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Structural studies of the O-antigenic polysaccharides from the enteroaggregative *Escherichia coli* strain 87/D2 and international type strains from *E. coli* O128

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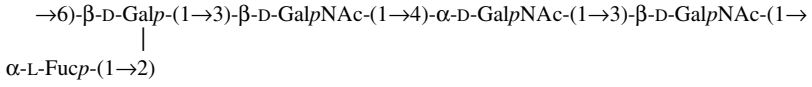
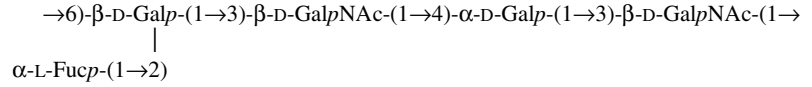
Abstract—The O-antigen of the lipopolysaccharide (LPS) from the enteroaggregative *Escherichia coli* strain 87/D2 has been determined by component analysis together with NMR spectroscopy. The polysaccharide has pentasaccharide repeating units in which all the residues have the *galacto*-configuration. The repeating unit of the O-antigen, elucidated using the O-deacylated LPS, is branched with the following structure:



Analysis of the ^{1}H NMR spectrum of the LPS revealed *O*-acetyl groups (~0.7 per repeating unit) distributed over two positions. Subsequent analysis showed that the galactose residue carries acetyl groups at either *O*-3 or *O*-4 in a ratio of ~2:1. The international reference strain from *E. coli* O128ab was investigated and the repeating unit of the O-antigens has the following structure:



Analysis of the ^{1}H NMR spectrum of the LPS revealed *O*-acetyl groups (approximately one per repeating unit) distributed over two positions. The integrals of the resonances for the *O*-acetyl groups indicated similarities between the O-antigen from *E. coli* O128ab and that of *E. coli* strain 87/D2, whereas the *O*-acetyl substitution pattern in the *E. coli* O128ac O-antigen differed slightly. Enzyme immunoassay using specific anti-*E. coli* O128ab and anti-*E. coli* O128ac rabbit sera confirmed the results.

Keywords: Escherichia coli; Enteroaggregative; Serology; Lipopolysaccharide; NMR; Biological repeating unit

1. Introduction

Enteroaggregative *Escherichia coli* (EAEC) is defined as *E. coli* that do not secrete LT or ST enterotoxins and adhere to HEp-2 cells in an aggregative (AA) pattern.[1,2] The basic strategy of EAEC seems to comprise colonization of the intestinal mucosa, probably predominantly that of the colon, followed by secretion of enterotoxins and cytotoxins.[1] Studies on human intestinal specimens indicate that EAEC induces mild, but significant, mucosal damage.[3] The clinical features of EAEC diarrhoea are increasingly well defined in outbreaks, sporadic cases and the volunteer model. A growing number of studies

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have supported the association of EAEC with diarrhoea in developing countries, most prominently in association with persistent diarrhoea.[4–8] Previous studies in children less than five years of age, all with diarrhoea or acute diarrhoea, have shown a significant difference of the EAEC prevalence compared to the controls.[9–13] The increasing number of such reports and the rising proportion of diarrhoeal cases in which EAEC is implicated suggest that this pathotype is an important emerging agent of paediatric diarrhoea. The serogroups that have been identified within the EAEC group are O3, O7, O15, O44, O77, O86, O111, O126 and O127. However, serotyping of EAEC is problematic. Due to their aggregative phenotype, many of the strains auto-agglutinate and are often described in the literature as non-typeable or as O-rough. It is also well established that the EAEC group is highly heterogeneous. In a study by Huppertz et al., EAEC from German children was analyzed and it was found that of the 14 typeable isolates all belonged to 14 different serogroups.[14] In a study by Jenkins et al., 93 out of 143 EAEC strains could be serotyped and belonged to as many as 47 different serogroups.[15] In recent years, the O-antigen structures of several EAEC strains have been determined.[16–22] In these cases, when the EAEC strain was serotyped and the O-antigen from the *E. coli* reference strain was studied, it was shown that the structure of the EAEC O-antigen was identical to the type strain, but for EAEC strain 180/C3 which subsequently was serotyped as *E. coli* O5ac.[21] Here, we report on the structures of the O-antigens from EAEC strain 87/D2 (serotyped as *E. coli* O128ab) and the international reference strains from *E. coli* O128ab and O128ac.

