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Clostridium difficile cell-surface polysaccharides composed of pentaglycosyl and hexaglycosyl phosphate repeating units

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Abstract—*Clostridium difficile* is a Gram-positive bacterium that is known to be a cause of enteric diseases in humans. It is the leading cause of antibiotic-associated diarrhea and pseudomembranous colitis. Recently, large outbreaks of *C. difficile*-associated diarrhea have been reported internationally, and there have been reports of increases in severe disease, mortality and relapse rates. At the moment, there is no vaccine against *C. difficile*, and the medical prevention of *C. difficile* infection is mostly based on the prophylactic use of antibiotics; however, this has led to an increase in the incidence of the disease. Here, we describe the chemical structure of *C. difficile* cell-surface polysaccharides. The polysaccharides of three *C. difficile* strains were structurally analyzed; ribotype 027 (North American pulsotype 1) strain was observed to express two polysaccharides, one was composed of a branched pentaglycosyl phosphate repeating unit: [→4)-α-L-Rha*p*-(1→3)-β-D-Glc*p*-(1→4)-[α-L-Rha*p*-(1→3]-α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→P] and the other was composed of a hexaglycosyl phosphate repeating unit: [→6)-β-D-Glc*p*-(1→3)-β-D-Gal*p*NAc-(1→4)-α-D-Glc*p*-(1→4)-[β-D-Glc*p*-(1→]-β-D-Gal*p*NAc-(1→3)-α-D-Man*p*-(1→P]. The latter polysaccharide was also observed to be produced by strains MOH900 and MOH718. The results described here represent the first literature report describing the covalent chemical structures of *C. difficile* cell-surface polysaccharides, of which PS-II appears to be a regular *C. difficile* antigen. These *C. difficile* teichoic-acid-like polysaccharides will be tested as immunogens in vaccine preparations in a rat and horse model.

Keywords: *Clostridium difficile*; Teichoic-acid polysaccharide; Structural characterization

1. Introduction

Clostridium difficile is a Gram-positive bacterium that is known to be the cause of enteric diseases in many animal species, including humans. In humans, *C. difficile*-associated diarrhea (CDAD) is the most commonly diagnosed cause of hospital-associated and antimicrobial-associated diarrhea.[1] Risk of CDAD has traditionally been higher among elderly patients and those that have undergone hospitalization, gastrointestinal surgery, or those exposed to antibiotics.[2] In the United States, the estimated number of cases of *C. difficile*-associated disease exceeds 250,000 per year,[3] with health care costs approaching US $1 billion annually.[4]

In the past five years, an unexpected increase in the incidence of CDAD has been observed. This has also been associated with higher rates of severe CDAD, treatment failure, and death.[5] Severe cases are being more frequently identified in younger patients and those without traditional risk factors. Much of this change has been associated with international dissemination of an outbreak clone, designated ribotype 027 (also known as NAP1 and BI).[6] Prevention of *C. difficile* is based on patient isolation, improved sanitation, improved

Abbreviations: GC, gas-chromatography; CDAD, *Clostridium difficile* associated disease; MS, mass-spectrometry; NMR, nuclear magnetic resonance; PS, polysaccharide

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infection control, and antimicrobial restriction, all of which are associated with high healthcare costs, and has been of variable success. Treatment of *C. difficile* infections is also problematic because the response to metronidazole, the main first-line treatment, is becoming unpredictable.[7] Vancomycin, the alternative choice, is expensive and its use raises concern about the emergence of vancomycin-resistant enterococci and other vancomycin-resistant organisms.

CDAD is also an important problem in many animal species such as horses and pigs.[8,9] It may also be a cause of the disease in other species, and, accordingly, there is concern that *C. difficile* may be transmissible from animals to humans. Indeed, types of *C. difficile* isolated from animals are often the same as those found in people, including the outbreak strain ribotype 027. This concern has increased due to the recent finding of *C. difficile* in retail meat samples.[10]

The reported increase in the incidence of CDAD, its recurrence rates, and its impact on morbidity and mortality, as well as the costs associated with treatment and appropriate isolation procedures to limit its spread, make clear the need for effective prevention approaches of CDAD. One particular strain, designated ribotype 027 or North American pulsotype 1 (NAP1) has emerged as an important cause of sporadic and epidemic disease internationally. This strain produces three main toxins: toxin A, toxin B, and CDT (binary toxin). It also has a deletion in a purported toxin regulating gene that appears to increase toxin production, at least in vitro.[11] Therefore, there is a growing need to develop a vaccine for humans against *C. difficile* to prevent CDAD or its recurrence. Additionally, vaccination is needed in animals to prevent animal disease and to reduce the shedding of *C. difficile* so as to reduce the risk of zoonotic transmission.

