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Note

Structure of the O-specific polysaccharide from a marine bacterium *Cellulophaga tyrosinoxydans*

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ABSTRACT

The O-polysaccharide was isolated from the lipopolysaccharide of *Cellulophaga tyrosinoxydans* and studied by chemical analyses along with ^{1}H and ^{13}C NMR spectroscopy, including 2D ^{1}H, ^{1}H COSY, TOCSY, ROESY, ^{1}H, ^{13}C HSQC, HMBC and H2BC experiments. The following new structure of the O-polysaccharide of *C. tyrosinoxydans* containing L-fucose (Fuc), N-acetyl-D-glucosamine (GlcNAc), 4-acetamido-4,6-dideoxy-D-glucose (Qui4NAc) and two L-rhamnose residues (Rha) was established:

β-D-Qui*p*4NAc
1
↓
2
→4)-α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→

The genus *Cellulophaga*, member of the family *Flavobacteriaceae* of the phylum *Bacteroidetes*, was proposed by Johansen et al.[1] The genus accommodates Gram-negative, strictly aerobic, heterotrophic, rod-shaped, yellow/orange pigmented and gliding bacteria isolated from different marine environments. At the time of writing, the genus *Cellulophaga* comprises seven validly described species, namely *Cellulophaga baltica*, *Cellulophaga fucicola*, *Cellulophaga lytica*,[1] *Cellulophaga algicola*,[2] *Cellulophaga pacifica*,[3] *Cellulophaga tyrosinoxydans*[4] and *Cellulophaga geojensis*.[5] Earlier we published the structures of the O-specific polysaccharide of lipopolysaccharides from *C. baltica*,[6] *C. fucicola*[7] and *C. pacifica*.[8] Acidic character of the studied *Cellulophaga* O-polysaccharides is defined by the presence of uronic acid (GlcA in *C. baltica*), nonulosonic acid (Pse in *C. fucicola*) and 2-acetamido-2-deoxy-D-mannuronic acid (ManNAcA in *C. pacifica*). In this paper we present the new structure of *C. tyrosinoxydans* O-polysaccharide, which contains rarely occurred sugar 4-acetamido-4,6-dideoxy-D-glucose.

The O-polysaccharide (OPS) of *C. tyrosinoxydans* was obtained by mild acid degradation of the lipopolysaccharide (LPS), isolated from dried bacterial cells by hot phenol/water extraction.[9] Sugar analysis by GLC of the alditol acetates derived after full acid hydrolysis of the OPS revealed Rha, Fuc and GlcN. Moreover, methanolysis of the OPS followed by GLC-MS analysis of acetylated methyl glycosides showed the presence of 4-acetamido-4,6-dideoxy-hexose (identified as Qui4NAc, vide infra).

The ^{13}C NMR spectrum of the OPS (Fig. 1, Table 1) contained, *inter alia*, signals for five anomeric carbons at δ 101.0–104.8, indicating a pentasaccharide repeating unit, two nitrogen-bearing carbons of amino sugars at δ 56.8 and 58.0, one non-substituted CH_2OH group at δ 62.0 (C-6 of GlcN, from data of DEPT-135 experiment), four methyl groups of 6-deoxy sugars at δ 16.9–18.1, two N-acetyl groups at δ 23.4 and 23.6 (CH_3), and δ 175.8 and 176.0 (CO).

Correspondingly, the ^{1}H NMR spectrum of the OPS showed, *inter alia*, five signals for anomeric protons in the low-field region at δ 4.72–5.27, two intense signals for CH_3–CO groups at δ 2.03–2.06 and four signals for CH_3–C groups at δ 1.21–1.29.

