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Note

Structure of the O-antigen of *Budvicia aquatica* 20186, a new bacterial polysaccharide that contains 3,6-dideoxy-4-*C*-[(*S*)-1-hydroxyethyl]-D-*xylo*-hexose (yersiniose A)

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ABSTRACT

The following structure of the O-specific polysaccharide (O-antigen) of *Budvicia aquatica* 20186 was elucidated by sugar analysis along with 1D and 2D ^{1}H and ^{13}C NMR spectroscopy:

→4)-α-L-Rha*p*-(1→3)-α-D-Gal*p*-(1→2)-α-Yer*p*-(1→3)-β-D-Gal*p*NAc-(1→

where Yer stands for 3,6-dideoxy-4-*C*-[(*S*)-1-hydroxyethyl]-D-*xylo*-hexose (yersiniose A).

A new, hydrogen sulfide-producing member of the family Enterobacteriaceae, the genus *Budvicia* with its single species *Budvicia aquatica*, was isolated and described by Aldova et al.[1] in 1983. Simultaneously, Richard and co-workers[2] studied a group of six strains isolated from stream water in Sweden and one strain isolated from a shrew in Spain. DNA relatedness studies on these strains and a formal genus and species proposal were subsequently reported collaboratively by the Czech group and a group at the Pasteur Institute in Paris.[3]

Phylogenetic analysis of the type strain of *B. aquatica* (20186HG01 = ATCC25567) indicates that *Budvicia* forms a distinct lineage in the family well separated from other Enterobacteriaceae genera.[4] Relatedness of 59 strains tested to the type strain of *B. aquatica* is 71–100% (87% on average) in 60 °C DNA hybridization reactions, and the divergence in related sequences is 0.0–0.2%. Relatedness of *B. aquatica* to other members of Enterobacteriaceae is 8% or less.[3] Strains of *B. aquatica* are found in surface water not associated with sewage. The first known case of infection was described in a 85-year-old woman exposed to the aftermath of the hurricane Katrina, who tested positive for *B. aquatica* from both blood and urine samples.[5]

Composition and structure of the lipopolysaccharide (LPS) on the cell surface of Gram-negative bacteria may be a useful taxonomic criterion. The LPS of *B. aquatica* has been studied scarcely. Earlier,[6] we have reported the structure of the O-specific polysaccharide chain (OPS, O-antigen) of the LPS of *B. aquatica* 97U124, which has a glycerol teichoic acid-like structure unusual for Gram-negative bacteria. In this work, we established the OPS structure of the type strain *B. aquatica* 20186, which contains a branched sugar, yersiniose A.

The OPS was released by mild acid hydrolysis of the LPS and purified by GPC on Sephadex G-50. Sugar analysis by GLC of the alditol acetates derived after full hydrolysis of the OPS revealed Rha, Gal and GalNAc. An additional monosaccharide constituent, 3,6-dideoxy-4-*C*-[(*S*)-1-hydroxyethyl]-D-*xylo*-hexose (yersiniose A, Yer), was identified by further studies of the OPS using NMR spectroscopy (see below). The D configuration of Gal and L configuration of Rha were determined by GLC analysis of the (*S*)-2-octyl glycosides; the absolute configurations of the other monosaccharides were established using known regularities of glycosylation effects on ^{13}C NMR chemical shifts.[7]

The ^{13}C NMR spectrum of the OPS (Fig. 1) showed signals for four anomeric carbons at δ 93.2–103.3, three methyl groups (C-6 of Rha, C-6 and C-2′ of Yer) at δ 13.5, 16.6 and 18.3, a methylene group (C-3 of Yer) at δ 29.8, two hydroxymethyl groups (C-6 of Gal and GalNAc) at δ 62.2 and 62.6, one nitrogen-bearing carbon (C-2 of GalNAc) at δ 52.5, other ring carbons at δ 65.1–81.3, and an *N*-acetyl group at δ 23.6 (CH_3) and 175.8 (CO). The absence of signals in the region of δ 82–88 characteristic of furanoses

