuberosum (STA) and Trifolium vulgaris (WGA) [β-N-acetylglucosamine (GlcNAc)], Glycine max (SBA) [β-N-acetyl-galactosamine (GalNAc)], Erythrina crystallina (ECA) [β-galactose (Gal) and β-GalNAc], Bondarzewia simplex (BS-1) [α-Gal and α-GalNAc], peanut agglutinin (PNA) [β-Gal-(1,3)-N-GalNAc], Sambucus nigra (SNA) [α-5-acetyleneuraminic acid (NeuAc)-(2,6)-Gal, α-NeuAc-(2,3)-Gal and α-NeuAc-(2,6)-GalNAc]. Lectins were dissolved in PBS containing 0.02% CaCl\(_2\) and 0.02% MgCl\(_2\) at a concentration of 0.5 mg mL\(^{-1}\).

**Preparation of lipopoligosaccharide and polysaccharide**

Biomass was harvested and polysaccharide molecules were extracted using an established mini-phenol water (MPh) procedure (Prendergast et al., 2001). Briefly, biomass harvested from plates or broth was washed in PBS and extracted using phenol at 65 °C. The water phase was retained and extracted three times with ethyl ether to remove residual phenol. The samples were then lyophilized and resuspended in distilled water to 2 mg mL\(^{-1}\).

**Electrophoresis**

Electrophoretic analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of MPh extracts was carried out on a separating gel of 15% (w/v) polyacrylamide gel electrophoresis (SDS-PAGE) of MPh extracts. After SDS-PAGE, Western blotting was carried out according to a standard methodology (Towbin et al., 1979) whereby fractionated PRCs were electrotransferred onto nitrocellulose membranes (pore size 0.45 μm, Bio-Rad). Membranes were probed with typing antiserum (Penner & Hennessey, 1980) or anti-GM\(_1\) antibody (Matreya Inc., Pleasant Gap, PA) (1 : 500) followed by peroxidase-conjugated goat anti-rabbit immunoglobulin G (Bio-Rad) (1 : 500), or with horseradish peroxidase (HRP)-conjugated cholera toxin B subunit (CT) (Sigma). Immunoreactants were visualized using the HRP development system (Bio-Rad).
Thin-layer chromatography (TLC) with immunostaining was performed as described by Schwerer et al. (1995). Developed TLC plates (Merck, Whitehouse Station, NJ) were air-dried for 30 min in a vacuum dessicator, fixed in 0.2% polyisobutylmethacrylate (Sigma) in n-hexane (BDH Laboratory Supplies, Poole, UK) for 1.5 min, and dried as before. Non-specific binding was reduced by submerging the plates for 1 h in PBS containing 0.3% gelatine (Bio-Rad). Subsequently, lanes were overlaid with HRP-conjugated CT ligand. Plates were incubated at 4°C overnight, washed three times with cold PBS, and the immunoreactants were visualized using the HRP development system.

Results and discussion

Campylobacter jejuni growth in differing growth phases

Using the OD\textsubscript{600 nm} readings obtained during culture, a growth curve was constructed for batch liquid cultures in Brucella broth of C. jejuni NCTC 11168. For batch cultivation at 37°C, the doubling time of cultures in exponential phase was estimated as 97.5 min, with a maximum specific growth rate (U\textsubscript{max}) of 0.43, and the culture entered stationary phase by 18 h of growth (Fig. 1). However, at 42°C, the doubling time of cultures in exponential phase was 82.65 min, but the culture had also entered stationary phase by 18 h of growth. Therefore, 18-h growth from batch cultures was used to produce PRC samples from stationary cells for analysis. In addition, to allow growth of exponential phase cells in greater quantities, the doubling time and U\textsubscript{max} estimated for exponential growth during batch cultivation were used to establish dilution and growth-rate parameters when setting up the chemostat. Exponential phase, planktonic C. jejuni NCTC 11168 cells, generated in the chemostat, were harvested from the liquid medium of the reaction chamber, and PRCs extracted from the exponential cells for analysis.