Here, we describe the covalent chemical structures of two cell-surface polysaccharides produced by *C. difficile*. Three *C. difficile* strains were chosen for this investigation: ribotype 027 or North American pulsotype 1 (NAP1), which has emerged as an important cause of sporadic and epidemic disease internationally; MOH900, classified as ribotype W or NAP2, the most common strain in people in Ontario hospitals (Weese et al., unpublished data), which possesses genes encoding toxins A and B, but not CDT; and strain MOH718, an uncommon toxin variant that possesses genes encoding toxin B but not toxins A or CDT.

2. Experimental procedures

2.1. Bacterial growth conditions and isolation of carbohydrate material

A ribotype 027 isolate that was obtained from a person with *C. difficile* associated diarrhea was used for this study. Cells of strain ribotype 027, strain MOH900, and MOH718 were grown in CDMN broth (*C. difficile* Moxalactam Norfloxacin) at 37 °C for 24 h in an anaerobic chamber, washed with phosphate buffered saline, separated by centrifugation, and freeze-dried. The putative cell-surface carbohydrates were isolated from the bacterial cell surface by a 1% acetic acid (95 °C, 1 h) treatment for separation from the peptidoglycan. Subsequent purifications were carried out by size exclusion chromatography (Bio-Gel P-6), with previous calibration with blue dextrin. The detection of carbohydrate material was accomplished by the phenol-sulphuric acid assay.[12] In some instances, for nuclear magnetic resonance (NMR) studies, these carbohydrate preparations were further homogenized by anion exchange chromatography, which was performed on a 5 mL Hitrap Q column (Amersham) in water for 10 min, employing a linear gradient of 0–1 M NaCl over 60 min with UV detection at 220 nm. The fractions were desalted by gel chromatography on Sephadex G-15 column. For ribotype 027, 8.6 mg of PS-I and 11.5 mg of PS-II were isolated from 3 g of cells. For strains MOH900 and MOH718, 12.6 mg of PS-II was obtained from 3 g of cells.

2.2. Sugar composition analysis and linkage analysis

Monosaccharide composition analysis was performed by the alditol acetate method.[13] The glycosyl hydrolyses were carried out with 4 M-trifluoroacetic acid at 105 °C for 5 h followed by the reduction in H_2O with $NaBD_4$ overnight at room temperature, and subsequent acetylation by acetic anhydride at 100 °C for 2 h. The alditol acetate derivatives were analyzed by gas chromatography (GC) using a Varian 3400 gas chromatograph equipped with a 30 m DB-17 capillary column [210 °C (30 min)→40 °C at 2 °C/min], and by GC–mass spectrometry (GC–MS) in the electron-impact (EI) and chemical-ionization (CI) modes in a ThermoFinigan PolarisQ instrument.

Sugar linkage analysis was performed by first methylation according to the methylation procedure of Ciucanu and Kerek[14] ($NaOH/Me_2SO/CH_3I$), followed by hydrolysis, reduction, and acetylation as described above. The permethylated alditol acetate derivatives were characterized by GC–MS in the electron impact mode (DB-17 column, isothermally at 190 °C for 100 min). The absolute configurations of the individual components were determined by the formation and characterization by GC–MS of the respective 2-(*S*)- and 2-(*R*)-butyl chiral glycosides.[15] The carbohydrate preparations were hydrolyzed in 4 M TFA at 100 °C for 4 h, reacted with 2-(*S*)- and 2-(*R*)-butanol at 100 °C for 6 h, and acetylated with acetic anhydride at 100 °C for 1 h. The 2-(*S*)- and 2-(*R*)-butyl chiral glycosides were characterized, and compared with standards,

by GC–MS using a 30 m DB-23 capillary column isothermally at 200 °C.