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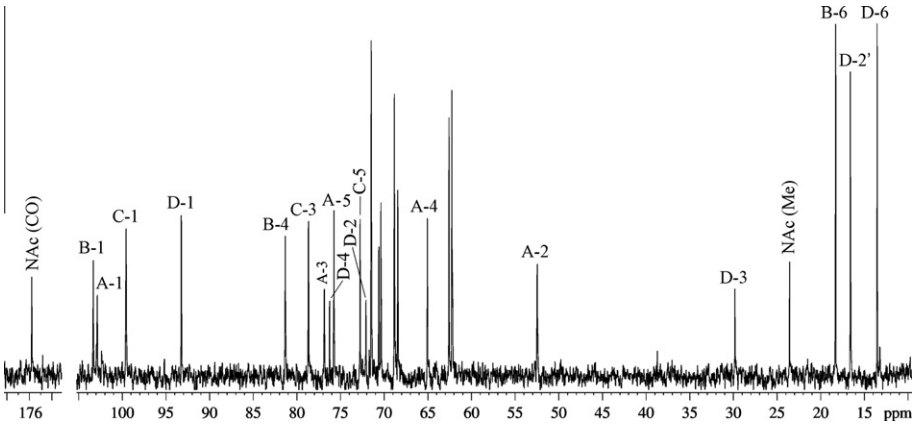

Figure 1. ^{13}C NMR spectrum of the OPS from *B. aquatica* 20186. Arabic numerals refer to carbons in sugar residues denoted as follows: **A**, GalN; **B**, Rha; **C**, Gal; **D**, Yer.

Table 1
Chemical shifts (δ, ppm) of the OPS from *Budvicia aquatica* 20186

Sugar residue	H-1 *C-1*	H-2 *C-2*	H-3 (3eq, 3ax) *C-3*	H-4 *C-4*	H-5 *C-5*	H-6 (6a, 6b) *C-6*	H-1′ *C-1′*	H-2′ *C-2′*
→3)-β-D-Gal*p*NAc-(1→	4.82	4.02	3.87	4.20	3.67	3.85, 3.79		2.04
	102.8	*52.5*	*76.9*	*65.1*	*75.7*	*62.2*	*175.8*	*23.6*
→4)-α-L-Rha*p*-(1→	5.00	4.02	3.96	3.68	3.85	1.35		
	103.3	*71.5*	*71.5*	*81.3*	*68.8*	*18.3*		
→3)-α-D-Gal*p*-(1→	5.15	3.94	3.90	4.05	4.02	3.74		
	99.5	*68.5*	*78.7*	*70.4*	*72.8*	*62.6*		
→2)-α-Yer*p*-(1→	5.15	4.11	1.94, 2.00		4.26	1.15	3.70	1.18
	93.2	*72.1*	*29.8*	*76.3*	*68.8*	*13.5*	*70.6*	*16.6*

^{13}C chemical shifts are given in italics.

indicated that all monosaccharide residues are in the pyranose form. The ^{1}H NMR spectrum of the OPS contained signals for four anomeric protons at δ 4.82–5.15, three methyl groups (H-6 of Rha, H-6 and H-2′ of Yer) at δ 1.11, 1.15 and 1.35, one methylene group (H-3 of Yer) at δ 1.94 and 2.00, other sugar protons at δ 3.67–4.26, and one *N*-acetyl group at δ 2.04.