Polysaccharide production is considered crucial for the formation of biofilms by bacteria, as it allows the construction of the matrix that bacterial cells inhabit (Sutherland, 2001; Moran & Ljungh, 2003). In order to study biofilm formation during polysaccharide production by C. jejuni, glass slides were both submerged and inserted at the air-liquid interface of batch and chemostat cultures to provide a solid surface. Campylobacter jejuni biofilms did form in batch cultures, consistent with previous observations (Joshua et al., 2006) and, in the chemostat, C. jejuni NCTC 11168 preferentially formed a biofilm on glass slides at the air-liquid interface. By stepwise staining with Coomassie Blue (staining protein) and Ruthenium Red (staining carbohydrate), it was possible to discriminate between cells and polysaccharide present in the biofilm matrix. As shown in Fig. 2, the channels which are common to biofilm structures (Espinosa-Urgel, 2003) were observed, consistent with the ability of C. jejuni to form a biofilm on glass slides. Whether biofilm formation influences the pathogenesis of C. jejuni remains unclear, but differences in cell surface hydrophobicity between planktonic and biofilm cells of C. jejuni, and increased survivability of C. jejuni in biofilms have been reported (Dykes et al., 2003; Joshua et al., 2006). However, owing to insufficient biomass production as a biofilm to provide adequate material for detailed polysaccharide analysis, further examination could not be performed.

Nevertheless, in addition to growth in the liquid media, PRC production by NCTC 11168 grown on two solid media, Columbia blood agar and Brucella agar, both of which are routinely used in laboratory culturing of C. jejuni, was used to produce biomass for PRC analysis.

Lectin binding by bacteria grown under different conditions

Lectin binding was employed to determine changes in surface carbohydrate expression by C. jejuni cells grown under different conditions. This is a microscale technique that has previously been applied to investigate carbohydrate expression by C. jejuni (O’Sullivan et al., 1990), as well as polysaccharide production during biofilm formation (Staudt et al., 2003). A selected number of lectins were chosen for testing because of their ability to bind GlcNAc,

Fig. 2. Combined Coomassie Blue and Ruthenium Red staining of Campylobacter jejuni biofilm present on glass slides recovered from the chemostat reaction chamber after 4 days of growth. The bar represents 10\textmu m.
GalNAc, Gal, and NeuAc, which have been chemically shown to be expressed in *C. jejuni* lipooligosaccharide and polysaccharides (Prendergast & Moran, 2000; St Michael et al., 2002; Karlyshev et al., 2005a, b; McNally et al., 2005; Papp-Szabo et al., 2005). Binding of the lectins to red blood cells served as a positive control (Table 1).

Comparing the growth of NCTC 11168 on solid media, differences were observed in the lectin binding patterns of cells, reflecting the influence of medium composition on glycosylation (Table 1). Cells grown on Columbia agar bound the WGA and STA lectins strongly (both reactive with β-GlcNAc), whereas the binding of lectins BS-1 and SBA (reactive with α-Gal/GalNAc and β-GalNAc) was weak. Colonies on Brucella agar showed two morphologies: large colonies that contained predominantly spiral rods and c. 10% coccolid forms; and small colonies that contained a higher proportion of coccolid forms (c. 40%). From previous studies, these two cell morphologies are considered as viable rod and degenerate coccolid forms, respectively (Moran & Upton, 1986). Both types of colonies were ubiquitous on the agar plates, with no one type localizing to a specific area, and samples of whole colonies were taken for analysis. Importantly, whereas cells from the large colonies showed moderate binding of the WGA lectin, cells from the small colonies did not bind any lectins. This difference in the lectin binding profile between the two colony types may reflect a different composition of cell PRCs between rod and coccolid forms.

Likewise, in liquid culture (Table 1), lectin binding patterns were different for exponential phase cells (chemostat-grown) for stationary phase cells (batch-grown). The chemostat-grown cells bound the WGA lectin moderately and the STA lectin poorly, whereas the batch liquid-grown cells bound only the WGA lectin poorly. This change in lectin binding profile between exponential and stationary phase cells potentially reflects a dependence of PRC expression on growth phase. Overall, the lectin binding patterns observed with liquid or solid media-grown cells were consistent with previously observed lectin typing patterns of *C. jejuni* (O’Sullivan et al., 1990). Collectively, the data suggest a dependence of PRC expression on the growth phase of the cells, in addition to the culture growth conditions. Therefore, more detailed electrophoretic and TLC characterization, as well as serological probing, of the PRCs produced under the different conditions was performed.