2.3. Nuclear magnetic resonance spectroscopy

^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker AMX 400 spectrometer at 293 K. Two-dimensional (2D) NMR correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), nuclear Overhauser enhancement spectroscopy (NOESY), heteronuclear spin quantum correlation (HSQC) spectroscopy and heteronuclear multiple bond correlation (HMBC) spectroscopy experiments were performed using the instrument's Bruker software. Prior to performing the NMR experiments, the samples were lyophilized three times with D_2O (99.9%). The HOD peak was used as the internal reference at δ_H 4.821 for ^{1}H NMR spectroscopy, and orthophosphoric acid (δ_P 0.0) as the external reference for ^{31}P NMR experiments. Just before the NMR experiments were carried out, a D_2O sample containing TMS (δ_H 0.00) was run to aid in the reference the HOD signal.

3. Results

The saccharide preparation obtained from the mild acid treatment of *C. difficile* ribotype 027 was subjected to size exclusion chromatography (Bio-Gel P-6). Albeit with a very poorly defined base-line separation, two distinct fractions, designated PS-I and PS-II, were obtained in the high molecular weight range.

3.1. Monosaccharide analysis of *C. difficile* ribotype 027 PS-I

The monosaccharide composition analysis of PS-I revealed that it was composed of rhamnose (Rha) and glucose (Glc) in a 1.8:3 relative ratio, respectively. The major linkage-types determined to be present in PS-I were terminal Rha*p* [Rha-(1→], 2-monosubstituted Glc*p* [→2)-Glc-(1→], 3-monosubstituted Glc*p* [→3)-Glc-(1→], and 3,4-disubstituted Glc*p* [→3,4)-Glc-(1→] in equimolar ratios. Traces of 4-monosubstituted Rha*p* [→4)-Rha-(1→] were also observed. The analysis of the 2-(*S*)- and 2-(*R*)-butyl chiral glycosides revealed that Glc was present as the D-enantiomer and Rha the L-enantiomer.

3.2. Nuclear magnetic resonance spectroscopy of *C. difficile* ribotype 027 PS-I

The ^{1}H NMR spectra of PS-I (Fig. 1) showed five anomeric resonances (labelled A through E), which was consistent with the number of linkage-types characterized by methylation analysis. 2D ^{1}H–^{1}H COSY, TOCSY, and ^{1}H–^{13}C HSQC experiments allowed the assignment of most of the ring protons and carbons of each unit present in PS-I (Table 1), and in which vicinal coupling constants

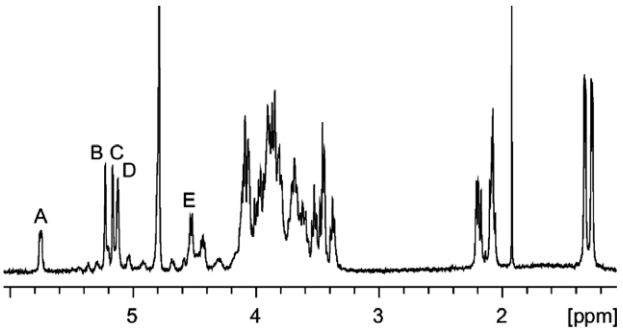


Figure 1. ^{1}H NMR spectrum of *C. difficile* ribotype 027 PS-I showing five anomeric resonances (designated A–E) and two doublets between 1.2 and 1.4 ppm belonging to two L-Rha*p* (6-deoxy-mannose) units. The resonances observed between 2.0 and 2.2 ppm could not be assigned. The singlet at 1.94 belongs to traces of sodium acetate.

allowed for the assignment of the D-Glc*p* and L-Rha*p* ring-protons spin systems. Anomeric resonances A(α), D(α), and E(β) belonged to D-Glc*p* units, and B(α) and C(α) anomeric signals to L-Rha*p* residues. The determination of the sequence of monosaccharide residues was made possible by a ^{1}H–^{13}C HMBC experiment, which allowed the assignment of the glycosyl linkages in PS-I. The ^{1}H–^{13}C correlations detected in PS-I were

H-1(D)/C-2(A) for [α-D-Glc*p*-(1→2)-α-D-Glc*p*]

H-1(E)/C-4(D) for [β-D-Glc*p*-(1→4)-α-D-Glc*p*]

H-1(C)/C-3(E) for [α-L-Rha*p*-(1→3)-β-D-Glc*p*]

H-1(B)/C-3(D) for [α-L-Rha*p*-(1→3)-α-D-Glc*p*]