2. Results and discussion

Analysis of the structure of O-antigen polysaccharides has in most cases been performed on materials that have been prepared by delipidation under mild acidic conditions to yield a polysaccharide (PS). It has also been possible to analyze the intact LPS and still obtain the structure of the O-antigen polysaccharide.[23] In some cases, good preparations are obtained upon O-deacylation in which base-labile ester-linked acyl-chains and *O*-acetyl groups are removed under basic conditions.[24] The material thus prepared is often referred to as an LPS-OH.[25]

The enteroaggregative *E. coli* strain 87/D2 was grown in a tryptone/yeast extract medium and the LPS was isolated from the bacterial membrane by hot phenol/water extraction. A material suitable for NMR spectroscopic analysis was obtained by O-deacylation. The ^{1}H NMR spectrum of *E. coli* strain 87/D2 LPS-OH showed, inter alia, resonances indicative of three *N*-acetyl groups at δ_H 2.01, 2.03 and 2.05 (Fig. 1).

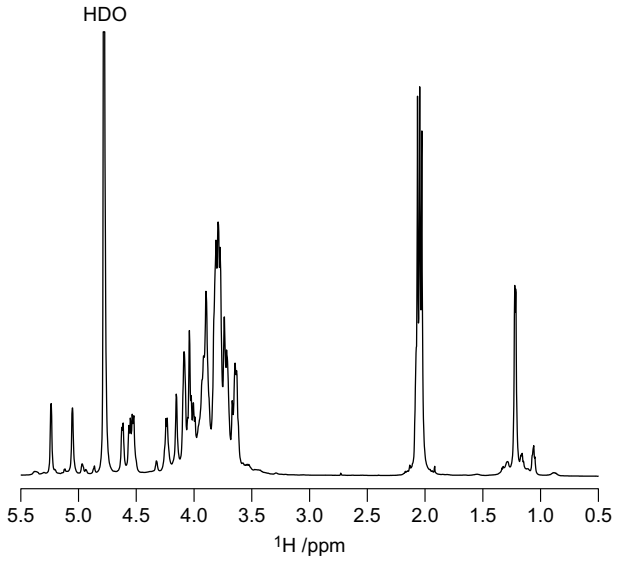
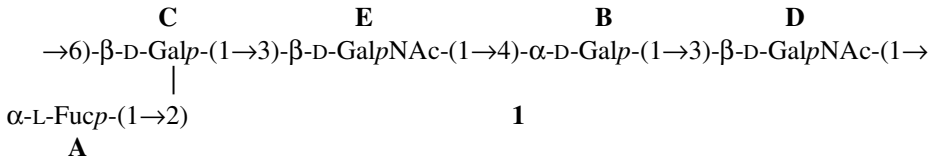


Figure 1. ^{1}H NMR spectrum of the LPS-OH from *E. coli* strain 87/D2.

Serotyping of enteroaggregative *E. coli* may be elusive.[26] *E. coli* strain 87/D2 was serotyped at Statens Serum Institute, Copenhagen, Denmark as O128ab:H27. Previously, there was a report by Sengupta et al. on the structural determination of the O-antigen polysaccharide from a strain of *E. coli* O128.[27] It contains branched pentasaccharide repeating units with two *N*-acetylgalactosamine residues and the H-type blood group antigen determinant.[28] Thus, the O-antigen from *E. coli* strain 87/D2 should differ from that previously reported for *E. coli* O128. Prior to any further studies on the former strain, we investigate the O-antigen structure of the international reference strains from *E. coli* O128ab and O128ac.

E. coli O128ab was grown in a Luria Bertani medium and the LPS was isolated from the bacterial membrane by hot phenol/water extraction. A material suitable for NMR spectroscopic analysis was also in this case obtained by O-deacylation. The ^{1}H NMR spectrum of *E. coli* O128ab LPS-OH showed, inter alia, resonances from two *N*-acetyl groups at δ_H 2.05. A hydrolysate of the LPS contained fucose, glucose, galactose, 2-amino-2-deoxyglucose, 2-amino-2-deoxygalactose and heptose in the ratio 22:14:34:3:20:6. The ^{1}H,^{13}C-HSQC NMR spectrum of the *E. coli* O128ab LPS-OH is shown in Figure 2. The ^{1}H and ^{13}C NMR resonances were assigned using 2D NMR techniques and the chemical shifts as well as sequential information obtained from ^{1}H,^{1}H-NOESY and ^{1}H,^{13}C-HMBC experiments are compiled in Table 1. Assuming that fucose has the L-configuration and galactose and galactosamine has the D-configuration, the structure of the repeating unit of the O-antigen polysaccharide from *E. coli* O128ab is given by **1**.

This is the same O-antigen structure that was presented for *E. coli* O128. However, in the ^{1}H NMR



spectrum of *E. coli* O128ab LPS, resonances from *O*-acetyl groups (approximately one per repeating unit) were observed at δ_H 2.16 and 2.18 in a ratio of ~2:1. Furthermore, a difference is present for key NMR assignments for the H-6 protons of the 2,6-substituted galactosyl residue.

