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Campylobacter jejuni strain discrimination and temperature-dependent glycome expression profiling by lectin microarray

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ABSTRACT

Gram-negative *Campylobacter jejuni* is the leading cause of bacterial gastroenteritis in humans worldwide and the most frequently identified infectious trigger in patients developing Guillain–Barré syndrome (GBS). While *C. jejuni* is pathogenic in humans, it is a commensal in avian hosts. Bacterial cell surface carbohydrates are important virulence factors and play roles in adherence, colonisation and infection. The mechanisms leading to infection or persistent colonisation of *C. jejuni* are not well understood but host temperature may provide an important stimulus for specific adaptation. Thus, examination of the modulation of the total surface glycome of *C. jejuni* in response to temperature may help shed light on commensal and pathogenic mechanisms for this species. *C. jejuni* strains 81116 and 81-176 were cultured at 37 and 42 °C to simulate human and avian host conditions, respectively, and whole cells were profiled on lectin microarrays constructed to include a wide range of binding specificities. *C. jejuni* 81116 profiles indicated that the previously characterised lipopolysaccharide (LPS)-like molecule and *N*-linked glycans were the predominantly recognised cell surface structures while capsular polysaccharide (CPS), lipooligosaccharides (LOS) and *N*-linked glycosylation were best recognised for strain 81-176 at 37 °C. The profiles of both strains varied and were distinguishable at both temperatures. At the higher temperature, reduced dominance of the LPS-like structure was associated with strain 81116 and a change in the relative distribution of CPS and LOS structures was indicated for strain 81-176. This change in LOS molecular mass species distribution between temperatures was confirmed by SDS–PAGE analysis. Additionally, opposite behaviour of certain lectins was noted between the plate agglutination assay and the microarray platform. Insights into the important glycosylation involved in *C. jejuni* host cell tropism at different growth temperatures were gained using the lectin microarray platform.

1. Introduction

Gram-negative *Campylobacter jejuni* is the leading cause of bacterial gastroenteritis in humans worldwide and is the most frequently identified primary antecedent infection associated with Guillain–Barré syndrome (GBS), the debilitating autoimmune neuropathy characterised by progressive paralysis.[1,2] Consequently, *C. jejuni* infection represents a significant health and economic burden worldwide.[2] While *C. jejuni* is pathogenic in humans, it is a commensal in many avian hosts and colonises in high numbers asymptomatically.[3] Consumption of contaminated food products, particularly poultry, is the main route of infection.[3] The mechanisms which lead to infection or persistent colonisation of *C. jejuni* in humans or chickens, respectively, are poorly understood. *C. jejuni* displays an extensive array of cell-surface glycoconjugates, having distinct biosynthetic loci for the expression of lipooligosaccharide (LOS)/lipopolysaccharide (LPS), and capsular polysaccharide (CPS), as well as protein *O*- and *N*-linked glycosylation.[4–6] As virulence factors, *C. jejuni* LOS and CPS play a role in adhesion and invasion of host cells and protection from host immune defences.[7–10] Flagellar *O*-glycosylation[9,10] and *N*-linked surface protein glycosylation[4] (Fig. 1) have also been identified as important virulence factors.[3] Antigenic variability of the cell surface glycoconjugates,[7–9] as well as structural mimicry of host glycans in the LOS of certain *C. jejuni* strains,[2] constitute immune evasion strategies. In particular, molecular mimicry of gangliosides in *C. jejuni* LOS is thought to elicit the production of cross-reactive anti-ganglioside antibodies which are involved in GBS pathogenesis.[1]