Because a 1D ^{31}P NMR spectrum of PS-I showed a resonance at δ_P −0.78 for a diester phosphate (no change in chemical shift at higher pH), we performed a ^{1}H–^{31}P HMBC experiment (Fig. 2), on a sample consisting of a mixture of both PS-I and PS-II, which afforded ^{1}H–^{31}P correlations between the ^{31}P signal at δ_P −0.78 and H-1 of A (2-substituted α-Glc) and H-4 of C (4-substituted α-L-Rha*p*) for a sequence of

$$\ldots \rightarrow 2)\text{-}\alpha\text{-D-Glc}p\text{-}(1 \rightarrow P \rightarrow 4)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow \ldots$$

Table 1. ^{1}H and ^{13}C NMR assignments for *C. difficile* ribotype 027 PS-I (δ, ppm; 25 °C)

Residue	1	2	3	4	5	6a/6b
α-Rha (C)	5.17	4.09	3.97	4.07	4.12	1.33
	101.4	71.2	70.9	78.9	68.6	17.8
β-Glc (E)	4.53	3.38	3.62	3.46	3.45	3.80/3.95
	102.4	75.2	83.0	69.0	77.1	62.2
α-Glc (D)	5.13	3.70	4.01	3.86	4.06	
	98.0	73.6	77.5	73.6	72.4	
α-Glc (A)	5.75	3.68	3.89	3.53	3.91	
	93.5	77.3	72.1	70.1	73.8	
α-Rha (B)	5.23	4.07	3.85	3.46	4.44	1.27
	101.9	71.1	71.0	73.0	69.4	17.5

^{31}P signal at −0.9 ppm. Unassigned H/C-6: 3.91/3.91:60.2; 3.81–3.87:61.2 ppm.

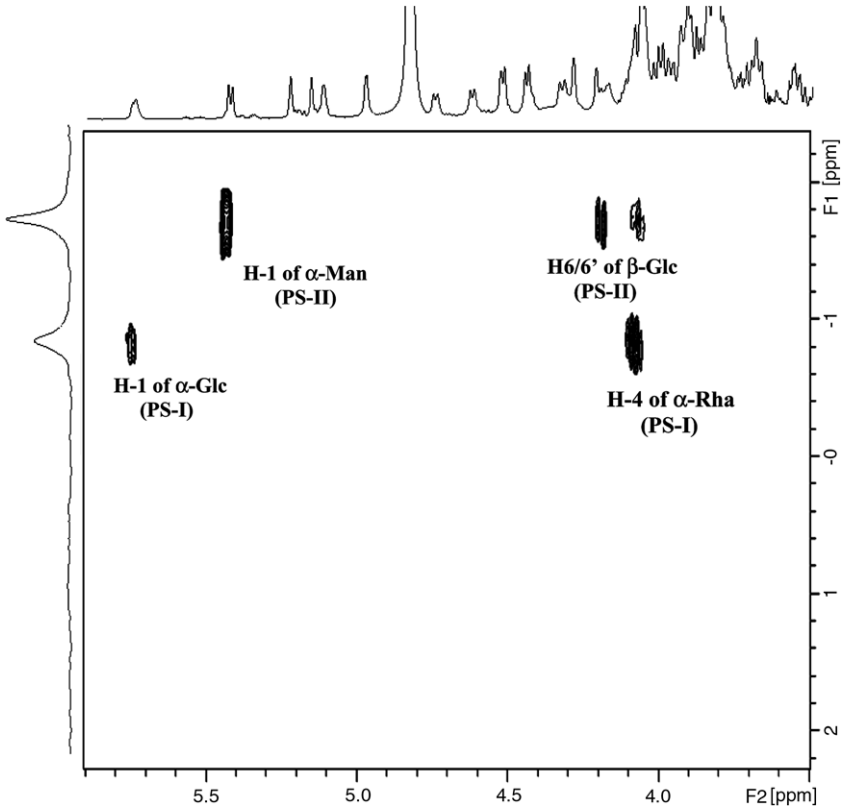


Figure 2. $^{1}H–^{31}P$ HMBC of a sample containing *C. difficile* ribotype 027 PS-I and PS-II showing the through-bond correlations between the glycosyl phosphate of PS-I (−0.78 ppm) and H-1 of α-D-Glc*p* and H-4 of α-L-Rha*p*; and the glycosyl phosphate of PS-II (−1.67 ppm) and H-1 of α-D-Man*p* and H-6/6′ of β-D-Glc*p*.