The structure of the O-antigen repeating unit of *E. coli* O128ac was also analyzed. A hydrolysate of the LPS contained fucose, glucose, galactose, 2-amino-2-deoxyglucose, 2-amino-2-deoxygalactose and heptose in the ratio 16:24:34:2:21:3. The ^{1}H,^{13}C-HSQC spectrum of *E. coli* O128ac as an LPS-OH indicated identity to that of *E. coli* O128ab. Thus, the O-antigen should have structure **1**. In the ^{1}H NMR spectrum of *E. coli* O128ac LPS, resonances from *O*-acetyl groups (approximately one per repeating unit) were observed at δ_H 2.15 and 2.17, but in a ratio of ~1:2 instead. However, this ratio differed between LPS preparations. The NMR results suggest that the differences between the O-antigens from *E. coli* O128ab and O128ac derive from their *O*-acetyl substitution patterns.

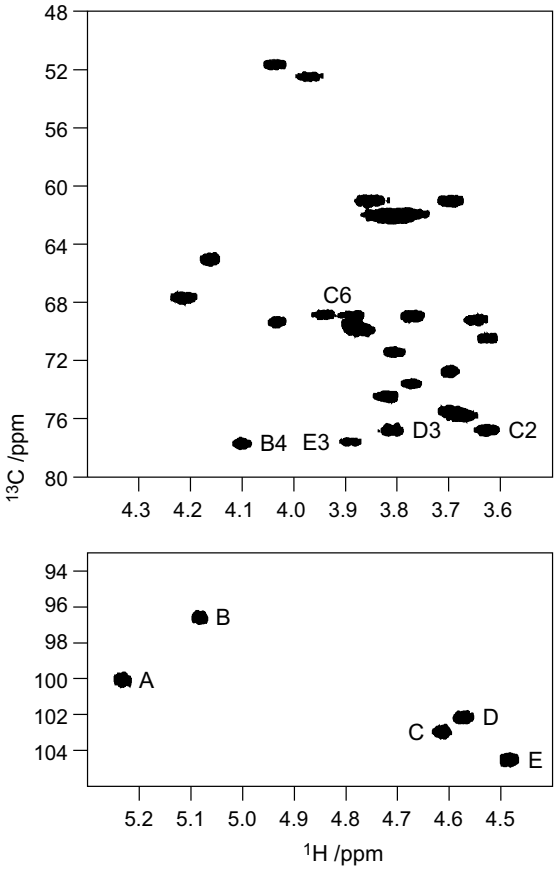


Figure 2. Part of the ^{1}H,^{13}C-HSQC NMR spectrum of the LPS-OH from *E. coli* O128ab.

Additionally, *E. coli* O128:K(67):H2 was also investigated. A material was prepared by delipidation of the LPS under mild acidic conditions to yield a PS devoid of *O*-acetyl groups as revealed by the ^{1}H NMR spectrum. The ^{1}H,^{13}C-HSQC spectrum revealed identity to that from the *E. coli* O128ab O-antigen. Consequently, the repeating unit of the O-polysaccharide also has structure **1**. In the ^{1}H NMR spectrum from the O-antigen polysaccharide presented by Sengupta et al., *O*-acetyl groups were not present. These two PS materials were prepared in comparable ways, which may account for their similarities. However, in the ^{1}H NMR spectrum of the *E. coli* O128:K(67):H2 LPS, resonances from *O*-acetyl groups (approximately one per repeating unit) were observed at δ_H 2.15 and 2.18 in a ratio of ~2:1. This finding is in complete agreement with the above since the strain has been serotyped O128ab:H2.

We now return to the analysis of the O-antigen polysaccharide from *E. coli* strain 87/D2. A hydrolysate of the LPS contained fucose, glucose, galactose, 2-amino-2-deoxyglucose, 2-amino-2-deoxygalactose and heptose in the ratio 22:12:22:4:35:4. Determination of the absolute configuration showed that fucose has the L-configuration whereas galactose and galactosamine the D-configuration. In the ^{1}H NMR spectrum, five resonances were observed in the anomeric region, viz., at 5.22, 5.04, 4.60, 4.54 and 4.51 ppm (Fig. 1). As indicated from above, the aminosugars are N-acetylated. The ^{1}H,^{13}C-HSQC NMR spectrum showed in the region for anomeric resonances five cross-peaks corresponding to hexopyranosyl residues (Fig. 3). Resonances at δ_C 50.4, 51.5 and 52.2 confirmed the presence of three aminosugars in the repeating unit of the O-antigen. The ^{1}H and ^{13}C NMR resonances were assigned using two-dimensional NMR techniques and the chemical shifts are compiled in Table 2. The assignments of the ^{13}C chemical shifts of the methyl groups of the *N*-acetyl groups in the aminosugars are based on chemical shift similarities to the corresponding monosaccharides. Residues **A** and **B** are α-linked as $J_{H-1,H-2}$ and $J_{H-1,C-1}$ coupling constants are ~4 Hz and ~174 Hz, respectively, and residues **C**–**E** are β-linked as the corresponding coupling constants are ~8 Hz and ~162 Hz,

