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Note

Structure of the O-polysaccharide from the lipopolysaccharide of *Providencia alcalifaciens* O60

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ABSTRACT

An O-polysaccharide (O-antigen) was isolated by mild acid degradation of the lipopolysaccharide of *Providencia alcalifaciens* O60 and studied by sugar and methylation analyses as well as ^{1}H and ^{13}C NMR spectroscopy, including 2D ROESY and $^{1}H,^{13}C$ HMBC experiments in D_2O and a ROESY experiment in a 9:1 H_2O–D_2O mixture to reveal correlations for NH protons. It was found that the polysaccharide is built up of linear pentasaccharide repeating units containing an amide of D-glucuronic acid with L-serine and has the following structure:

→4)-β-D-Glc*p*-(1→3)-α-D-Gal*p*-(1→4)-β-D-Gal*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→
L-Ser-(2–6)┘

The O-antigen studied is structurally and serologically closely related to the O-antigen of *Proteus vulgaris* O44.

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The genus *Providencia* of the *Enterobacteriaceae* family has been recently enlarged[1] to eight species, among which *Providencia stuartii*, *Providencia rettgeri*, *Providencia alcalifaciens,* and *Providencia rustigianii* are known to be opportunistic pathogens in humans.[2] *Providencia* infections include traveler's diarrhea,[3] food-borne gastroenteritis,[4] and urinary tract infections,[5] particularly in patients with long-term indwelling urinary catheters or extensive severe burns. As in other Gram-negative bacteria, the serological O-specificity of *Providencia* is defined by the fine structure of the O-antigen (O-polysaccharide), which is a part of the lipopolysaccharide (LPS). O-Antigen serotyping scheme for *Providencia* includes three species (*P. alcalifaciens*, *P. rustigianii*, *P. stuartii)* and consists of 63 O-serogroups.[6] At present, more than 30 O-polysaccharide structures have been established with the aim of the elucidation of the molecular basis of the serological classification of *Providencia* strains. In this paper we report on a new structure of the O-polysaccharide of *P. alcalifaciens* O60.

The LPS was isolated from dry bacterial cells by the phenol–water procedure and degraded under mild acid conditions. The subsequent fractionation of the carbohydrate portion by GPC on Sephadex G-50 resulted in a high-molecular-mass polysaccharide. Sugar analysis using GLC of the alditol acetates derived after acid hydrolysis of the polysaccharide revealed glucose, galactose, and 2-amino-2-deoxygalactose. In addition, glucuronic acid (GlcA) was identified by anion-exchange chromatography using a sugar analyzer, whereas amino acid analysis revealed serine. The L configuration of serine and D configuration of Glc, Gal, and GalN were determined by GLC of the acetylated (*S*)-2-octyl glycosides.

Linkage analysis by GLC–MS of the partially methylated alditol acetates derived from the methylated polysaccharide revealed major derivatives from 3- and 4-substituted hexoses, 3- and 4-substituted 2-amino-2-deoxyhexoses as well as minor compounds derived evidently from the LPS core.

The ^{13}C NMR spectrum of the polysaccharide (Fig. 1) demonstrated a pentasaccharide repeating unit. It contained signals for five anomeric carbons at δ 101.8–105.7, three nitrogen-bearing carbons (C-2 of Gal*p*N and Ser) at δ 52.8, 54.0, and 57.4, five $HOCH_2$–C groups at δ 61.4-62.8, two CO_2H groups (C-6 of Glc*p*A

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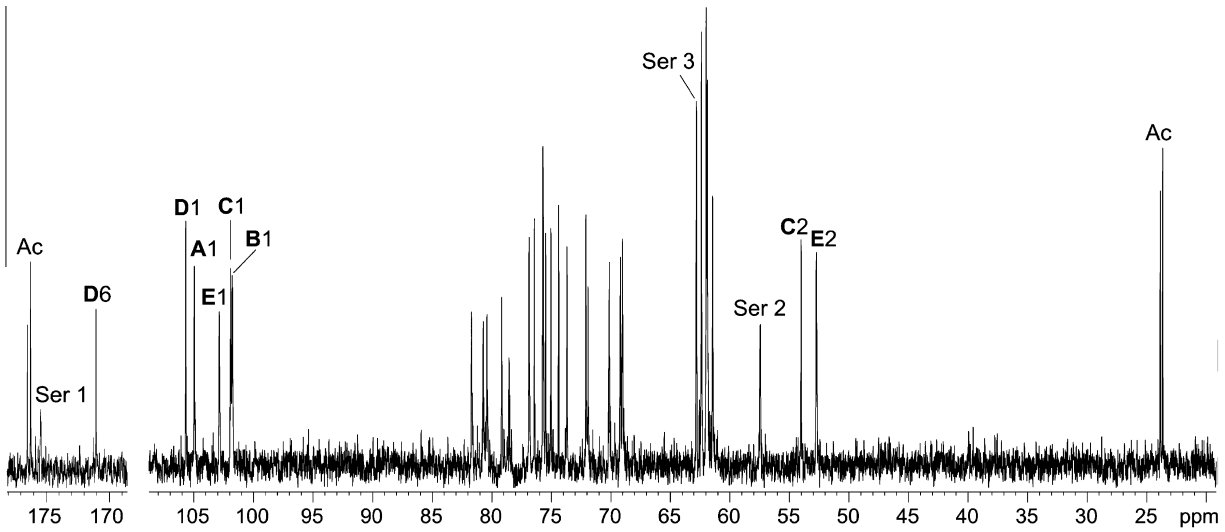