The results obtained from methylation analysis and HMBC spectroscopy experiments revealed that *C. difficile* ribotype 027 PS-I was composed of pentaglycosyl phosphate repeating block:

C **E** **D** **A**

…→4)-α-L-Rha*p*-(1→3)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→P…

3

↑

1

α-L-Rha*p*

B

To corroborate the monosaccharide sequence of PS-I obtained through the analysis of the $^{1}H–^{31}P$ HMBC experiment (Fig. 2), a 2D $^{1}H–^{1}H$ NOESY (Fig. 3) was performed on PS-I and revealed *inter*-NOE connectivities (schematically shown below) between H-1 (δ_H 5.23) of the terminal α-L-Rha*p* (B) and H-3 (δ_H 4.01) of the branched 3,4-substituted α-D-Glc*p* (D) for [α-D-Rha*p*-(1→3)-α-D-Glc*p*-(1→], between H-1 (δ_H 5.17) of the 4-substituted α-L-Rha*p* (C) and H-3 (δ_H 3.62) of the 3-substituted β-D-Glc*p* (E) for [→4)-α-L-Rha*p*-(1→3)-β-D-Glc*p*-(1→], between H-1 (δ_H 5.13) of the branched 3,4-substituted α-D-Glc*p* (D) and H-2 (δ_H 3.68) of the 2-substituted β-D-Glc*p* (A) for [→3/4)-α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→], and between H-1 (δ_H 4.53) of the 3-substituted α-D-Glc*p* (E) and H-4 (δ_H 3.86) of the branched 3,4-substituted α-D-Glc*p* (D) for [→3)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→]. The monosaccharide sequences implied by the *inter*-NOE connectivities described above substantiated the glycosyl sequences observed previously by the $^{1}H–^{13}C$ HMBC experiment. Other through-space interactions, many arising from *intra*-NOE connectivities, were also detected by the 2D $^{1}H–^{1}H$ NOESY experiment, but, of particular note, the *inter*-NOE interaction between the anomeric protons of the branched 3,4-substituted α-D-Glc*p* (D; δ_H 5.13) and of the 2-substituted α-D-Glc*p* (A; δ_H 5.75) revealed the proximity of these two protons in the (1→2) glycosidic linkage between these two α-D-Glc*p* units [→3/4)-α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→].

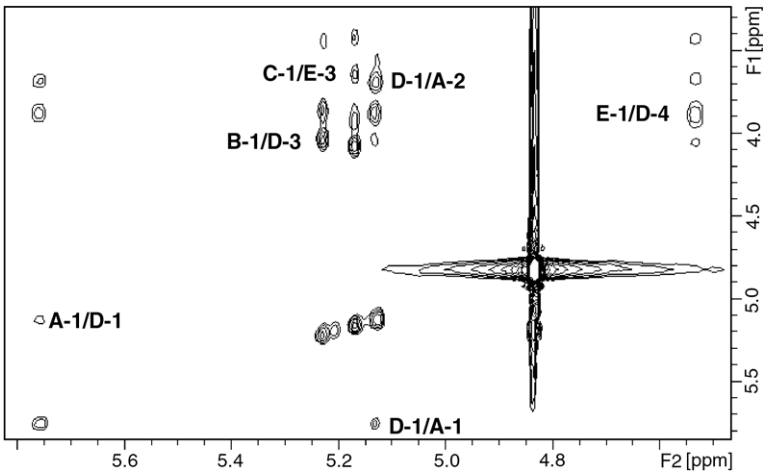
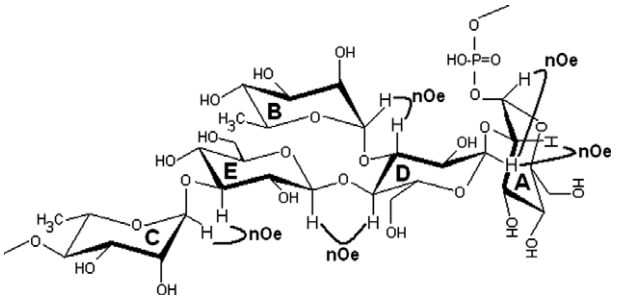


Figure 3. ^{1}H–^{1}H NOESY spectrum of *C. difficile* 027 PS-1 showing *inter*-NOE connectivities indicating the sequence of the glycosyl residues.



