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Note

Structure of the O-polysaccharide of *Photorhabdus luminescens* subsp. *laumondii* containing D-*glycero*-D-*manno*-heptose and 3,6-dideoxy-3-formamido-D-glucose

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ABSTRACT

The O-polysaccharide from the lipopolysaccharide of a symbiotic bacterium *Photorhabdus luminescens* subsp. *laumondii* TT01 from an insect-pathogenic nematode was studied by sugar analysis and ^{1}H and ^{13}C NMR spectroscopy and found to contain D-*glycero*-D-*manno*-heptose (DDHep) and 3,6-dideoxy-3-formamido-D-glucose (D-Qui3NFo). The following structure of the pentasaccharide repeating unit of the O-polysaccharide was established:

→2)-β-DDHep*p*-(1→3)-β-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-β-D-Glc*p*NAc-(1→
3
↑
1
α-D-Qui*p*3NFo

The genus *Photorhabdus* from the family *Enterobacteriaceae* includes three entomopathogenic species associated with nematodes of the genera *Heterorhabditis*.[1] No serological classification scheme for *Photorhabdus* species has been developed so far. Aiming at creation of the chemical basis for an O-antigen-based classification, we have elucidated structures of the O-polysaccharides of several strains of *Photorhabdus asymbiotica* subspp. *australis* and *asymbiotica* and found them to be closely related.[2] In this work, we established the structure of the O-polysaccharide of *Photorhabdus luminescens*, which differs significantly from the structures of *P. asymbiotica*.

The polysaccharide was obtained by mild acid hydrolysis of the lipopolysaccharide isolated from dried bacterial cells of *P. luminescens* subsp. *laumondii* TT01 by the phenol–water procedure. Sugar analysis of the polysaccharide by GLC–MS of the acetylated alditol acetates derived after full acid hydrolysis of the polysaccharide revealed galactose, a heptose having the same retention time as D-*glycero*-D-*manno*-heptose (DDHep), 3-amino-3-deoxyquinovose (Qui3N) and GlcN in a ratio of 1:0.76:0.53:0.93 (detector response). Further NMR spectroscopic studies showed that the monosaccharide ratio in the repeating unit is 1:1:1:2, respectively. A lower than expected content of Qui3N and GlcN in GLC analysis is evidently accounted for by a lower response factor for amino sugars (~0.5 of that for neutral sugars). Analysis by GLC of the acetylated (+)-2-octyl glycosides indicated the D configuration of Gal and GlcN. The absolute configuration of heptose as DDHep and the D configuration of Qui3N were established by analysis of ^{13}C NMR chemical shift data of the polysaccharide (Table 1) using known regularities in glycosylation effects.[3]

The ^{1}H NMR and ^{13}C NMR (Fig. 1) spectra of the polysaccharide showed signals of different intensities, including those for an *N*-formyl group (δ_H 8.27 and 8.03, δ_C 166.5 and 169.3 for the major *Z*- and minor *E*-isomers, respectively; compare published data[4]). 2D ^{1}H,^{1}H COSY, TOCSY, and ^{1}H,^{13}C HSQC (Fig. 2) experiments revealed spin systems for five pyranosidic residues (anomeric atom signals at δ_H 4.47–5.44 and δ_C 98.3–104.7), including two residues of GlcN and one residue each of Gal, DDHep and Qui3N (Table 1). Splitting of the signals for Qui3N showed that it is the sugar that carries the *N*-formyl group (Fo) occurring as two stereoisomers. The presence of signals for two *N*-acetyl groups [δ_H 2.03 and

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Table 1
^{1}H and ^{13}C NMR chemical shifts (δ, ppm) of the O-polysaccharide from *P. luminescens* subsp. *laumondii* TT01

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*	C-7 *H-7a, 7b*
→3)-β-D-Glc*p*NAc-(1→[a]	**A**	101.7 (101.6) *5.05* (*5.09*)	55.4 *3.86*	79.2 *3.70*	72.6 *3.70*	77.4 *3.39*	61.6 (61.2) *3.90, 3.85* (*3.82, 3.75*)	
→3)-α-D-Glc*p*NAc-(1→	**B**	98.3 *5.44*	53.5 *4.10*	81.7 *3.79*	69.4 *3.60*	71.1 *3.66*	62.0 *3.81, 3.81*	
→3)-β-D-Gal*p*-(1→	**C**	104.7 *4.47*	73.3 *3.66*	82.4 *3.82*	70.0 *4.11*	76.1 *3.80*	61.5 *3.85, 3.85*	
→2,3)-β-DDHep*p*-(1→	**D**	103.2 *4.86*	77.2 *4.42*	77.4 *3.79*	68.9 *3.78*	78.3 *3.45*	72.6 *4.02*	62.8 *3.75, 3.64*
α-D-Qui*p*3N(*Z*-Fo)-(1→ (major)	*Z*-**E**	100.1 *5.24*	71.4 *3.65*	54.1 *4.19*	74.4 *3.22*	69.6 *4.05*	17.8 *1.29*	
α-D-Qui*p*3N(*E*-Fo)-(1→ (minor)	*E*-**E**	100.5 *5.26*	70.7 *3.63*	58.7 *3.63*	73.7 *3.22*	69.5 *4.04*	17.9 *1.28*	

