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Note

Chemical structure of the O-polysaccharide isolated from *Pectobacterium atrosepticum* SCRI 1039

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ABSTRACT

The lipopolysaccharide (LPS) of the bacterium *Pectobacterium atrosepticum* SCRI 1039 was hydrolyzed and the products were separated. A study of the obtained O-polysaccharide by means of chemical methods, GLC, GLC–MS, and NMR spectroscopy allowed us to identify a branched polymer with a pentasaccharide repeating unit of the structure shown below, in which the fucose residue was partially O-acetylated at C-2, C-3 or C-4.

→3)-β-D-Glc*p*NAc-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→2)-α-D-Glc*p*-(1→
3
↑
1
α-L-Fuc*p* (*O*-Ac at C-2 or C-3 or C-4)

Pectobacterium atrosepticum is a Gram-negative enterobacterial phytopathogen. For many years it was known as *Erwinia carotovora* subps. *atroseptica*.[1] At the end of the last century several species and subspecies of *Erwinia* were reclassified to the *Pectobacterium* genus. In 2003, three new subspecies of *Pectobacterium carotovorum* were promoted to the species level (*Pectobacterium atrosepticum, Pectobacterium betavascularum* and *Pectobacterium wasabiae*).[2,3] Nevertheless, their taxonomy is still unclear because both classifications schemes are cited in the scientific literature.[4] Bacteria belonging to the *P. atrosepticum* species can cause soft rot and black leg effects in vegetables, especially in potatoes.[5] In this paper, the isolation and structure determination of the O-specific polysaccharide (OPS) of LPS from *P. atrosepticum* SCRI 1039 strain (serotype I) has been described.[6–8]

LPS was obtained from dry bacterial cells using the hot phenol–water extraction method.[9] Mild hydrolysis of the LPS with 1% acetic acid followed by lipid A centrifugation and fractioning of the carbohydrate portion by gel permeation chromatography (GPC) provided pure O-polysaccharide. GLC and GLC–MS analyses of obtained alditol acetates revealed the presence of Fuc, Rha, Glc, and GlcN as main components of OPS. Absolute configurations of monosaccharide constituents as acetylated (*S*)-(+)-butan-2-ol glycosides derivatives were assigned by GLC and GLC–MS. The L configuration of Fuc, and Rha, as well as D configuration of Glc and GlcN were identified.

The substitution positions of monosaccharides in the repeating unit of OPS were determined by methylation analysis. GLC and GLC–MS of the partially methylated alditol acetates indicated five different derivatives: 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-fucitol, 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl-rhamnitol, 1,2,3,5-tetra-*O*-acetyl-4-*O*-methyl-rhamnitol, 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl-glucitol, and 2-deoxy-1,3,5-tri-*O*-acetyl-4,6-di-*O*-methyl-2-acetamidoglucitol.

The complete structural characterization of OPS was achieved by 1D and 2D ^{1}H and ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum of OPS contained several signals in the anomeric region, a few typical signals of methyl groups of 6-deoxy residues (δ 1.21–1.32), and several signals characteristic for *N*- and *O*-acetyl groups in the region of δ 2.00–2.20 (Fig. 1a). The unequal intensities of the anomeric protons (Fig. 1a), and the presence of 'non anomeric' proton signals in the anomeric region of ^{1}H domain of ^{1}H,^{13}C HMQC