Figure 1. ^{13}C NMR spectrum of the O-polysaccharide from *P. alcalifaciens* O60. Arabic numerals refer to carbons in serine and sugar residues denoted by letters as shown in Table 1.

Table 1
^{1}H and ^{13}C NMR data (δ, ppm) of the O-polysaccharide from *P. alcalifaciens* O60

Residue		C-1 *H-1*	C-2 *H-2*	C-3 *H-3*	C-4 *H-4*	C-5 *H-5*	C-6 *H-6a,6b*
→4)-β-D-Glc*p*-(1→	**A**	104.9 *4.71*	74.4 *3.40*	75.7 *3.69*	80.4 *3.61*	75.7 *3.55*	61.4 *3.64, 3.85*
→3)-α-D-Gal*p*-(1→	**B**	101.8 *4.96*	69.0 *4.01*	80.7 *4.10*	70.1 *4.29*	71.9 *4.36*	62.0 *3.68, 3.68*
→4)-β-D-Gal*p*NAc-(1→	**C**	101.9 *4.50*	54.0 *3.94*	72.1 *3.76*	78.5 *4.04*	76.9 *3.70*	61.9 *3.95, 3.95*
→4)-β-D-Glc*p*A-(1→	**D**	105.7 *4.58*	73.7 *3.40*	75.1 *3.64*	79.2 *3.93*	75.5 *4.01*	171.0
→3)-β-D-Gal*p*NAc-(1→	**E**	102.9 *4.58*	52.8 *4.03*	81.7 *3.88*	69.2 *4.12*	76.4 *3.73*	62.4 *3.80, 3.74*
L-Ser		175.4	57.4 *4.50*	62.8 *3.93, 3.96*			

Additional chemical shifts for the *N*-acetyl groups are δ_H 2.03, 2.04, δ_C 23.7, 23.9 (both CH_3), 176.2 and 176.5 (both CO).

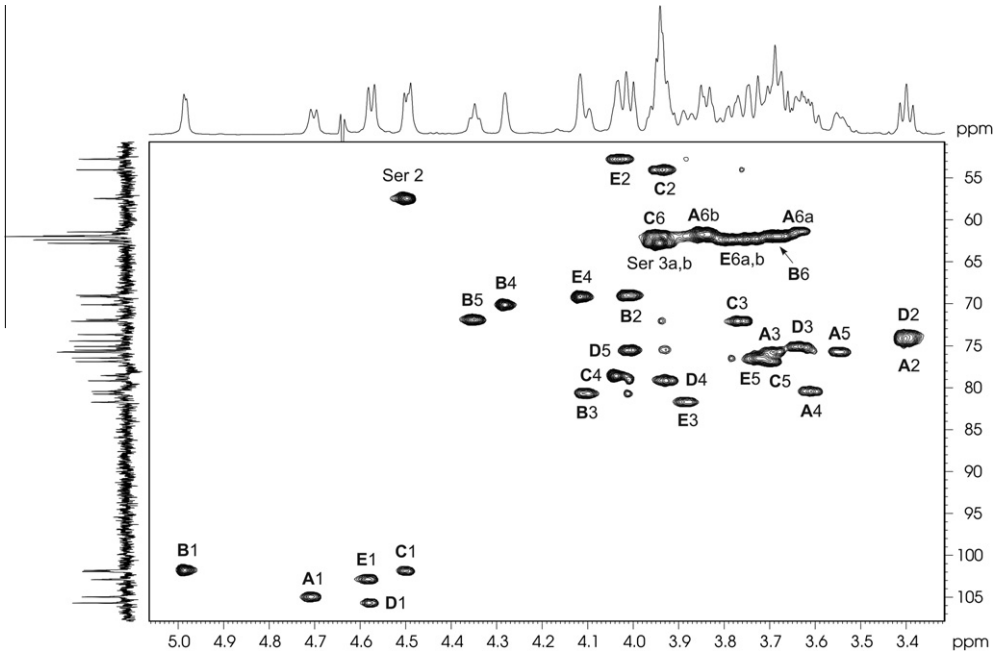

Figure 2. Part of an ^{1}H,^{13}C HSQC spectrum of the O-polysaccharide from *P. alcalifaciens* O60. The corresponding parts of the ^{1}H and ^{13}C NMR spectra are shown along the horizontal and vertical axes, respectively. Arabic numerals refer to H/C pairs in serine and sugar residues denoted by letters as shown in Table 1.

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