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Structural analysis of the O-polysaccharide from the lipopolysaccharide of *Pseudomonas putida* BIM B-1100

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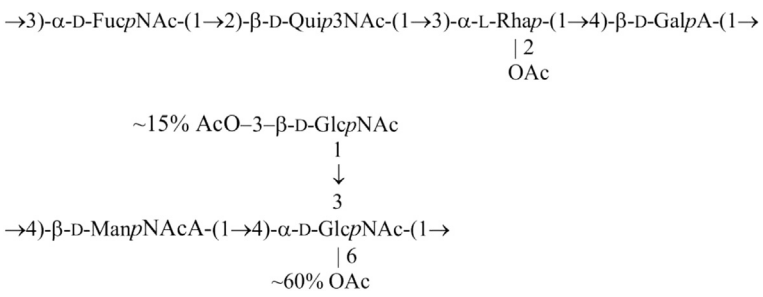
ABSTRACT

Two specific polysaccharides, together with an →4)-α-D-Glc*p*-(1→ glucan (bacterial glycogen), were obtained from a lipopolysaccharide preparation isolated from the bacterium *Pseudomonas putida* BIM B-1100 by phenol/water extraction. The following structures of the polysaccharides were established by composition analysis, Smith degradation, ESI-MS, and 1D and 2D NMR spectroscopy:



Pseudomonas is a wide-spread bacterial genus inhabiting various ecological niches. Lipopolysaccharide (LPS) is a significant component of the cell wall of these bacteria, which contributes to the integrity and stability of the outer membrane, takes part in cell defense from external stress factors, and promotes the viability of pseudomonads under various conditions. LPS blocks elimination of pathogenic pseudomonads from the host and enhances their resistance to medicines [1]. Immunomodulating and immunogenic properties of LPS provide a basis for development of vaccine preparations [2,3]. LPS plays an important role in interaction of bacteria with other bacteria, including other representatives of the same genus, and may act as bacteriophage receptors. Bacteriophages specific to the S-form LPS display a narrow host range owing to a large variability of the O-antigen structure whereas bacteriophages recognizing the R-form LPS show a broader host range due to the conservative nature of the LPS core [4–6].

A new biotechnologically promising strain, BIM B-1100, was isolated from the surface of strawberry leaves grown in the Central Botanical Garden of Minsk. Analysis of the nucleotide sequence of 16S rRNA indicated that this strain should be taxonomically affiliated to the genus *Pseudomonas*, the species *Pseudomonas putida*. It is resistant to the lytic effect by 24 bacteriophages (*Pseudomonas* phages BIM BV-1 to BIM BV-24 from the Belarusian National Collection of Microorganisms). Studies of LPS are important for elucidation of phage resistance mechanisms of representatives of the genus *Pseudomonas* and evaluation of bacteriophages as alternative drugs. In this work, we established chemical structures of polysaccharides isolated from a LPS preparation isolated from *P. putida* BIM B-1100.

Bacterial cells of *P. putida* BIM B-1100 were extracted with aq 45% phenol and the LPS thus obtained was degraded under mild

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acid conditions. A water-soluble carbohydrate portion was fractionated by GPC on Sephadex G-50 to give a high-molecular-mass polysaccharide preparation (PS). Composition analysis by GLC of the alditol acetates obtained after full acid hydrolysis of the PS revealed Rha, FucNAc, Qui3NAc, Glc and GlcNAc in the ratios ~1:1:1:3:2, respectively. Determination of the absolute configurations of the monosaccharides by GLC of the acetylated glycosides with (*S*)-2-octanol demonstrated D-Glc, D-GlcN, and L-Rha. The absolute configuration of D-FucNAc, D-Qui3NAc, D-GalA, and D-ManNAcA (see below) was inferred from the ^{13}C NMR chemical shifts using the known regularities in the glycosylation effects [7].