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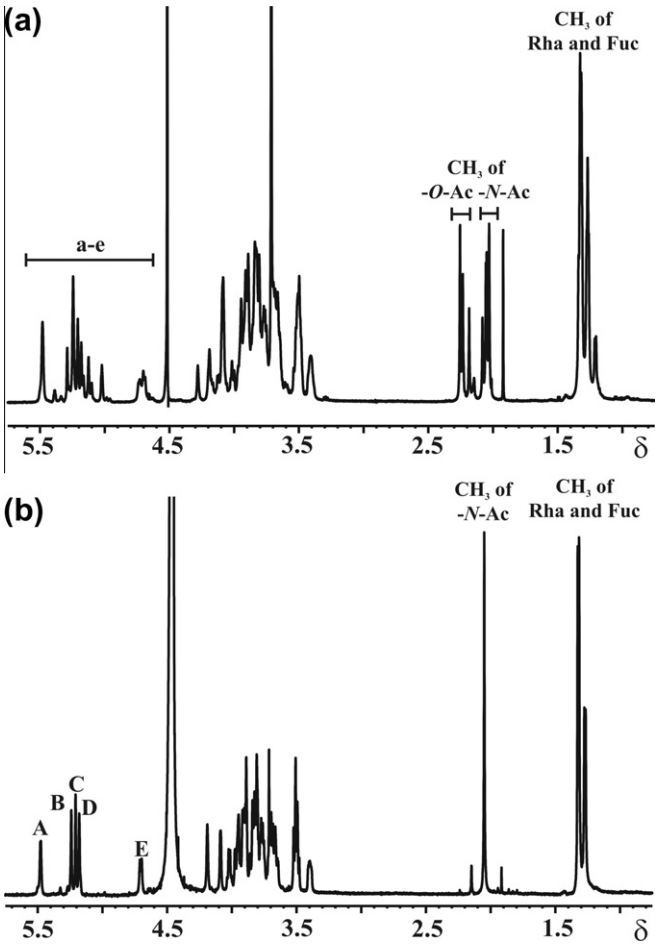

Figure 1. ^{1}H NMR spectra: (a) O-PS isolated from *P. atrosepticum* SCRI 1039, (b) de-O-acetylated O-PS isolated from *P. atrosepticum* SCRI 1039. The letters refer to the anomeric signals of carbohydrate residues as defined in Tables 1 and 2.

spectrum (data not shown), indicated partial O-acetylation. Therefore, the OPS sample was de-O-acetylated using hydrazine. The ^{1}H NMR spectrum recorded for de-O-acetylated OPS showed five signals in the anomeric region at δ 5.479 (**A**), 5.240 (**B**), 5.207 (**C**), 5.179 (**D**), and 4.702 (**E**) in an approximate ratio of 1:1:1:1:1 (Fig. 1b). The signals were labeled from **A** to **E** according to decreasing chemical shifts values of the anomeric protons (Fig. 1b, Table 1). Moreover, three signals of methyl groups of 6-deoxy sugars (at δ 1.317, 1.265, and 1.320), as well as the signal of *N*-acetyl group (δ 2.049) were present in the spectrum.

All constituent residues were identified on the basis of NMR data and results of sugar analysis. The small couplings of the anomeric signals of residues **B**, and **D** ($^{3}J_{H\text{-}1,H\text{-}2}$ <1.5 Hz) suggested the *manno* configuration of the residues. Moreover both two spin systems were terminated with CH_3 groups in COSY and TOCSY spectra, which clearly pointed to Rha residues. The *gluco* configurations of **A** and **E** residues were assigned on the basis of $^{3}J_{H,H}$ coupling constant pattern. Residue **E** was identified as GlcN—the nitrogen-carrying carbon C-2 (δ 55.42), thus residue **A** was recognized as Glc residue. The remaining **C** residue was identified as Fuc according to the compositional analysis results. This assumption was proved by $^{3}J_{H,H}$ coupling constant pattern and the presence of CH_3 group at the end of spin system in COSY and TOCSY spectra.