Table 1. ^{1}H and ^{13}C NMR chemical shifts (ppm) at 25 °C of the resonances from the O-antigen polysaccharide of *E. coli* O128ab and inter-residue correlations from $^{1}H,^{1}H$-NOESY and $^{1}H,^{13}C$-HMBC spectra

Sugar residue		^{1}H /^{13}C						Correlation to atom (from anomeric atom)	
		1	2	3	4	5	6	NOE	HMBC
α-L-Fuc*p*-(1→	**A**	5.23 [3.9] (0.03) 100.0 {176} (6.9)	3.76 (−0.01) 68.9 (−0.2)	3.62 (−0.24) 70.5 (0.2)	3.70 (−0.11) 72.7 (−0.1)	4.21 (0.01) 67.7 (0.6)	1.20 (−0.01) 16.2 (−0.1)	H-2, **C**	C-2, **C** H-2, **C**
→4)-α-D-Gal*p*-(1→	**B**	5.08 [4.0] (−0.14) 96.6 {172} (3.4)	3.64 (−0.14) 69.5 (0.2)	3.88 (0.07) 69.7 (−0.4)	4.10 (0.15) 77.7 (7.4)	3.80 (−0.23) 71.4 (0.1)	3.70, 3.86 61.0 (−1.0)	H-3, **D** H-4, **D**	C-3, **D** H-3, **D**
→2,6)-β-D-Gal*p*-(1→	**C**	4.61 [7.6] (0.08) 103.0 {163} (5.6)	3.62 (0.17) 76.8 (3.8)	3.82 (0.23) 74.4 (0.6)	3.88 (−0.01) 69.7 (0.0)	3.77 (0.12) 73.6 (−2.3)	3.88, 3.94 68.8 (7.0)	H-3, **E**	C-3, **E**
→3)-β-D-Gal*p*NAc-(1→[a]	**D**	4.57 [8.4] (−0.11) 102.2 {164} (5.9)	4.04 (0.14) 51.6 (−3.2)	3.81 (0.04) 76.8 (4.8)	4.16 (0.18) 65.0 (−3.9)	3.68 (−0.04) 75.8 (−0.2)	∼3.80 62.0 (0.1)	H-6, **C**	C-6, **C**
→3)-β-D-Gal*p*NAc-(1→[a]	**E**	4.48 [8.2] (−0.20) 104.6 {161} (8.3)	3.97 (0.07) 52.4 (−2.4)	3.89 (0.12) 77.6 (5.6)	4.03 (0.05) 69.3 (0.4)	3.70 (−0.02) 75.5 (−0.5)	∼3.80 62.0 (0.1)	H-4, **B**	C-4, **B** H-4, **B**

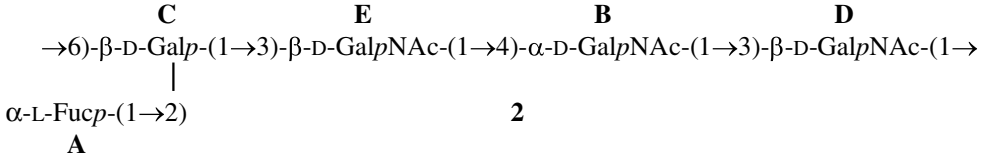
$J_{H-1,H-2}$ values are given in Hz in square brackets and $J_{H-1,C-1}$ values in braces. Chemical shift differences as compared to the corresponding monosaccharides are given in parenthesis.

[a] Chemical shifts for NAc are δ_H 2.05; δ_C 23.2 and 175.1.

respectively. The chemical shifts of resonances from C-2 atoms show that residues **B**, **D** and **E** are the galactosamine residues.

Substitution positions and the linkage patterns for the sugar residues may be identified by ^{13}C glycosylation shifts.[29] Residue **A** shows a significant carbon-13 chemical shift deviation only for its C-1 resonance. Thus, it is a terminal α-L-Fuc*p* residue. Residue **B** has a large glycosylation shift of $\Delta\delta_C$ 6.2 for C-4, which reveals it to be →4)-α-D-Gal*p*NAc-(1→. Residue **C** was assigned to →2,6)-β-D-Gal*p*-(1→ due to the glycosylation shifts at C-2 and C-6, $\Delta\delta_C$ 3.8 and 7.0, respectively. Residues **D** and **E** both have significant positive glycosylation shifts for their C-3 resonances, $\Delta\delta_C$ 4.1 and 5.3, respectively, demonstrating them both to be →3)-β-D-Gal*p*-NAc-(1→. Notably, chemical shift displacements were observed for C-4 in **D**, $\Delta\delta_C$ –4.2, and a low glycosylation shift for C-1 in **B**, $\Delta\delta_C$ 2.8. These chemical shift deviations should be due to the proximity between the anomeric proton in residue **B** and H-4 in residue **D** (cf. NOE in Table 2), also referred to as a γ-gauche effect.[30]