Assignment of the ^{1}H and ^{13}C (Fig. 1) NMR spectra of the OPS (Table 1) using 2D COSY, TOCSY, ^{1}H,^{13}C HSQC and HMBC experiments revealed four monosaccharide spin systems. Three spin systems were assigned to Rha, Gal and GalNAc. The forth spin systems belonged to yersiniose (Fig. 2), which was identified by the following intraresidue correlations: (i) in the TOCSY spectrum, a CH_2 group (H-3eq and H-3ax) at δ 1.94 and 2.00 with H-2 and H-1 at δ 4.11 and 5.15, respectively; CH_3 groups (H-6 and H-2′) with H-5 and H-1′ at δ 1.15/4.26 and 1.1.8/3.70, respectively; (ii) in the ROESY spectrum, H-3eq with H-2 and H-3ax with H-5 at δ 1.94/4.11 and 2.00/4.26; a CH_3 group (H-6) with H-1′ at δ 1.15/3.70; (iii) in the HMBC spectrum, protons of two CH_3 groups (H-6 and H-2′) with C-5 and C-1′ at δ 1.15/68.8 and 1.18/70.6 and a tertiary carbon (C-4) at δ 1.15/76.3 and 1.18/76.3, respectively; carbons of CH_3 groups (C-6 and C-2′) with H-5 and H-1′ at δ 13.5/4.26 and 16.6/3.70; carbon of a CH_2 group (C-3) with H-1′ at δ 3.70. These data were in agreement with the lack of the H-4 proton and the $^3J_{H,H}$ coupling constants determined from the ^{1}H NMR spectrum: $J_{1,2}$ 4.1 Hz, $J_{2,3eq}$ 4.5 Hz, $J_{2,3ax} \approx J_{3ax,3eq}$ 12.5 Hz, $J_{5,6} \approx J_{1',2'}$ 6.5 Hz. A large $J_{2,3ax}$ value of 12.5 Hz showed that the H-2 proton is axial and, hence, yersiniose exists in the $^4C_1$ conformation. A comparison of the ^{13}C NMR chemical shifts with published data of the 1′-epimers (yersiniose A and B)[8] indicated that the OPS contains the (*S*)-1′-epimer, that is yersiniose A (particularly, compare $\delta_{C\text{-}2'}$ 16.7 and 18.0, $\delta_{C\text{-}4}$ 76.6 and 77.4 of yersiniose A and B, respectively,[8] with $\delta_{C\text{-}2'}$ 16.6 and $\delta_{C\text{-}4}$ 76.3 of yersiniose in the OPS).

The configurations of the glycosidic linkages were determined by a ROESY experiment, which showed H-1/H-3 and H-1/H-5 correlations for a β-linked sugar (GalNAc) and H-1/H-2 correlations for α-linked sugars (Rha, Gal and Yer) (Fig. 3). They were confirmed by the C-5 chemical shifts compared with those of the corresponding α- and β-pyranoses[9] or -pyranosides.[8]

Downfield displacements of the signals for C-3 of Gal to δ 78.7, C-3 of GalNAc to δ 76.9, C-4 of Rha to δ 81.3 and C-2 of Yer to δ 72.1 from their positions in the spectra of the corresponding non-substituted monosaccharides at δ 70.4, 72.4, 73.5[9] and 65.6,[8] respectively, demonstrated that the OPS is linear and revealed the glycosylation pattern in the repeating unit. This pattern was confirmed and the monosaccharide sequence was analyzed by 2D ROESY and ^{1}H,^{13}C HMBC experiments. The ROESY spectrum (Table 2 and Fig. 3) showed transglycosidic correlations for H-1 of all sugar residues but the expected Yer H-1,GalNAc H-3 cross-peak overlapped with the intraresidue Gal H-1,H-3 cross-peak. The HMBC spectrum established the GalNAc→Rha→Gal→Yer sequence (Table 2) but there were cross-peaks neither for Yer H-1 nor Yer C-1. However, the Yer→GalNAc linkage was inferred unambiguously from the ^{13}C NMR chemical shift data, which showed substitution of GalNAc at position 3, and taking into account the linear character of the OPS.

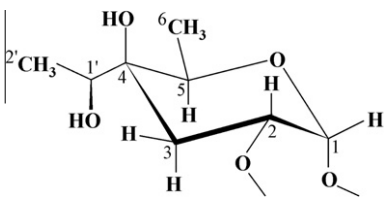

Figure 2. Structure of yersiniose A.