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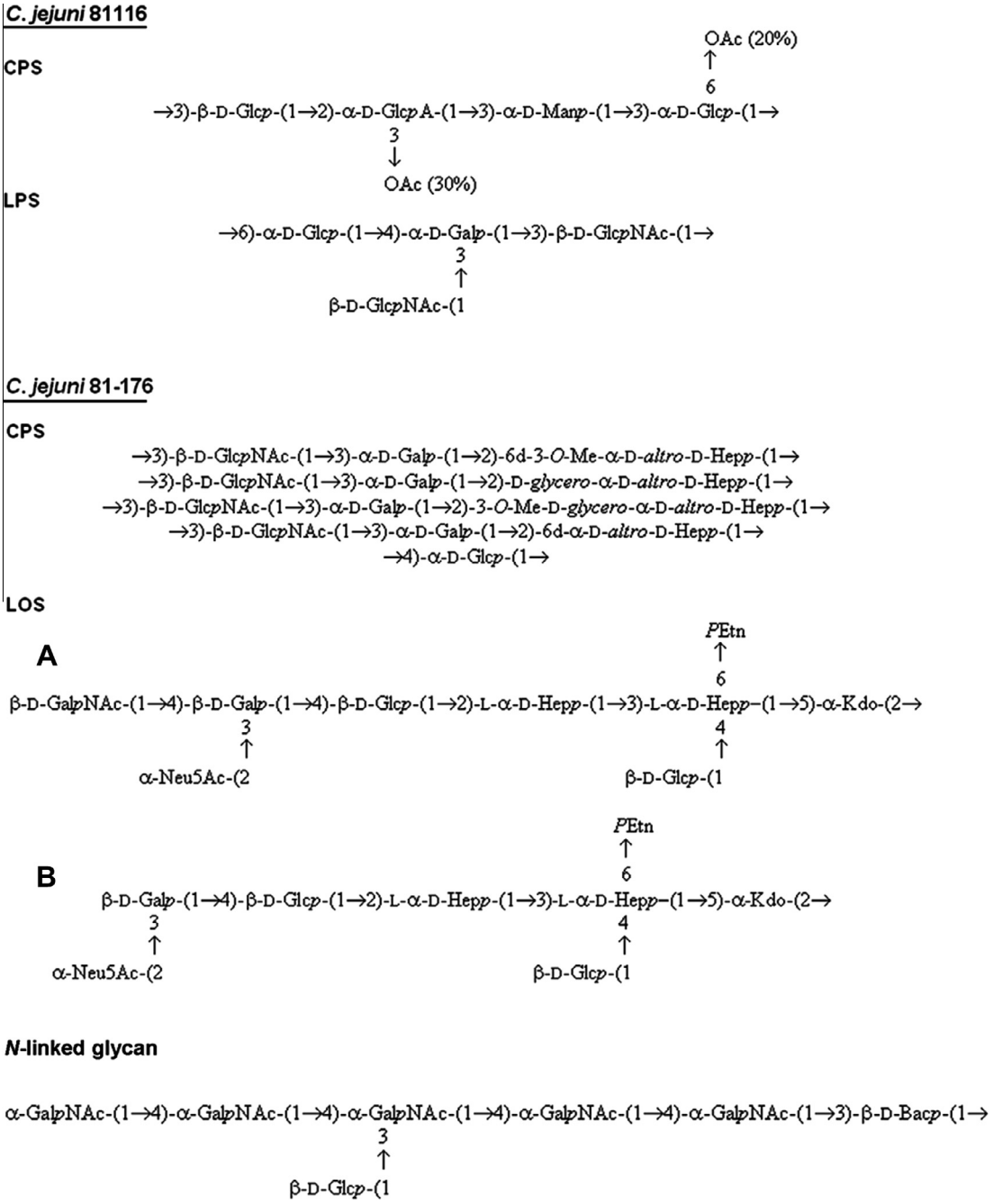

Figure 1. Polysaccharide structures from capsular (CPS) and lipopolysaccharide (LPS) from *C. jejuni* 81116,[16,17] CPS[13,60] and predominant 3.6 and 3.8 kDa lipooligosaccharide (LOS) core structures from *C. jejuni* 81-176.[7,52] The Gal residue of *C. jejuni* 81-176 can be substituted by non-stoichiometric amounts of *O*-methyl phosphoramidate (MeOPN) at C-2.[58] (A) GM_2-like predominant component of 3.8 kDa LOS core and minor component of 3.6 kDa LOS core. (B) GM_3-like predominant component of the 3.6 kDa LOS core. The conserved *N*-linked glycan structure[4] of *C. jejuni* is depicted at the bottom of the figure, where Bac is bacillosamine (2,4-diacetamido-2,4,6-trideoxyglucopyranose) and is linked to asparagine.

Considerable intra-species variation can exist within *C. jejuni*.[3] The extensively studied, genome-sequenced strains *C. jejuni* 81-176 and 81116, originally isolated from human hosts, are genetically and phenotypically distinct.[11,12] The highly virulent 81-176 strain, which belongs to serogroup HS:23/36 and was originally isolated from a raw milk-borne case of colitis, is hyper-invasive to intestinal cells *in vitro*.[11] It produces two independent CPSs, one of which is phase variable[8,13] (Fig. 1), and phase variable gene expression enables the potential production of LOS structures which mimic the gangliosides GM_2, GM_3, GD_{1b} and GD_2.[7] *C. jejuni* 81116 types as both HS:6 and HS:7, was first isolated in a waterborne case of gastroenteritis and has comparatively low invasiveness.[14,15] This strain was found to express two independent polysaccharides, one LPS-related and the other CPS-derived[16,17] (Fig. 1), and may also express an LOS-like molecule.[6]

Among the many physiological differences between the human and chicken host environment, core body temperature (37 and 42 °C in humans and chickens, respectively) is known to affect *C. jejuni* genetically and phenotypically.[3] Differential transcription of numerous *C. jejuni* genes, including genes involved in modulation of the cell surface glycosylation, have been observed due to temperature shift from 37 to 42 °C.[18] Temperature-related