The sequence of the sugar residues in the O-antigen repeating unit of *E. coli* strain 87/D2 was determined from $^{1}H,^{1}H$-NOESY (Fig. 4) and $^{1}H,^{13}C$-HMBC experiments (Table 2). Sequential correlations were present between all sugar residues in the repeating unit. Consequently, the structure of the repeating unit of the O-antigen polysaccharide from *E. coli* strain 87/D2 is given by **2**:



2

In the ^{1}H NMR spectrum of *E. coli* strain 87/D2 LPS, resonances from 0.7 *O*-acetyl groups were observed at δ_H 2.16 and 2.19 in a ratio of ∼2:1, similar to those from the *E. coli* O128ab LPS.

From *E. coli* strain 87/D2, a PS material was also prepared by delipidation under mild acidic conditions. The ^{1}H NMR spectrum showed the presence of, inter alia, resonances from *O*-acetyl groups δ_H 2.18 (0.4) and 2.20 (0.3) in accordance with the above results. In the ^{13}C NMR spectrum, resonances from *O*-acetyl groups

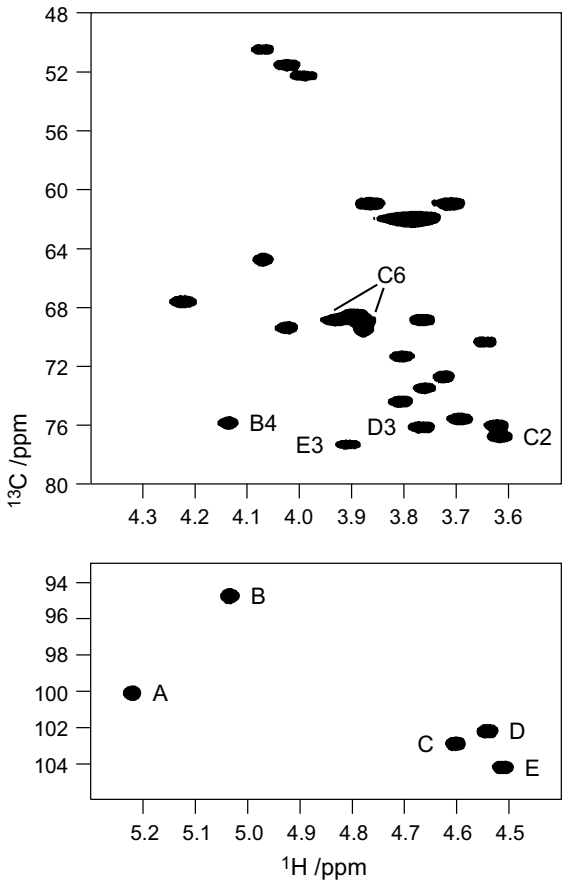


Figure 3. Part of the ^{1}H,^{13}C-HSQC spectrum of the LPS-OH from *E. coli* strain 87/D2.

were observed, inter alia, at δ_C 21.3 and 21.5 in a ratio of ~2:1, as well as 174.1 and 174.4 in a ratio of ~1:2. Furthermore, resonances at δ_C 99.7 and 100.5 as well as at δ_C 102.0 and 102.4 indicated that the chemical shifts of C-1 in residues **A** and **D**, respectively, were altered as a result of *O*-acetyl substitution, that is, the residues substituting the branching sugar. ^{1}H and ^{13}C NMR chemical shift displacements upon *O*-acetyl substitution of sugar residues are indicative of the position esterified.[31] 2D NMR analysis using ^{1}H,^{1}H-TOCSY and multiplicity-edited ^{1}H,^{13}C-HSQC experiments were used to unravel the substitution patterns of the *O*-acetyl groups. In the ^{1}H,^{1}H-TOCSY spectra spin systems were identified that correspond to H-1 to H-4 with δ_H 4.74, 3.87, 5.00 and 4.08, respectively, as well as H-1 at δ_H 4.71 and H-4 at δ_H 5.29. From the ^{1}H,^{13}C-HSQC spectrum, the cross-peaks corresponding to the ^{13}C chemical shifts were extracted, namely C-1 to C-4, in the first case having δ_C 103.2, 73.5, 77.2 and 67.6, respectively. In the second case, the chemical shifts were present at δ_C 103.2 for C-1 and δ_C 72.4 for C-4. Thus, these chemical shifts render **C** as the *O*-acetyl substituted residue. In addition, ^{13}C-resonances were present for 6-substituted residues at δ_C 69.3, 68.4 and 68.1. Application of the ^{1}H and ^{13}C NMR chemical shift displacements due to *O*-acetyl substitution, starting from the chemical shifts of the branching sugar residue **C**, revealed excellent agreement between those calculated from substituent effects and the experimentally observed ones. In Figure 5, resonances are shown from the significantly deshielded ring protons, $\Delta\delta_H \sim 1.3$, on the acetyloxylated carbons, $\Delta\delta_C \sim 2.5$, resulting from acetylation of either *O*-3 or *O*-4 in residue **C**. Consequently, three different forms are present as a result of *O*-acetyl modification, namely in residue **C** there are 3-OAc and 4-OAc substitutions at the two adjacent positions (the former being more occupied) as well as a non-substituted variant.