**Electrophoretic analyses of PRCs**

Silver staining of polyacrylamide gels indicated that *C. jejuni* produced lipooligosaccharide (PRC 1) both at 37 °C and at 42 °C in exponential and stationary phase cells (in chemostat and batch liquid cultures), and on all solid media tested, as indicated by the low-Mr bands seen in Fig. 3a (arrow A). The two bands indicated in Fig. 3a, by arrow B are examples of aggregation of lipooligosaccharide owing to high concentration. This was confirmed by excision and re-electrophoresis of the bands.

Polyacrylamide gels stained with Alcian Blue revealed the presence of polysaccharide (PRC 2) in some but not all samples. Cells grown on Columbia agar, in the chemostat (exponential phase cells) and in batch liquid culture (stationary phase cells), produced polysaccharide with a pattern of 4–5 mid-Mr bands (Fig. 3b, arrow D). This banding pattern was also seen for polysaccharides from *C. jejuni* 81-176, 81116 and ATCC 43446 when grown on Columbia agar (data not shown). However, the banding pattern seen with stationary phase cells (batch liquid samples) of NCTC 11168 (Fig. 3b, arrow E) was more ladder-like than that for the Columbia agar or for chemostat samples, both of which had a more irregular pattern of bands. This was true at both 37 °C and 42 °C. Moreover, this difference in the

### Table 1. Lectin typing of cellular samples from *Campylobacter jejuni* NCTC 11168 grown on various media

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Columbia agar</th>
<th>Brucella agar</th>
<th>Chemostat-grown exponential cells</th>
<th>Batch culture stationary cells</th>
<th>Red blood cells (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WGA</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>STA</td>
<td>+++</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>BS-1</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td>+</td>
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<tr>
<td>SBA</td>
<td>+</td>
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<tr>
<td>ECA</td>
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<td>SNA</td>
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<td>PBS</td>
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*Red blood cells were used as a positive control.


2Reaction strength: –, none; +, weak; ++, moderate; ++++, strong.
characteristics of this polysaccharide was reflected in loss of reactivity of batch liquid samples with lectins STA, BS-1 and SBA that are reactive with β-GlcNAc, α-Gal/GalNAc and β-GalNAc. This potentially indicates phase variation in the capsular polysaccharide produced by NCTC 11168, as has been observed in C. jejuni 81–176 (Bacon et al., 2001).

When silver-stained, cells grown in liquid culture (both exponential and stationary phase cells) at 37°C produced a higher-Mr polysaccharide (PRC 3), which had an electrophoretic banding pattern resembling that of lipopolysaccharide (Fig. 3a, arrow C), although the identity of the molecule is unclear. Furthermore, this banding pattern was absent from PRC samples of cells grown at 42°C, indicating that expression of this molecule may be temperature-dependent.

Thin-layer chromatographic analyses of PRCs
Analysis by TLC using diphenylamine staining, which stains carbohydrate, showed that all MPh-extracted samples contained a band with an Rf value of 0.36, which has previously been shown to correspond to lipooligosaccharide (Fig. 4) (Prendergast et al., 2001). This confirmed the results of electrophoretic analysis. All samples showed bands that were red in colour except for the Columbia agar-grown sample, which was blue (Fig. 4), indicating that different carbohydrate-related structures are present in the samples.

Serological profiling of PRCs
In C. jejuni strains such as C. jejuni NCTC 11168, 81–176, 81116 and ATCC 43446, a mid-Mr, antigenic polysaccharide was reactive with homologous typing antisera upon Western blotting of MPh-extracted PRCs (data not shown). This is consistent with the electrophoretic observations that these strains contain a capsular polysaccharide, and confirms the dominant role of polysaccharide in the serospecificity of these strains.