3.3. Monosaccharide analysis of *C. difficile* ribotype 027 PS-II

Monosaccharide composition analyses carried out on PS-II showed the presence of Glc, mannose (Man), and *N*-acetyl-glucosamine (GalNAc) in a 2.8:1:1.8 relative ratio, respectively. Small amounts of Rha were also detected in PS-II. All units were observed to have the D-absolute configuration. Sugar linkage-type analysis performed on PS-II showed many variably linked monosaccharides units. The major linkage-types determined to be present in PS-II were terminal Glc*p* [Glc-(1→], 4-monosubstituted Glc*p* [→4)-Glc-(1→], 3-monosubstituted Man*p* [→3)-Man-(1→], 3-substituted Gal*p*NAc [→3)-GalNAc-(1→], and 3,4-disubstituted Gal*p*NAc [→3,4)-GalNAc-(1→] with the respective ratios of 1:1:1:0.8:0.7. Co-eluting with the 4-monosubstituted Glc residue, trace amounts of an *m*/*z* ion characteristic of 6-monosubstituted Glc*p* [→6)-Glc-(1→] (*m*/*z* 189) were also detected in PS-II. Additionally, small amounts of the linkage-types observed to be present in PS-I were also detected in the linkage-type analyses of PS-II.

3.4. Nuclear magnetic resonance spectroscopy *C. difficile* ribotype 027 PS-II

Because the analysis described above pointed to the fact that PS-II also carried small amounts of units assignable to PS-1, PS-II was subjected to further purification by anion exchange chromatography. A PS-II almost completely devoid of PS-I residues, as shown by monosaccharide analysis, was obtained and subsequently used for NMR.

A ^{31}P NMR experiment carried out on PS-II revealed that it also carried a diester phosphate component with a resonance at δ_P −1.67. The ^{1}H NMR of PS-II (Fig. 4) yielded six anomeric resonances, consistent with the number of linkage types present in PS-II. The assignment of most of the ring protons and carbons of each glycose unit present in PS-II was furnished by 2D ^{1}H–^{1}H COSY, TOCSY, and ^{1}H–^{13}C HSQC experiments (Table 2), with anomeric resonance A belonging to a Man*p* unit, B, E and F to Glc*p* units, and C and D to Gal*p*NAc residues. As with PS-I, the sequence of the monosaccharide residues was made possible by a ^{1}H–^{13}C HMBC experiment with the following ^{1}H–^{13}C correlations being detected:

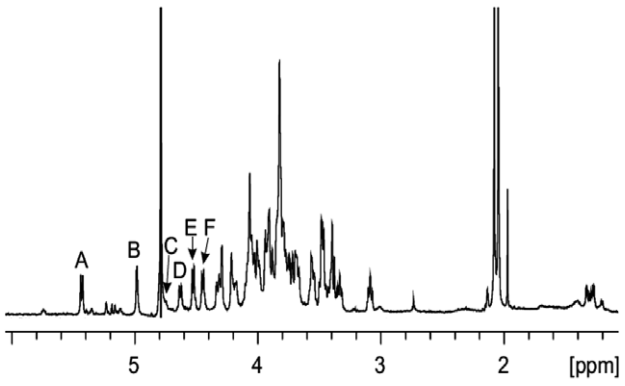


Figure 4. ^{1}H NMR spectrum of *C. difficile* ribotype 027 PS-II showing six anomeric resonances (designated A–F) and two singlets between 2.0 and 2.1 ppm belonging to two D-Gal*p*NAc units. The singlet at 1.95 belongs to traces of sodium acetate.

Table 2. ^{1}H and ^{13}C NMR data for *C. difficile* ribotype 027 PS-II (δ, ppm; 25 °C)

Residue	1	2	3	4	5	6a/6b
β-Glc (E)	4.53	3.34	3.49	3.48	3.57	4.07/4.19
	105.5	73.8	76.4	70.2	75.4	65.7
β-GalNAc (D)	4.64	4.05	3.93	4.22	3.78	
	102.3	52.5	80.9	68.7	76.2	
α-Glc (B)	4.99	3.56	4.01	3.70	4.33	3.68/3.84
	99.6	72.3	72.3	79.6	70.8	60.4
β-GalNAc (C)	4.76	4.09	4.00	4.30	3.81	
	100.7	53.1	79.6	75.5	76.5	
α-Man (A)	5.44	4.07	4.07	3.82	3.83	
	97.0	69.2	79.1	65.6	74.8	
β-Glc (F)	4.45	3.09	3.49	3.38	3.41	3.74/3.93
	106.0	74.1	76.4	70.7	76.7	61.7