Acetyl groups are an important part of O-antigen structures when present due to their associated immunogenicity.[32] Removal of the *O*-acetyl groups leads to loss of antigenicity as well as alteration of serogroup specificity.[33,34] In the recent analysis of the genome of *E. coli* O128 (ATCC 12810) performed by Shao et al.,[35] subsequent to the O-antigen determination of *E. coli* O128 by Sengupta et al., they proposed the presence of a putative *O*-acetyl tranferase, denoted WbsI, due to the high level of homology to microbial acetyl transferases. As the presented structure at the time did not contain any *O*-acetyl groups, they simply concluded that its action was yet to be determined. Our results presented herein for four *E. coli* O128 strains, all of which contain *O*-acetyl groups, now clarify that there must be an active *O*-acetyl transferase expressed in *E. coli* O128, as postulated by Shao et al.

To further investigate the similarity between the structures of the O-antigens present in the three strains, an indirect enzyme immunoassay was used. Titrations of the rabbit anti-*E. coli* O128ab (Fig. 6a) and anti-*E. coli* O128ac (Fig. 6b) sera against the corresponding LPS as well as against the 87/D2 LPS show a higher reactivity of the 87/D2 LPS with the O128ab antibodies as compared to the anti-O128ac serum. However, the reactivity is weaker as compared to the corresponding O128ab LPS confirming the structural difference between the two strains. The anti-*E. coli* O128ac serum seems to be more specific for the homologous O128ac LPS (Fig. 6b) showing that the specificity of the antibodies is dependent on the O-acetylation pattern. The higher activity of the anti-O128ab as compared to the anti-O128ac antibodies against the 87/D2 LPS may be due to the similarities in the acetylation patterns in the two LPS.

It is surmised that the repeating units of these O-antigens have an *N*-acetyl-D-galactosamine residue at their reducing ends.[26] Consequently the H-type disaccharide antigen is then present at the terminal non-reducing end of the O-antigen polysaccharide where it should be readily accessible to antibody recognition. There is a close structural relationship between the O-antigens of *E. coli* O128ab and O128ac, the difference being in

Table 2. ^{1}H and ^{13}C NMR chemical shifts (ppm) at 25 °C of the resonances from the O-antigen polysaccharide of *E. coli* strain 87/D2 and inter-residue correlations from $^{1}H,^{1}H$-NOESY and $^{1}H,^{13}C$-HMBC spectra

Sugar residue		^{1}H /^{13}C						Correlation to atom (from anomeric atom)	
		1	2	3	4	5	6	NOE	HMBC
α-L-Fuc*p*-(1→	**A**	5.22 [4.0]	3.76	3.64	3.72	4.22	1.21	H-2, **C**	C-2, **C**
		(0.02)	(−0.01)	(−0.22)	(−0.09)	(0.02)	(0.00)		
		100.1 {174}	68.8	70.3	72.7	67.6	16.4		H-2, **C**
		(7.0)	(−0.3)	(0.0)	(−0.1)	(0.5)	(0.1)		
→4)-α-D-Gal*p*NAc-(1→[a]	**B**	5.04 [3.8]	4.07	3.89	4.13	3.80	3.71, 3.87	H-3, **D**	C-3, **D**
		(−0.24)	(−0.12)	(−0.06)	(0.08)	(−0.33)		H-4, **D**	
		94.8 {173}	50.4	68.4	75.8	71.3	61.0		H-3, **D**
		(2.8)	(−0.8)	(0.0)	(6.2)	(−0.1)	(−1.1)		
→2,6)-β-D-Gal*p*-(1→	**C**	4.60 [7.7]	3.62	3.80	3.88	3.76	3.87, 3.93	H-3, **E**	C-3, **E**
		(0.07)	(0.17)	(0.21)	(−0.01)	(0.11)			
		102.9 {161}	76.8	74.4	69.5	73.5	68.8		H-3, **E**
		(5.5)	(3.8)	(0.6)	(−0.2)	(−2.4)	(7.0)		
→3)-β-D-Gal*p*NAc-(1→[b]	**D**	4.54 [8.4]	4.02	3.76	4.07	3.62	∼3.78	H-6, **C**	C-6, **C**
		(−0.14)	(0.12)	(−0.01)	(0.09)	(−0.10)			
		102.2 {163}	51.5	76.1	64.7	76.0	62.0		
		(5.9)	(−3.3)	(4.1)	(−4.2)	(0.0)	(0.1)		
→3)-β-D-Gal*p*NAc-(1→[c]	**E**	4.51 [8.5]	3.99	3.91	4.02	3.69	∼3.78	H-4, **B**	C-4, **B**
		(−0.17)	(0.09)	(0.14)	(0.04)	(−0.03)			
		104.2 {162}	52.2	77.3	69.4	75.6	62.0		H-4, **B**
		(7.9)	(−2.6)	(5.3)	(0.6)	(−0.4)	(0.1)		

