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Note

Structure of the polysaccharides from the lipopolysaccharide of *Azospirillum brasilense* Jm125A2

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ABSTRACT

Two polysaccharides were obtained by mild acid degradation of the lipopolysaccharide of associative nitrogen-fixing bacteria *Azospirillum brasilense* Jm125A2 isolated from the rhizosphere of a pearl millet. The following structures of the polysaccharides were established by sugar and methylation analyses, Smith degradation, and ^{1}H and ^{13}C NMR spectroscopy:

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

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

Structure **1** has been reported earlier for a polysaccharide from *A. brasilense* S17 (Fedonenko YP, Konnova ON, Zdorovenko EL, Konnova SA, Zatonsky GV, Shaskov AS, Ignatov VV, Knirel YA. *Carbohydr Res* 2008;343:810–6), whereas to our knowledge structure **2** has not been hitherto found in bacterial polysaccharides.

Azospirillum is a genus of Gram-negative bacteria within the family Rhodospirillaceae. It was first described by Tarrand et al. in 1978 and at that time comprised two species: *Azospirillum brasilense* and *Azospirillum lipoferum.*[1] Currently, the genus *Azospirillum* includes 16 species, most of which have been found in the rhizosphere of important crops and forage cereals in various climatic zones.[2,3] Lipopolysaccharides (LPSs) and capsular polysaccharides of these bacteria are involved at the initial stages of formation of associations with plants.[3,4] On the basis of the serological specificity of LPSs and the structure of the O-specific polysaccharides (OPSs), about 30 strains of *A. brasilense* and *A. lipoferum*, including type strains Sp7 and Sp59b, have been classified into three serogroups.[5–7] The OPSs of serogroups I and III are linear homopolymers of D-rhamnose or branched polysaccharides having an L-rhamnan backbone and side-chain glucose residues, respectively.[6,7] In most strains of serogroup II, two or more structurally different OPSs coexist, and the same OPS structures are shared by serologically heterogeneous strains.[7–10]

In this paper, we report structures of two OPSs of *A. brasilense* Jm125A2 isolated from the rhizosphere of pearl millet (*Pennisetum americanum*) in the USA.[11] Dot-blot analysis demonstrated a weak cross-reactivity of antibodies raised to strain Jm125A2 with cells of *A. brasilense* SpBr14,[12] whose OPS structure has been reported recently.[13]

The LPS was obtained from bacterial mass by hot phenol–water extraction and degraded under mild acidic conditions. A lipid sediment was removed by centrifugation, and the OPS was separated from low-molecular compounds by GPC of the carbohydrate-containing supernatant on Sephadex G-50. Sugar analysis by GLC

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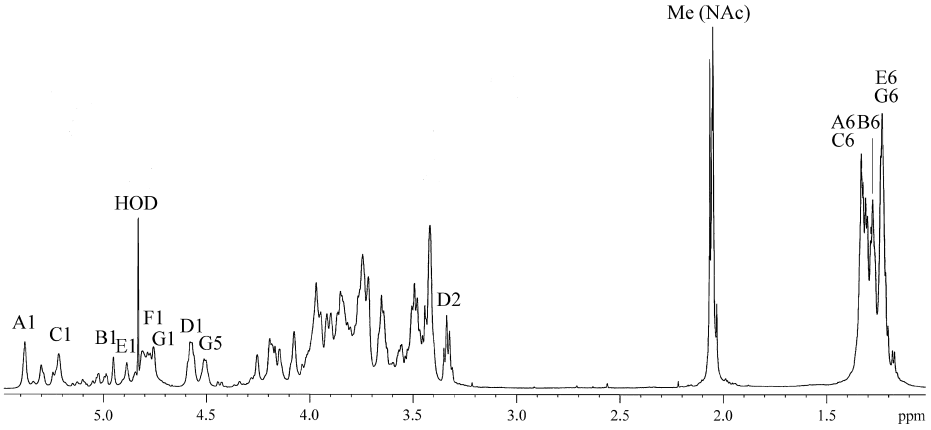

Fig. 1. ^{1}H NMR spectrum of the OPS from *A. brasilense* Jm125A2. Arabic numerals refer to carbons in sugar residues denoted as shown in Chart 1.

of the alditol acetates derived after full acid hydrolysis of the OPS revealed rhamnose (Rha), glucose (Glc), 2-acetamido-2-deoxyglucose (GlcNAc), and 3-acetamido-3-deoxyfucose (Fuc3NAc) in the ratio ~5:2.6:1:1.2 (detector response). GLC of the acetylated (*S*)-2-octyl glycosides demonstrated the D configuration of Glc and the L configuration of Rha. The absolute configurations of GlcN and Fuc3N were inferred by analysis of glycosylation effects on ^{13}C NMR chemical shifts using known regularities (see below).