^{31}P signal at −1.7 ppm. Unassigned H:C-6: 3.85/3.93:61.0; 3.81/3.90:61.9; 3.83/3.83:62.0 ppm. J_{P-H} at H-1 of Man 7.6 Hz. NAc signals observed at 2.05:23.4, 176.2; 2.08:23.3, 175.9 ppm.

H-1(C)/C-3(A) for [β-D-Gal*p*NAc-(1→3)-α-D-Man*p*]
H-1(B)/C-4(C) for [α-D-Glc*p*-(1→4)-β-D-Gal*p*NAc]
H-1(D)/C-4(B) for [β-D-Gal*p*NAc-(1→4)-α-D-Glc*p*]
H-1(E)/C-3(D) for [β-D-Glc*p*-(1→3-β-D-Gal*p*NAc]
H-1(F)/C-3(C) for [β-D-Glc*p*-(1→3)-α-D-Gal*p*NAc]

The ^{1}H–^{31}P HMBC experiment (Fig. 2) showed that the ^{31}P signal at −1.67 ppm of PS-II showed correlations to H-1 of A (3-substituted α-D-Man*p*) and H-6 and 6′ of E (6-substituted β-D-Glc*p*) for a sequence of

…3)-α-D-Man*p*-(1→P→6)-β-D-Glc*p*-(1→ …

Collectively, the results obtained from GC–MS analysis and NMR spectroscopy experiments revealed that *C. difficile* ribotype 027 PS-II was composed of hexaglycosyl phosphate repeating block:

E **D** **B** **C** **A**

…→6)-β-D-Glc*p*-(1→3)-β-D-Gal*p*NAc-(1→4)-α-D-Glc*p*-(1→4)-β-D-Gal*p*NAc-(1→3)-α-D-Man*p*-(1→P…

3
↑
1

β-D-Glc*p*

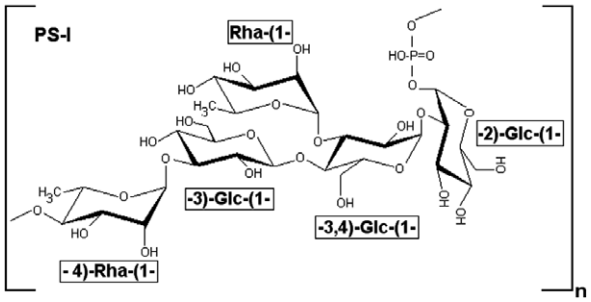
F

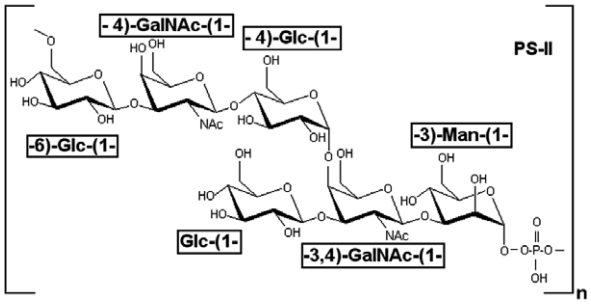
3.5. Analysis of *C. difficile* strains MOH 900 and MOH 718 polysaccharides

GC–MS and NMR analyses of the carbohydrate material obtained from *C. difficile* strains MOH900 and MOH718 revealed that they produced a polysaccharide similar to PS-II of *C. difficile* ribotype 027. Figure 5 shows the GC–MS profile of the permethylated alditol acetates of *C. difficile* strain MOH900 PS-II. No units that composed PS-I of *C. difficile* ribotype 027 were found to be present in strains MOH900 and MOH718.