The ^{1}H and ^{13}C NMR spectra of the OPS were assigned using 2D homonuclear COSY, TOCSY, ROESY, heteronuclear ^{1}H, ^{13}C HSQC, HMBC and H2BC experiments (Table 1). The COSY and TOCSY spectra revealed five isolated proton spin systems. The one spin system of *galacto* configuration (Fuc, **A**) was identified by H-1/H-2 up to H-4 correlations. Also COSY and TOCSY spectra showed cross peaks of H-1/H-2 up to H-6 for the two sugar residues with *gluco* configuration (GlcNAc, **B**, and Qui4NAc, **D**) and cross peaks of H-1/

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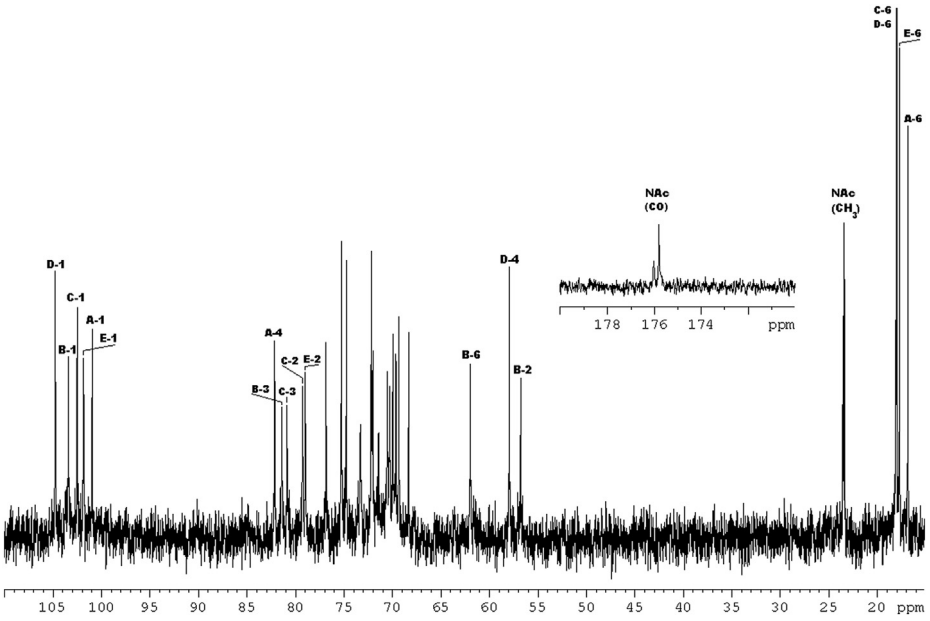

Fig. 1. ^{13}C NMR spectrum of the OPS of *C. tyrosinoxydans*. Arabic numerals refer to the carbons in the sugar residues denoted as described in Table 1.

Table 1
^{1}H and ^{13}C NMR data of the OPS of *Cellulophaga tyrosinoxydans* (δ, ppm)

Sugar residue		1	2	3	4	5	6 (6a,6b)
O-specific polysachharide							
A: →4)-α-L-Fuc*p*-(1→	^{1}H	5.03	3.75	3.95	3.89	4.39	1.22
	^{13}C	101.0	69.3	70.0	82.2	68.3	16.9
B: →3)-β-D-Glc*p*NAc-(1→	^{1}H	4.76	3.89	3.72	3.59	3.52	3.98, 3.81
	^{13}C	103.4	56.8	81.4	69.6	76.9	62.0
C: →2,3)-α-L-Rha-(1→	^{1}H	5.27	4.42	3.94	3.59	3.72	1.27
	^{13}C	102.5	79.3	80.9	72.0	70.5	18.0
D: β-D-Qui*p*4NAc-(1→	^{1}H	4.72	3.38	3.54	3.59	3.58	1.21
	^{13}C	104.8	75.3	74.8	58.0	72.2	18.1
E: →2)-α-L-Rha-(1→	^{1}H	4.91	4.10	3.92	3.50	4.16	1.29
	^{13}C	101.9	79.0	71.4	73.3	70.3	17.7

The chemical shifts for the N-acetyl groups of OPS are: δ_H 2.03–2.06; δ_C 23.4–23.6 (2 Me), and 175.8–176.0 (2 CO).

H-2 and H-2 up to H-6 for sugar residues with *manno* configuration (Rha^1, **C**, and Rha^2, **E**).