$J_{H-1,H-2}$ values are given in Hz in square brackets and $J_{H-1,C-1}$ values in braces. Chemical shift differences as compared to the corresponding monosaccharides are given in parenthesis.

[a] Chemical shifts for NAc are δ_H 2.03; δ_C 22.9 and 175.0.

[b] Chemical shifts for NAc are δ_H 2.01; δ_C 23.2 and 175.1.

[c] Chemical shifts for NAc are δ_H 2.05; δ_C 23.4 and 175.3.

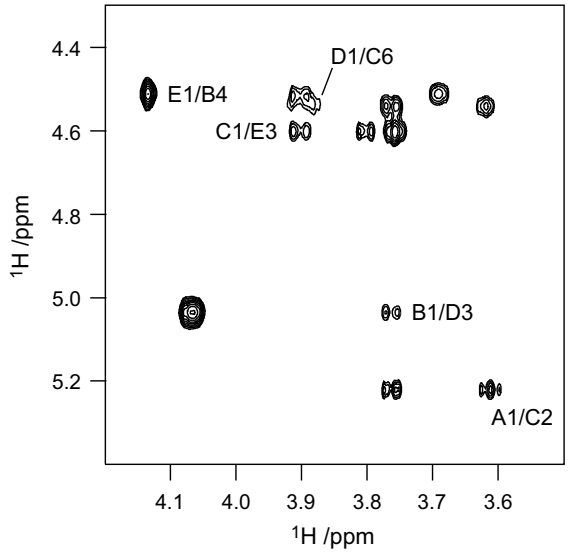


Figure 4. Part of the $^{1}H,^{1}H$-NOESY spectrum of the LPS-OH from *E. coli* strain 87/D2.

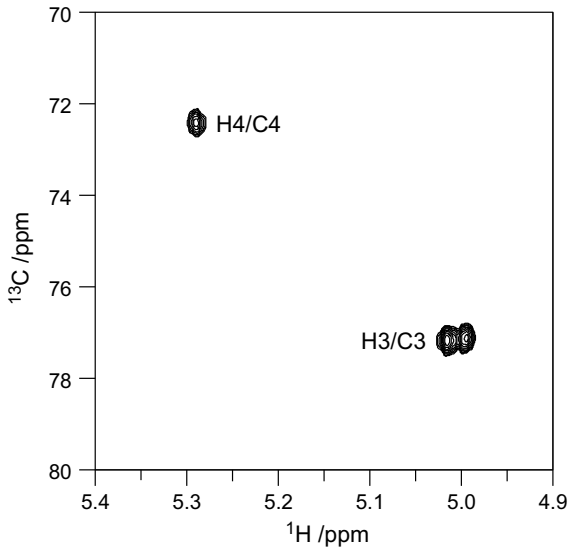


Figure 5. Selected region of the $^{1}H,^{13}C$-HSQC spectrum of the PS from *E. coli* strain 87/D2 showing downfield-shifted resonances as a result of O-acetylation in residue C.

the substitution pattern of the *O*-acetyl groups or the relative degree of substituent modification. Whether these differences are due to *O*-acetyl migration or the activity of the *O*-acetyl transferase, or both, remain to be elucidated. The *E. coli* strain 87/D2, belonging to the serogroup O128ab, differs to the international type strain in that it has an *N*-acetylgalactosamine residue

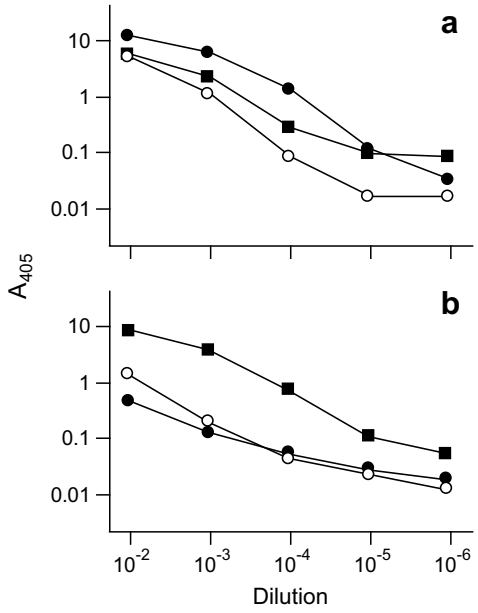


Figure 6. Enzyme immunoassay with rabbit—anti-*E. coli* O128ab (a) and O128ac (b) sera titrated against LPS isolated from *E. coli* strain 87/D2 (○), O128ab (●), and O128ac (■).

as the second sugar in the repeating unit instead of a galactose residue.