The ^{1}H and ^{13}C NMR spectra showed signals of different intensities, thus indicating that the PS is structurally heterogeneous, *inter alia* due to non-stoichiomteric O-acetylation (there were signals for O-acetyl groups at δ_H 2.11–2.17 and δ_C 21.7). O-Deacetylation of the PS resulted in elimination of a part of heterogeneity. The ^{13}C NMR spectrum of the O-deacetylated polysaccharide (DPS) (Fig. 1) contained signals for eight anomeric carbons at δ 96.9–106.0, three *C*H$_3$-C groups (C-6) of Rha, FucN, and Qui3N at δ 16.7–18.4, three HO*C*H$_2$-C groups (C-6 of Glc and 2 GlcN) at δ 61.2–62.6, five nitrogen-bearing carbons (C-2 of 2 GlcN, FucN and ManNA, C-3 of Qui3N) at δ 49.1–57.5, other sugar ring carbons in the region δ 68.4–80.9, and N-acetyl groups (CH$_3$ at δ 23.4–23.5, CO at δ 175.1–176.6). These data were consistent with the composition of the PS determined by sugar analysis. The absence of signals from the region of δ 83–88 characteristic of furanosides [8] showed that all monosaccharide residues are in the pyranosidic form. The ^{1}H NMR spectrum of the DPS (Fig. 1) contained signals for eight anomeric protons at δ 4.48–5.53, three CH$_3$-C groups (H-6 of Rha, FucN, and Qui3N) at δ 1.21–1.31, other sugar protons in the region δ 3.23–4.50, and N-acetyl groups at δ 1.98–2.08.

The ^{1}H and ^{13}C NMR spectra of the DPS were assigned using 2D ^{1}H,^{1}H COSY, ^{1}H,^{1}H TOCSY, ^{1}H,^{1}H ROESY, ^{1}H,^{13}C HSQC, and ^{1}H,^{13}C HMBC experiments (Table 1), and spin systems for one residue each of Glc (**H**), Rha (**C**), FucNAc (**A**), Qui3NAc (**B**), GalA (**D**), ManNAcA (**E**), and two GlcNAc residues (**F** and **G**) were identified. The assignment was based on correlations of H-1 and H-2 to H-6 for Glc, GlcNAc, and Qui3NAc, H-1 to H-2 and H-3 to H-6 for Rha or to H-5 for ManNAcA, H-1 to H-4 for GalA and FucNAc in the TOCSY spectrum. The assignment within each spin system was performed using COSY, and relative configurations of the monosaccharides were determined based on $^{3}J_{H,H}$ coupling constants. The H-6 signals for ManNAcA and GalA were found by H-5/C-6 correlations in the HMBC spectrum.

Relatively low-field positions of the C-5 signals at δ 74.4–77.4 in the ^{13}C-NMR spectrum of the DPS, as compared with published data for the corresponding monosaccharides [8,9], showed that Qui3NAc (**B**), GalA (**D**), ManNAcA (**E**), and GlcNAc (**G**) are β-linked. Similarly, the α configuration of FucNAc (**A**), Rha (**C**), GlcNAc (**F**), and Glc (**H**) was inferred by relatively high-field positions of the C-5 signals at δ 68.4–72.8. The configurations were confirmed by relatively large $^{3}J_{1,2}$ coupling constant of >7 Hz for the β-linked monosaccharides and <4 Hz for the α-linked monosaccharides.

Relatively low-field positions of the signals for C-2 of Qui3NAc (**B**), C-3 of FucNAc (**A**) and Rha (**C**), C-4 of GalA (**D**), ManNAcA (**E**), and Glc (**H**), C-3 and C-4 of GlcNAc (**F**), as compared with their positions in the corresponding non-substituted monosaccharides