The HSQC experiment (Fig. 2, Table 1) revealed that carbon C-2 of the sugar **B** at δ 56.8 and carbon C-4 of the sugar **D** at δ 58.0 bear nitrogen. H-2/C-2 and H-6/C-6 correlations at δ_H/δ_C 3.89/56.8 and 3.98, 3.81/62.0, respectively, suggested that the sugar **B** with *gluco* configuration was a derivative of GlcNAc. H-4/C-4 and H-6/C-6 correlations at δ_H/δ_C 3.59/58.0 and 1.21/18.1, respectively, allowed sugar **D** to identify as a derivative of Qui4NAc.

The $^{1}J_{C1-H1}$ coupling constant values determined from the gated-decoupling spectrum of the OPS showed that all sugar residues are in the pyranoid form.[10] Also the $^{1}J_{C1-H1}$ coupling constant values as well as $^{3}J_{H1-H2}$ coupling constant values revealed that **A** (169 Hz, 3 Hz), **C** (176.4 Hz) and **E** (169 Hz) sugar residues are α-linked, but **B** (168.1 Hz, 8.7 Hz) and **D** (166.2 Hz, 7.3 Hz) sugar residues are β-linked.[11] Chemical shift of C-5 of sugar residues **C** and **E** at δ_C 70.5 and 70.3, respectively, also confirmed α-configuration of these monosaccharides.[12]

Significant down-field displacement of the signals for C-4 of Fuc, C-3 of GlcNAc, C-2 and C-3 of Rha^1 (**C**) and C-2 of Rha^2 (**E**) residues at δ 82.2, 81.4, 79.3, 80.9 and 79.0, respectively, in the ^{13}C NMR spectrum of the OPS, as compared with their position in the spectra of the corresponding non-substituted monosaccharides,[12] demonstrated the modes of sugar glycosylation.

The sequence of the sugar residues was established using ^{1}H, ^{1}H ROESY and ^{1}H, ^{13}C HMBC experiments. The 2D ROESY experiment revealed strong inter-residue cross peaks between the anomeric protons and protons at the linkage carbons at δ 5.03/3.72; 4.76/3.94; 5.27/4.10; 4.72/4.42 and 4.91/3.89, which were assigned to **A** H-1/**B** H-3; **B** H-1/**C** H-3, **C** H-1/**E** H-2; **D** H-1/**C** H-2 and **E** H-1/**A** H-4 correlations, respectively. Accordingly, the HMBC spectrum (Fig. 3, Table 1) also displayed the following inter-residue cross peaks: **A** H-1/**B** C-3; **B** H-1/**C** C-3; **C** H-1/**E** H-2; **D** H-1/**C** C-2; **E** H-1/**A** C-4 at δ_H/δ_C 5.03/81.4; 4.76/80.9; 5.27/79.0; 4.72/79.3 and 4.91/82.2, respectively. These data defined the sequence of the monosaccharides and demonstrated that Fuc residue is 4-substituted, GlcNAc residue is 3-substituted, Rha^2 residue is 2-substituted while Rha^1 residue is 2,3-disubstituted and it is a branched point. The Qui4NAc residue has no substitution and so it is a terminal monosaccharide.

Determination of the absolute configuration by GLC of the acetylated (S)-2-octyl glycosides demonstrated that GlcNAc residue has the D configuration but Rha and Fuc have L configuration. The absolute configuration of Qui4NAc was established on the basis of the values of chemical shifts and glycosylation effects[12,13] in the ^{13}C NMR spectrum of the OPS (Table 1). Large α-effects of 8.6 ppm for C-1 of Qui4NAc and of 7.2 ppm for C-2 of L-Rha indicated different absolute configurations of Qui4NAc and of L-Rha in β(1→2)-linked disaccharide fragment (on the contrary, relatively small α-effects of about 4 ppm for C-1 of Qui4NAc and ~5 ppm for C-2 of L-Rha would be observed in case of their same absolute configurations). Hence, Qui4NAc has D configuration. Besides, in all other cases of