3. Experimental

3.1. Bacterial strains and conditions of growth

The *E. coli* O128ab and *E. coli* O128ac strains were obtained from The International *Escherichia* and *Klebsiella* Centre (WHO), Statens Serum Institute, Copenhagen, Denmark. These bacteria were grown in a Luria Bertani (LB) medium. *E. coli* O128:K(67):H2 (CCUG 11427) was obtained from the Culture Collection University of Gothenburg, Sweden. This strain is the original test strain 56-54 (Cigleris) and is of serotype O128ab:H2. The *E. coli* strain 87/D2 was isolated in 1991 from an infant with persistent diarrhoea in León, Nicaragua. These bacteria were grown in a tryptone/yeast extract medium as previously described.[20]

3.2. Preparation of lipopolysaccharide and lipid-free polysaccharide

The LPS was extracted and purified as previously described.[20] The *E. coli* O128 LPS was delipidated using 1% acetic acid at 100 °C for 1 h and purified as previously described.[16] Lipid-free polysaccharide from *E. coli* strain 87/D2 was prepared by treatment of the LPS with 0.1 M sodium acetate, pH 4.2, at 100 °C for 5 h and purified as previously described.[36] The O-deacylation of the LPS was performed by treatment with 12% aqueous ammonia at 37 °C for 15 h. Subsequent dialysis and lyophilization yielded O-deacylated LPS.

3.3. Component analyses

The LPS was hydrolyzed with 2 M trifluoroacetic acid at 120 °C for 2 h. After reduction with NaB^2H_4 and acetylation, the sample was analyzed by GLC. The absolute configurations of the sugars present in the O-antigen of *E. coli* strain 87/D2 were determined by derivatization as their acetylated (+)-2-butyl glycosides essentially as described.[37] The presence of glucose in the hydrolysates to a larger extent than anticipated from the core region of the LPS may, at least in part, be a result of contaminating α-(1→6)-linked glucans sometimes found in LPS preparations.

3.4. GLC analyses

Alditol acetates and acetylated 2-butyl glycosides were separated on an HP-5 column using a temperature programme of 180 °C for 1 min, 3 °C min^{-1} to 210 °C, 10 min at 210 °C except for the 2-butyl galactosides, which were separated on a DB-225 column with a temperature programme of 180 °C for 1 min, 3 °C min^{-1} to 210 °C, 1 min at 210 °C, 4 °C min^{-1} to 230 °C, 10 min at 230 °C and the 2-butyl fucosides, which were separated on a DB-225 column with a temperature programme of 140 °C for 1 min, 0.5 °C min^{-1} to 160 °C, 1 min at 160 °C, 8 °C min^{-1} to 210 °C, 1 min at 210 °C, 2 °C min^{-1} to 230 °C, 10 min at 230 °C. Hydrogen was used as carrier gas. The columns were fitted to a Hewlett–Packard model 5890 series II gas chromatograph equipped with a flame ionization detector.

3.5. NMR spectroscopy

NMR spectra of LPS, LPS-OH and PS materials in D_2O solutions were recorded at 25 and 70 °C using a Varian Inova 600 spectrometer equipped with a 5 mm PFG triple-resonance probe and on Bruker AVANCE 400 and 500 MHz spectrometers, the latter equipped with a 5 mm PFG triple-resonance CryoProbe. Data processing was performed using vendor-supplied software. Chemical shifts are reported in ppm using internal sodium 3-trimethylsilyl-(2,2,3,3-$^2H_4$)-propanoate (TSP, δ_H 0.00) or external 1,4-dioxane in D_2O (δ_C 67.40) as references. 2D NMR experiments were used to assign signals including multiplicity-edited $^1H,^{13}C$-HSQC experiments.[38,39] Inter-residue correlations were obtained by a $^1H,^1H$-NOESY experiment[40] with a mixing time of 50 ms and a $^1H,^{13}C$-HMBC experiment[41] with a 50 ms delay for the evolution of long-range couplings. The chemical shifts were compared to those of the corresponding monosaccharides.[42]

3.6. Immunochemical analyses

The rabbit anti-*E.* coli O128ab and O128ac specific antisera were obtained from The International *Escherichia* Centre (WHO), Statens Serum Institute, Copenhagen, Denmark. The enzyme immunoassay (EIA) was performed as described previously.[21,43]

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