Chemical shifts for *Z*-Fo (major) are δ_H 8.27 and δ_C 166.5; *E*-Fo (minor) δ_H 8.03 and δ_C 169.3; NAc δ_H 2.03 and 2.10; δ_C 23.3, 23.9 (both Me), 175.4 and 175.7 (both CO).
[a] Shown are chemical shifts of the major repeating unit with α-Qui3N(*Z*-Fo) (*Z*-**E**). When different, chemical shifts of the minor repeating unit with α-Qui3N(*E*-Fo) (*E*-**E**) are indicated in parentheses.

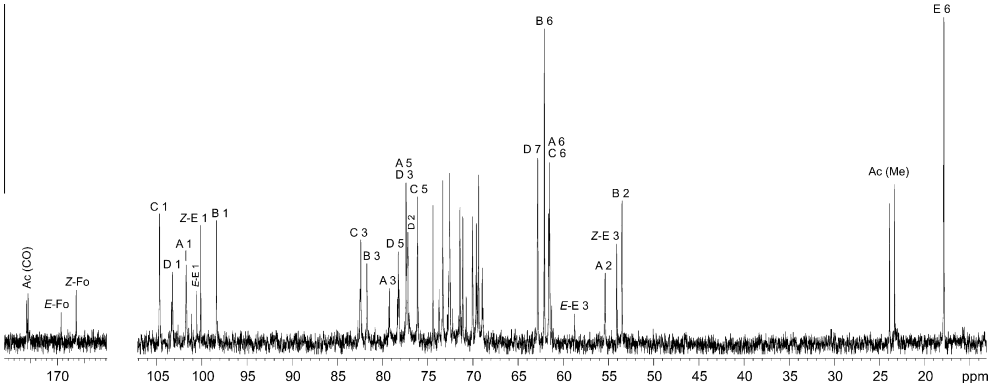

Figure 1. ^{13}C NMR spectrum of the O-polysaccharide of *P. luminescens* subsp. *laumondii* TT01. Arabic numerals refer to carbons in sugar residues denoted as shown in Table 1.

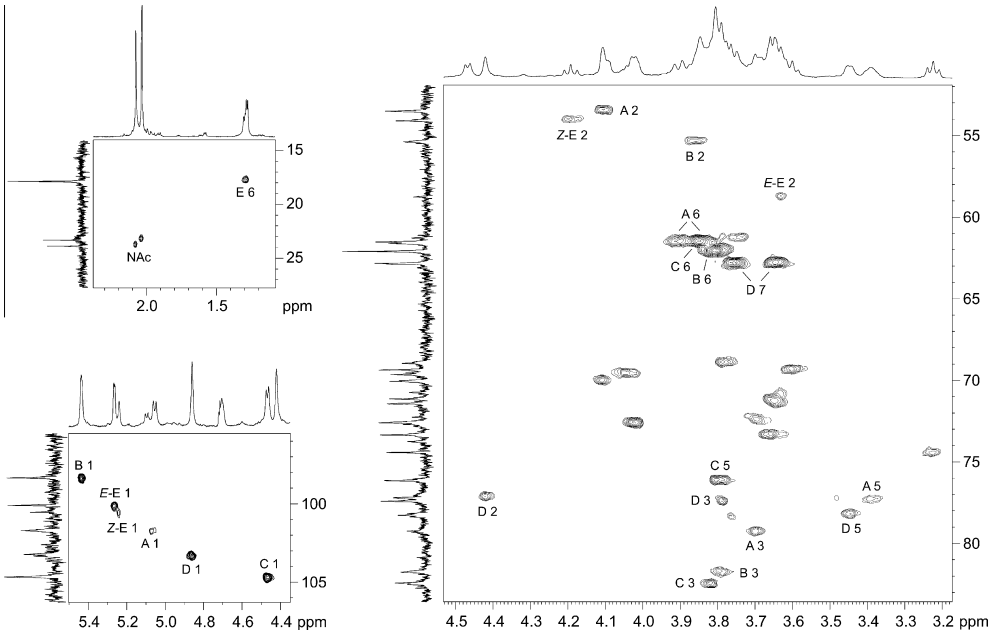

Figure 2. Parts of a ^{1}H,^{13}C HSQC spectrum of the O-polysaccharide of *P. luminescens* subsp. *laumondii* TT01. The corresponding parts of the ^{1}H and ^{13}C NMR spectra are shown along the horizontal and vertical axes, respectively. Arabic numerals refer to H/C pairs in sugar residues denoted as shown in Table 1.