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Note

Structure and gene cluster organization of the O-antigen of *Providencia alcalifaciens* O45:H25

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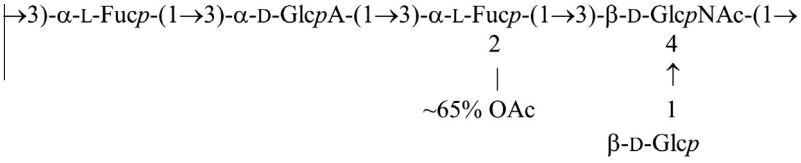
ABSTRACT

O-Polysaccharide was obtained by mild acid degradation of the lipopolysaccharide of *Providencia alcalifaciens* O45:H25 and studied by sugar analysis, Smith degradation, and ^{1}H and ^{13}C NMR spectroscopy. The following structure of the pentasaccharide repeat of the O-polysaccharide was established:



The O-antigen gene cluster of *P. alcalifaciens* O45 was sequenced and found to be in full agreement with the O-polysaccharide structure established.

Bacteria of the genus *Providencia* are widespread in nature and may cause urinary tract infections and enteric diseases in humans.[1] A combined O-antigen-based serotyping scheme for medically important *Providencia* species, *P. alcalifaciens, P. stuartii,* and *P. rustigianii*, consists of 63 O-serogroups.[2] The O-antigen represents a polysaccharide chain (O-polysaccharide) of the lipopolysaccharide (LPS) and consists of a number of oligosaccharide repeats (O-units) in S-type LPS or a single O-unit in SR-type LPS. Aiming at elaboration of the chemical basis for this classification, O-polysaccharide structures have been established for more than half of the O-serogroups.[3,4] In this work, we elucidated the structure of the O-polysaccharide of the S-type LPS of *P. alcalifaciens* O45:H25. Earlier, structure of a peptidoglycan-like surface polysaccharide has been established in *P. alcalifaciens* O45:H26,[5] which possessed an O-polysaccharide-lacking R-type LPS, most likely, owing to S→R dissociation during storage of the strain.

O-Polysaccharide structure elucidation: The LPS was isolated from bacterial cells of *P. alcalifaciens* O45:H25 by the phenol–water procedure[6] and degraded under mild acidic conditions. Fractionation of the carbohydrate portion by GPC on Sephadex G-50 resulted in O-polysaccharide (PS-1) and oligosaccharide fractions. Sugar analysis using GLC of the alditol acetates derived after full acid hydrolysis of PS-1 revealed fucose, glucose, and GlcNAc in the ratio ~2:2:1 (detector response), respectively. In addition, glucuronic acid (GlcA) was identified by GLC of the acetylated methyl glycosides. The D configuration of Glc and GlcNAc and the L configuration of Fuc were determined by GLC of the acetylated (*S*)-2-octyl glycosides.[7] The D configuration of GlcA was inferred by analysis of glycosylation effects on ^{13}C NMR chemical shifts using known regularities[8] (see below).

The ^{13}C NMR spectrum of PS-1 (Fig. 1, bottom) showed a structural heterogeneity, most likely, owing to non-stoichiometric O-acetylation (there was a signal for an *O*-acetyl group at δ 21.8). Mild alkaline O-deacetylation of PS-1 resulted in a regular polysaccharide (PS-2) consisting of pentasaccharide repeats. Its ^{13}C NMR spectrum (Fig. 1, top) contained, inter alia, signals for five anomeric carbons at δ 100.1–102.7, two C–CH_3 groups (C-6 of Fuc) at δ 16.6 and 16.7, two C–CH_2OH groups (C-6 of Glc and GlcNAc) at δ 61.1 and 63.5, one C–CO_2H group (C-6 of GlcA) at δ 177.5, one nitrogen-bearing carbon (C-2 of GlcNAc) at δ 57.4, and one *N*-acetyl group at δ 23.6 (CH_3) and 175.8 (CO). The absence of signals from

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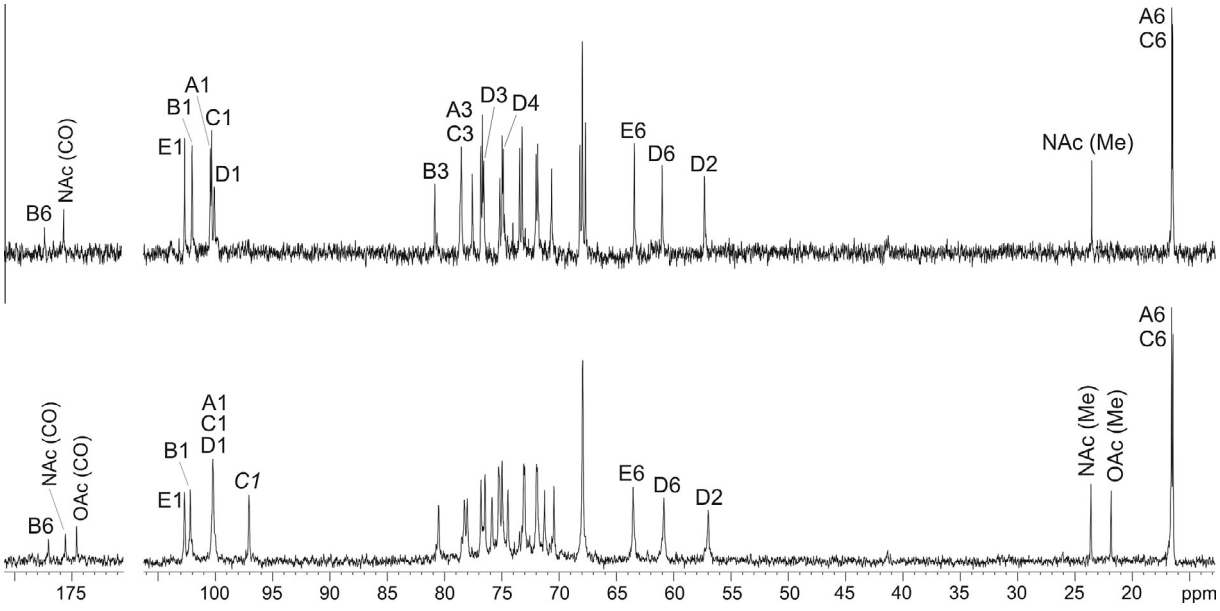