Western blotting of MPh-extracted PRCs from NCTC 11168 using anti-GM1 antibodies or CT as ligand exhibited only low-Mr bands on the nitrocellulose membrane, with no high- or mid-Mr bands detectable (Fig. 5B, lanes 4 and 5).
Blue-stained polycrylamide gels (Karlyshev & Wren, 2001). Supporting the occurrence of capsular polysaccharide in C. jejuni, a lipid anchor for this polysaccharide has recently been identified in C. jejuni 81–176 (Corcoran et al., 2006), and capsule-like structures have been observed with electron microscopy (Karlyshev et al., 2001). In the present study, a polysaccharide (PRC2) was produced by C. jejuni NCTC 11168 that appeared as mid-Mr bands on Alcian Blue-stained polycrylamide gels, similar to those of capsular polysaccharide (Karlyshev et al., 2000). Also, this polysaccharide was not reactive with anti-GM1 antibodies or CT ligand on Western blots, consistent with the reported structure of a capsular polysaccharide in NCTC 11168 (St Michael, 2002), but was reactive with HS2 typing antiserum. However, although capsular polysaccharide was produced both on Columbia agar and in liquid culture, its structure appeared growth phase-dependent and may reflect phase-variable capsule expression (Bacon et al., 2001). Thus, further structural studies on the capsular polysaccharide of NCTC 11168 are required.

Another, previously undescribed, polysaccharide (PRC3) was produced by cells grown in liquid medium but not on solid media. When silver-stained, PRC3 had a high-Mr, ladder-like electrophoretic banding pattern that resembled that of lipopolysaccharide. Moreover, the mobility and staining properties of this PRC are similar to those of the neutral polysaccharide of C. jejuni 81116 (Muldoon et al., 2002), which has been tentatively identified as a capsule-independent lipopolysaccharide-like molecule responsible for serological cross-reaction in serotyping (Kilcoyne et al., 2006). Nevertheless, PRC3 was not reactive with anti-GM1 antibodies or CT ligand, nor with HS2 typing antiserum. The structure of the polysaccharide was independent of growth phase, as electrophoretic patterns were identical for exponential and stationary phase cells, but was temperature-dependent, being expressed at 37°C but not at 42°C. As routine laboratory culture of C. jejuni is performed on solid agar media, and because PRC3 is produced only in liquid culture by planktonic cells, this may in part explain why this polysaccharide has remained undetected in this strain. Consistent with the results of this study with C. jejuni NCTC 11168 showing the occurrence of a third PRC, preliminary observations of a Calcofluor White-reactive moiety, independent of capsular polysaccharide, have been reported in C. jejuni 81–176 (McLennan et al., 2005). Nevertheless, in C. jejuni 81–176, in addition to lipopolysaccharide and serodominant capsular polysaccharide, Papp-Szabo et al. (2005) have described a cell surface α-(1,4)-glucan that was inferred to be a second capsular polysaccharide, but data concerning the capsular lipid anchor were not provided.

In summary, our data suggest that a variety of PRCs are produced by C. jejuni and are influenced by growth conditions and growth phase. Hence, experimental pathogenic

Conclusions

Our analyses indicate that there appear to be three major PRCs produced by C. jejuni NCTC 11168. Lipopolysaccharide (PRC 1) was produced at 37°C and at 42°C by exponential and stationary phase cells in liquid cultures and on all solid media tested. Furthermore, the lipopolysaccharide of NCTC 11168 exhibited ganglioside mimicry that contained a GM1-like epitope. Previously, it was suggested that C. jejuni produced only lipopolysaccharide and antigenic capsular polysaccharide (Karlyshev et al., 2000), which was observed as mid-Mr bands on Alcian Blue-stained polycrylamide gels (Karlyshev & Wren, 2001). Supporting the occurrence of capsular polysaccharide in C. jejuni, a lipid anchor for this polysaccharide has recently been identified in C. jejuni 81–176 (Corcoran et al., 2006), and capsule-like structures have been observed with electron microscopy (Karlyshev et al., 2001). In the present study, a polysaccharide (PRC2) was produced by C. jejuni NCTC 11168 that appeared as mid-Mr bands on Alcian Blue-stained polycrylamide gels, similar to those of capsular polysaccharide (Karlyshev et al., 2000). Also, this polysaccharide was not reactive with anti-GM1 antibodies or CT ligand on Western blots, consistent with the reported structure of a capsular polysaccharide in NCTC 11168 (St Michael, 2002), but was reactive with HS2 typing antiserum. However, although capsular polysaccharide was produced both on Columbia agar and in liquid culture, its structure appeared growth phase-dependent and may reflect phase-variable capsule expression (Bacon et al., 2001). Thus, further structural studies on the capsular polysaccharide of NCTC 11168 are required.