GLC–MS analysis of the partially methylated alditol acetates derived from the methylated OPS indicated the presence of 2,3,4,6-tetra-O-methyl-glucose, 2,4-di-O-methylrhamnose, 3,4-di-O-methylrhamnose, 4-O-methylrhamnose, 2-deoxy-4,6-di-O-methyl-2-(N-methyl)acetamidoglucose (from 3-substituted GlcNAc), and 3-deoxy-2,4-di-O-methyl-3-(N-methyl)acetamidofucose (from terminal Fuc3NAc). Therefore, the OPS contains 2-substituted, 3-substituted, and 2,3-disubstituted Rha, 3-substituted GlcNAc, and terminal Glc and Fuc3NAc residues.

Analysis using ^{1}H NMR (Fig. 1), ^{13}C NMR (Fig. 2A), and 2D NMR spectroscopy, including ^{1}H,^{1}H ROESY and ^{1}H, ^{13}C HMBC experiments, revealed the presence of two structurally distinct repeating units. One of them was tetrasaccharide **1** (Chart 1), whose structure had been established earlier for the OPS of *A. brasilense* S17.[8] In order to determine the structure of the other repeating unit, Smith degradation of the OPS was performed. As a result, the repeats **1** were destroyed to give a polysaccharide (PS) consisting of repeating units **2**. A comparison of ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra of the PS and initial OPS showed that the latter included the same repeating units **2**, which, therefore, were not affected by Smith degradation.

The ^{13}C NMR spectrum of the PS (Fig. 2B) showed signals of three anomeric carbons at δ 98.2–103.1, one HO*C*H$_2$–C group (C-6 of GlcNAc) at δ 62.2, two nitrogen-bearing carbons (C-2 of GlcNAc and C-3 of Fuc3NAc) at δ 52.2 and 56.8, two *C*H$_3$–C groups (C-6 of Fuc3NAc and Rha) at δ 16.6 and 17.4, other sugar ring carbons in the region of δ 67.6–83.1, and two N-acetyl groups (*C*H$_3$ at δ 23.3 and 23.5, *C*O at δ 175.2 and 175.6). Accordingly, the ^{1}H NMR spectrum of the OPS showed signals for three anomeric protons at δ 4.77–4.88, two *C*H$_3$–C groups (H-6 of Rha and Fuc3NAc) at δ 1.23 and 1.25, other

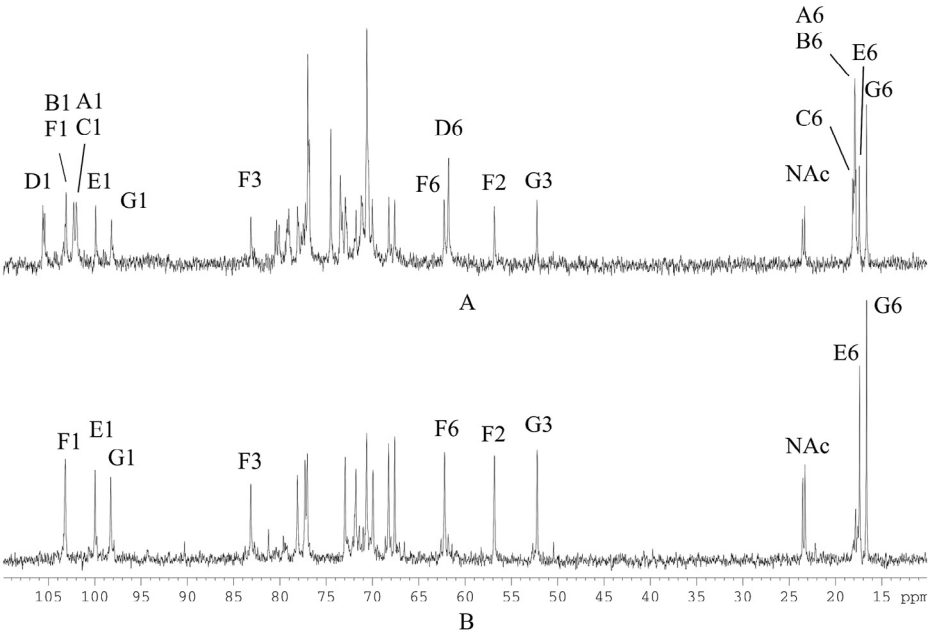

Fig. 2. ^{13}C NMR spectra of the OPS from *A. brasilense* Jm125A2 (A) and PS obtained after Smith degradation of the OPS (B). Arabic numerals refer to carbons in sugar residues denoted as shown in Chart 1.