The anomeric configurations of all residues were identified by $^{1}J_{H\text{-}1,C\text{-}1}$ coupling constants. $^{1}J_{H\text{-}1,C\text{-}1}$ >170 Hz, revealed α configuration of Fuc (174 Hz), Rha (173 and 174 Hz) and Glc (176 Hz). Only GlcN residue possessed β anomeric configuration ($^{1}J_{H\text{-}1,C\text{-}1}$ = 164 Hz). The pyranoside form of the rings of all monosaccharides were assigned by the lack of carbon atom signals in the region of δ ~83–88 of the ^{13}C NMR spectrum.[10,11]

Table 1
^{1}H and ^{13}C chemical shifts of the de-O-acetylated O-polysaccharide from *P. atrosepticum* SCRI 1039

Residue	Chemical shifts ^{1}H and ^{13}C (ppm)					
	H1 C1	H2 C2	H3 C3	H4 C4	H5 C5	H6 C6
→2)-α-Glc	5.479	3.664	3.807	3.498	3.678	3.831/3.831
(A)	98.01	76.86	72.45	69.77	72.69	60.82
→2)-α-Rha	5.240	4.084	3.913	3.510	3.833	1.320
(B)	100.33	78.22	70.24	72.75	69.77	17.03
α-Fuc	5.207	3.942	3.885	3.800	4.012	1.317
(C)	102.69	70.02	66.27	72.45	68.06	17.03
→2,3)-α-Rha	5.179	4.189	3.966	3.507	3.706	1.265
(D)	101.37	78.03	75.94	72.75	70.21	17.08
→3)-β-GlcN	4.702	3.763	3.922	3.695	3.396	3.771/3.905
(E)	102.04	55.42	78.53	71.29	75.86	60.93

Spectra were recorded of a solution in $^{2}H_2O$ at 600 MHz and 55 °C relative to internal acetone (δ_H 2.225; δ_C 31.45). Underlined values indicate sites of glycosylation.

The ^{13}C spectrum (not shown) presented five anomeric carbon signals at δ 102.69 (**C**), 102.04 (**E**), 101.37 (**D**), 100.33 (**B**), and 98.01 (**A**). The spectrum also contained the remaining ring sugar carbon resonances, one signal of the methyl carbon of the *N*-acetyl group (δ 22.84), and signals of C-6 of three 6-deoxy monosaccharides at δ 17.03 (double intensity), and 17.08. Additionally, one signal of the carbonyl carbon atoms at δ 174.03 was observed in HMBC spectrum (not shown). It was derived from the *N*-acetyl group of GlcNAc.

^{1}H,^{1}H DQF-COSY, and TOCSY, as well as ^{1}H,^{13}C HMQC spectra allowed the complete assignment of all ^{1}H and ^{13}C chemical shifts (Table 1). Low-field shifted signals of carbon atoms of **A**2 (δ 76.86), **B**2 (δ 78.22), **D**2 and **D**3 (δ 78.03 and 75.94), **E**3 (δ 78.53) compared with respective reference data revealed positions of glycosylation.[12,13] The remaining Fuc residue which did not contain low-field shifted carbon signals, was assigned as a terminal monosaccharide.

The ROESY spectrum revealed the sequence of the sugar residues in the repeating unit (Fig. 2). The following *inter*-residual proton contacts were identified in the spectrum: **A**-1/**E**-3, **B**-1/**A**-2 and **A**-3, **C**-1/**D**-3, **D**-1/**B**-2, and **E**-1/**D**-2. The compositional analyses results and NMR data allowed to propose the structure of the de-O-acetylated O-specific polysaccharide from *P. atrosepticum* SCRI 1039 as:

(**E**) (**D**) (**B**) (**A**)

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

3

↑

1

α-L-Fuc*p*

(C)

The data presented above and a set of NMR spectra allowed assignment of the partially O-acetylated OPS structure. The ^{1}H NMR spectrum of the OPS showed several overlapping signals in the anomeric region (Fig. 1a). The HMQC experiment (spectrum not shown) helped to identify 11 cross-peaks in the anomeric region. Five of them **a** (δ 5.482/98.02), **b** (δ 5.247/100.30), **c** (δ 5.207/102.68), **d** (δ 5.182/101.37), and **e** (δ 4.701/102.05) were