Figure 1. ^{13}C NMR spectra of the O-polysaccharide (PS-1) (bottom) and O-deacetylated polysaccharide (PS-2) (top) from *P. alcalifaciens* O45:H25. Arabic numerals refer to carbons in sugar residues denoted by letters as shown in Table 1. In the spectrum of PS-1, an upfield displacement to δ 97.1 of a part of the signal for C-1 of Fuc **C** annotated *C1* was caused by β-effect of 2-O-acetylation of this sugar residue.

the region δ 82–88 indicated that all sugar residues are pyranosidic.[9] The ^{1}H NMR spectrum of PS-2 showed signals for five anomeric protons at δ 4.51–5.29, two C–CH_3 groups (H-6 of Fuc) at δ 1.18 and 1.20, and one *N*-acetyl group at δ 2.03.

The ^{1}H and ^{13}C NMR spectra of PS-2 were assigned using ^{1}H,^{1}H COSY, TOCSY, ROESY (Fig. 2), ^{1}H,^{13}C HSQC (Fig. 3), and HMBC experiments (Table 1). The COSY and TOCSY spectra revealed spin-systems for three *gluco*-configurated sugar residues (GlcNAc **A**, GlcA **C,** and Glc **E**) and two fucose residues (**B** and **D**). As judged by the $J_{1,2}$ coupling constants of 7–8 Hz, GlcNAc and Glc were β-linked, whereas GlcA and both Fuc residues were α-linked ($J_{1,2}$ <4 Hz).

The ^{13}C NMR chemical shifts for C-2–C-6 of β-Glc*p* in PS-2 were close to those of the unsubstituted monosaccharide,[10] whereas significant downfield displacements by 7.3–8.2 ppm were observed for the signals for C-3 of GlcA and both Fuc residues, as compared with their positions in the corresponding non-substituted monosaccharides.[11] These displacements were due to α-glycosylation effects and defined the glycosylation pattern in the O-unit. Indicative downfield displacements by 1.8 and 4.8 ppm occurred also for C-3

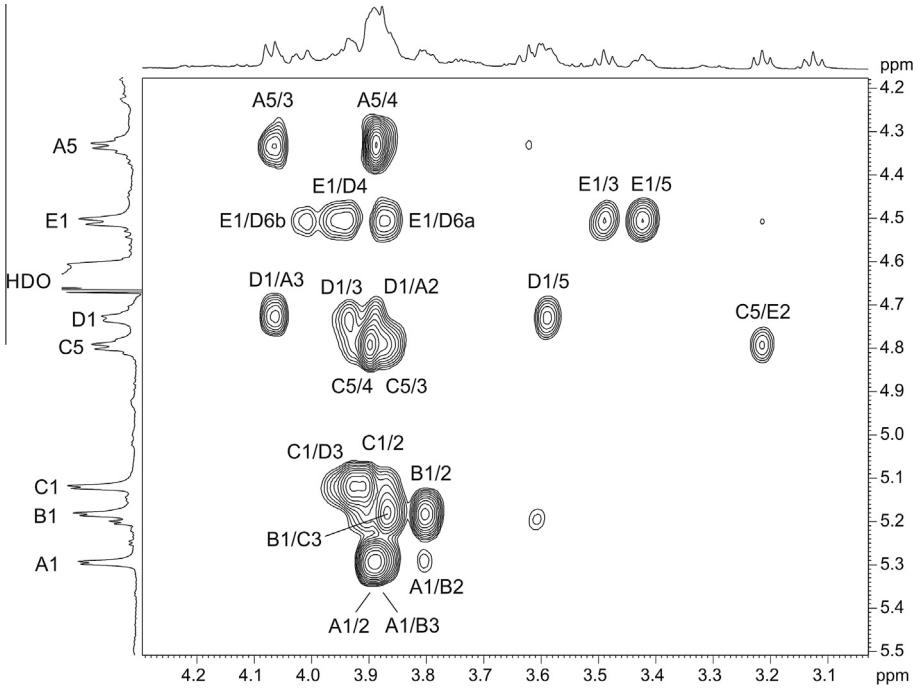

Figure 2. Part of a 2D ROESY spectrum of the O-deacetylated polysaccharide (PS-2) from *P. alcalifaciens* O45:H25. The corresponding parts of the ^{1}H NMR spectrum are shown along the axes. Arabic numerals refer to protons in sugar residues denoted by letters as shown in Table 1.