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In summary, our data suggest that a variety of PRCs are produced by C. jejuni and are influenced by growth conditions and growth phase. Hence, experimental pathogenic

Conclusions

Our analyses indicate that there appear to be three major PRCs produced by C. jejuni NCTC 11168. Lipopolysaccharide (PRC 1) was produced at 37°C and at 42°C by exponential and stationary phase cells in liquid cultures and on all solid media tested. Furthermore, the lipopolysaccharide of NCTC 11168 exhibited ganglioside mimicry that contained a GM1-like epitope. Previously, it was suggested that C. jejuni produced only lipopolysaccharide and antigenic capsular polysaccharide (Karlyshev et al., 2000), which was observed as mid-Mr bands on Alcian Blue-stained polycrylamide gels (Karlyshev & Wren, 2001). Supporting the occurrence of capsular polysaccharide in C. jejuni, a lipid anchor for this polysaccharide has recently been identified in C. jejuni 81–176 (Corcoran et al., 2006), and capsule-like structures have been observed with electron microscopy (Karlyshev et al., 2001). In the present study, a polysaccharide (PRC2) was produced by C. jejuni NCTC 11168 that appeared as mid-Mr bands on Alcian Blue-stained polycrylamide gels, similar to those of capsular polysaccharide (Karlyshev et al., 2000). Also, this polysaccharide was not reactive with anti-GM1 antibodies or CT ligand on Western blots, consistent with the reported structure of a capsular polysaccharide in NCTC 11168 (St Michael, 2002), but was reactive with HS2 typing antiserum. However, although capsular polysaccharide was produced both on Columbia agar and in liquid culture, its structure appeared growth phase-dependent and may reflect phase-variable capsule expression (Bacon et al., 2001). Thus, further structural studies on the capsular polysaccharide of NCTC 11168 are required.

Another, previously undescribed, polysaccharide (PRC3) was produced by cells grown in liquid medium but not on solid media. When silver-stained, PRC3 had a high-Mr, ladder-like electrophoretic banding pattern that resembled that of lipopolysaccharide. Moreover, the mobility and staining properties of this PRC are similar to those of the neutral polysaccharide of C. jejuni 81116 (Muldoon et al., 2002), which has been tentatively identified as a capsule-independent lipopolysaccharide-like molecule responsible for serological cross-reaction in serotyping (Kilcoyne et al., 2006). Nevertheless, PRC3 was not reactive with anti-GM1 antibodies or CT ligand, nor with HS2 typing antiserum. The structure of the polysaccharide was independent of growth phase, as electrophoretic patterns were identical for exponential and stationary phase cells, but was temperature-dependent, being expressed at 37°C but not at 42°C. As routine laboratory culture of C. jejuni is performed on solid agar media, and because PRC3 is produced only in liquid culture by planktonic cells, this may in part explain why this polysaccharide has remained undetected in this strain. Consistent with the results of this study with C. jejuni NCTC 11168 showing the occurrence of a third PRC, preliminary observations of a Calcofluor White-reactive moiety, independent of capsular polysaccharide, have been reported in C. jejuni 81–176 (McLennan et al., 2005). Nevertheless, in C. jejuni 81–176, in addition to lipopolysaccharide and serodominant capsular polysaccharide, Papp-Szabo et al. (2005) have described a cell surface α-(1,4)-glucan that was inferred to be a second capsular polysaccharide, but data concerning the capsular lipid anchor were not provided.

In summary, our data suggest that a variety of PRCs are produced by C. jejuni and are influenced by growth conditions and growth phase. Hence, experimental pathogenic
Influence of growth conditions on polysaccharide production by *C. jejuni*

Studies when investigating the role of polysaccharides and glycolipids in *C. jejuni* should consider the ability of this bacterium to produce different PRCs under differing growth conditions.

Acknowledgements

This work was funded by the National Development Plan under the Higher Education Authority (HEA) PRTL-3 programme and the Irish Health Research Board. We thank Prof. T. Kosunen (University of Helsinki, Finland) for providing typing sera, and Dr G. Fleming (NUi Galway, Ireland) for his advice on the chemostat work.

References