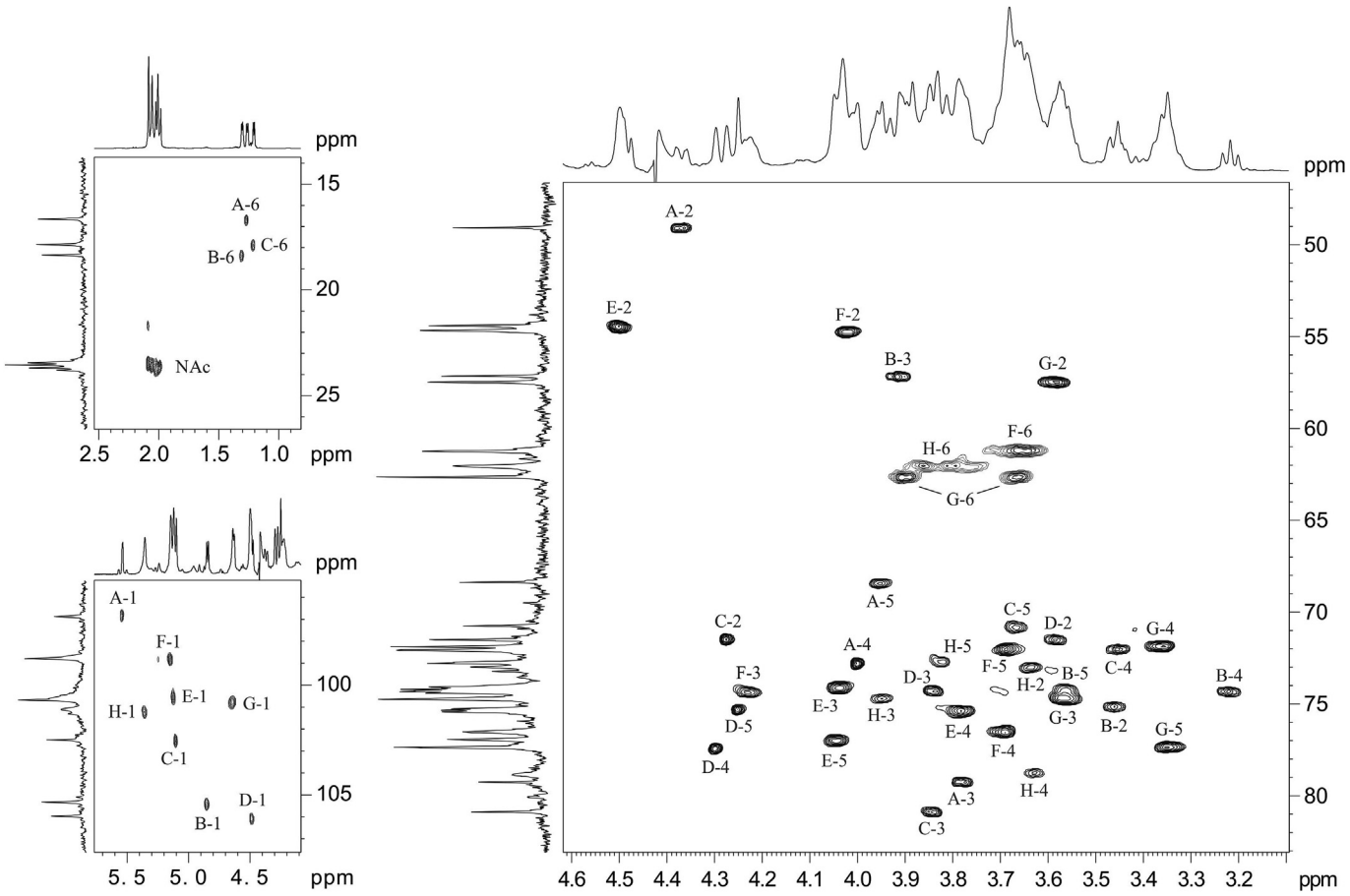

Fig. 1. Parts of a 2D ^{1}H,^{13}C HSQC spectrum of the O-deacetylated polysaccharides (DPS) from *P. putida* BIM B-1100. The corresponding parts of the ^{13}C and ^{1}H NMR spectra are displayed along the vertical and horizontal axes, respectively. Arabic numerals refer to the H/C pairs in the monosaccharide residues denoted as indicated in Chart 1 and Table 1.