4. Discussion

The structural results presented here represent the first report describing the covalent chemical structures of *C. difficile* cell-surface polysaccharides. *C. difficile* ribotype 027, the strain recently identified as being responsible for the onset of a current outbreak of *C. difficile*-related infections, was shown to express two highly complex cell-surface teichoic-acid-like polysaccharides (PS-I and PS-II). The other two strains of *C. difficile*, MOH900 and MOH718, were found to produce only PS-II. Each PS was composed of frequently found monosaccharides with several linkage-types connected by a glycosyl phosphate bridge:





In 1982, Poxton and Cartmill[16] described the composition of two carbohydrate preparations extracted from *C. difficile* strain NCTC 11223. The material obtained by cold trichloroacetic acid treatment of cells was observed to contain glucose, mannose, galactosamine, and phosphate, a similar composition to that of PS-II described here, and the other, extracted by phenol treatment of cells, contained glucose, glucosamine, phosphate, and fatty acids, units typically present in lipotheichoic-acid polysaccharides of Gram-positive bacteria. We are now carrying out the extraction of sac-

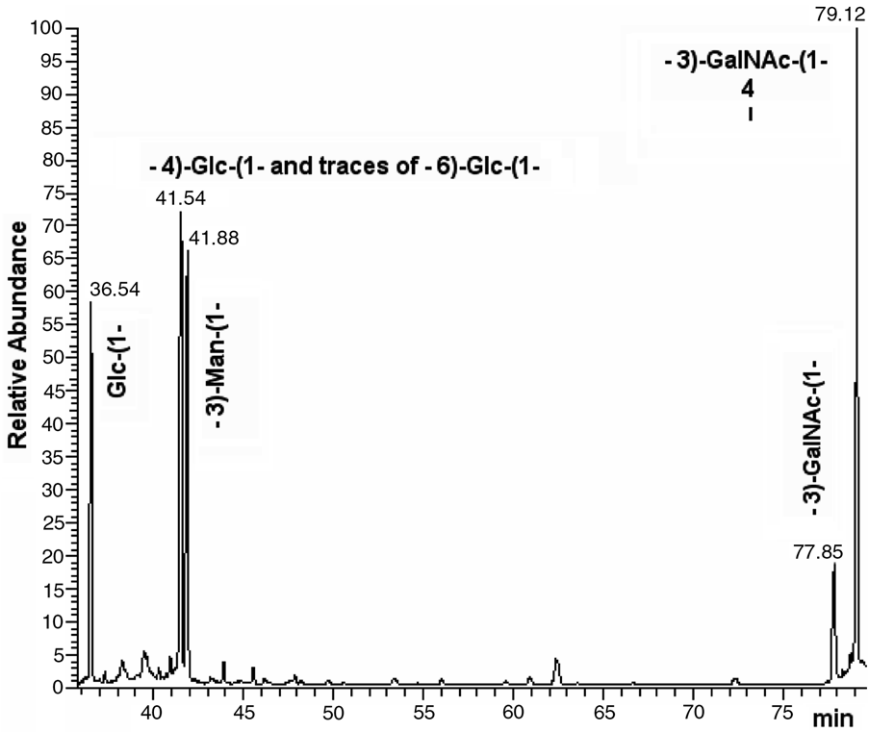


Figure 5. GC–MS profile of the permethylated alditol acetates of *C. difficile* MOH 900 PS-II showing that this strain produced a polysaccharide with similar structural units to that of PS-II found in *C. difficile* ribotype 027.

charide material with a water–phenol mixture to determine if the strains in our laboratory also express this lipotheichoic-acid-like molecule. In the same work, Poxton and Cartmill also observed that both the carbohydrate preparations showed partial immunological identity and that both cross-reacted with *Clostridium sordellii* antiserum.

The fact that the three distinctive *C. difficile* strains investigated here expressed a similar cell-surface polysaccharide, PS-II, and that the NCTC 11223 strain[16] also carried similar components, strongly suggests that PS-II may be a common carbohydrate antigen of *C. difficile*, which may be advantageous in the development of a carbohydrate-based anti-*C. difficile* vaccine. In addition to producing PS-II, *C. difficile* ribotype 027 was shown to elaborate a second teichoic-acid polymer, PS-I. This additional cell-surface carbohydrate antigen may play a role in the increased pathogenesis of *C. difficile* ribotype 027, such as an improved resistance to attack by phagocytes.

Our future plans include the detection of IgM, IgG, and IGA antibodies that recognize the purified preparations of PS-I and PS-II in sera of previously infected humans and animals, and the immunological evaluation of these polysaccharides, as sole antigens, as neoglycoconjugates, as an anti-*C. difficile* prophylactic and therapeutic vaccine.

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