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Note

Structural characterization of polysaccharides expressed by *Burkholderia oklahomensis* E0147

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ABSTRACT

Burkholderia oklahomensis E0147 is a US isolated bacterium believed to express a similar O-antigen to type A structure of the highly pathogenic species, *Burkholderia pseudomallei*. Both species are genetically closely related. Lipopolysaccharide was collected from E0147 and structurally characterized to test this hypothesis. Glycosyl composition and linkage analyses in conjunction with 1D and 2D ^{1}H and ^{13}C NMR spectroscopy showed that the O-antigen was a repeating disaccharide with the following structure:

[3)-β-D-Glc*p*-(1→3)-2OAc-α-L-6dTal*p*-(1→]$_n$

NMR spectroscopy also revealed the presence of a co-extracted exopolysaccharide previously described in *B. pseudomallei*, with the structure:

[3)-2OAc-β-D-Gal*p*-(1→4)-α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→5)-β-D-Kdo*p*-(2→]$_n$.

Burkholderia oklahomensis is a Gram-negative, saprophytic bacterium known from four strains isolated in the United States.[1] Three strains were isolated in central Oklahoma, one from a farming accident where a deep wound was contaminated with soil and the other two were taken from environmental samples on that same farm thereafter.[2] The Georgian strain E0147 was isolated from a man who crashed his car into a mud bank and subsequently developed ulcers in his left eye from which E0147 was isolated eight weeks later.[3] The two clinical isolates were originally mistaken as native cases of melioidosis, a multifarious disease endemic to Southeast Asia and northern Australia and caused by *Burkholderia pseudomallei*.[4]

Four distinct O-antigens have been described in *B. pseudomallei*, types A, B, B2, and rough.[5] Type A is found in approximately 85% of all isolates and is to date the only O-antigen that has been structurally characterized. It is an unbranched polysaccharide consisting of 3)-β-Glc*p*-(1→3)-α-6dTal*p*-(1→ disaccharide repeating units with the 6-deoxytalose (6dTal) residue variably methylated and acetylated.[6,7] This backbone has also been described in the closely related *Burkholderia mallei* and *Burkholderia thailandensis* with variable side group modification patterns.[8–10]

Recently, *B. oklahomensis* E0147 was shown to express a serologically positive O-antigen with an identical ladder pattern to *B. pseudomallei* type A LPS.[5,11] We report here the confirmation of the type A 3)-β-Glc*p*-(1→3)-α-6dTal*p*-(1→ backbone in E0147. A second polysaccharide was also detected with structure [3)-2OAc-β-Gal*p*-(1→4)-α-Gal*p*-(1→3)-β-Gal*p*-(1→5)-β-Kdo*p*-(2→]$_n$. This exopolysaccharide (EPS) has been previously described in *B. pseudomallei* and *Burkholderia cepacia*.[12–14]

Previously, the comparison of the O-antigen biosynthesis gene clusters of *B. pseudomallei* K96243 and *B. oklahomensis* E0147 revealed homology of 82% at the nucleotide level.[11] However the *wbiD* gene, which encodes a methyltransferase, is truncated in *B. oklahomensis* E0147 due to a thymine insertion at position 1,236 relative to *B. pseudomallei* K96243's gene BPSL2677. This gene product is believed to be responsible for adding a methyl group to the 2-*O* position on the 6-deoxytalose residue.[15] This suggests that the *B. oklahomensis* E0147 may produce an O-antigen with backbone Glucose-6-deoxytalose (Glc-6dTal) similar to those described by Knirel et al.,[6] which lacked O-methylation.

The monosaccharide composition of the *B. oklahomensis* LPS was determined by GC/MS as previously described,[10] revealing glucose (Glc), galactose (Gal), and 6-deoxytalose (6dTal) as the major components. Lesser amounts of rhamnose (Rha), ribose, mannose,

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heptose, xylose, and 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) were also detected. Linkage analyses showed that 3-linked Glc*p* (glucopyranose) and 3- and 4-linked Gal*p* (galactopyranose) were predominant with lesser linkages including 3-linked 6dTal*p* and terminal Gal*p*, Glc*p*, and 6dTal*p*. One-dimensional proton NMR of the LPS showed the presence of six protons in the anomeric region (Fig. 1), suggesting that the O-antigen was considerably more complex than other *Burkholderia* type A LPS structures. Repeated proton NMR analysis on the sample showed that the relative intensities of these peaks varied, suggesting the presence of two polysaccharide chains, one containing two sugar residues (peaks at 5.23 and 4.61 ppm) and the other containing four residues (Fig. 2; peaks at 5.08, 4.89, 4.73, and 4.65 ppm).

The signal at 5.23 ppm was about twice as intense as the signal at 4.61 ppm. A 2D ^{1}H- ^{13}C-HSQC spectrum showed that this peak arose from two different protons, which were identified as H-1 and H-2 of α-6dTal, H-2 being shifted downfield due to acetylation. The peak at 4.61 ppm was identified by COSY (Fig. 3), TOCSY (data not shown), and HSQC (Fig. 4) as H-1 of β-Glc, of which NOESY (Fig. 5) revealed a cross peak to H-3 of 6dTal. NOESY also showed a correlation between H-1 of 6dTal and H-3 of β-Glc, elucidating the basic repeating structure of the first polysaccharide as →3)-β-D-Glc*p*-(1→ 3)-2OAc-α-L-6dTal*p*-(1→. Three modification patterns were found associated with the 6dTal residue. Unlike previous studies with *B. pseudomallei* and *B. thailandensis*, an unmodified 3-α-6d-Tal*p* residue was not detected [6,7,10], nor was there

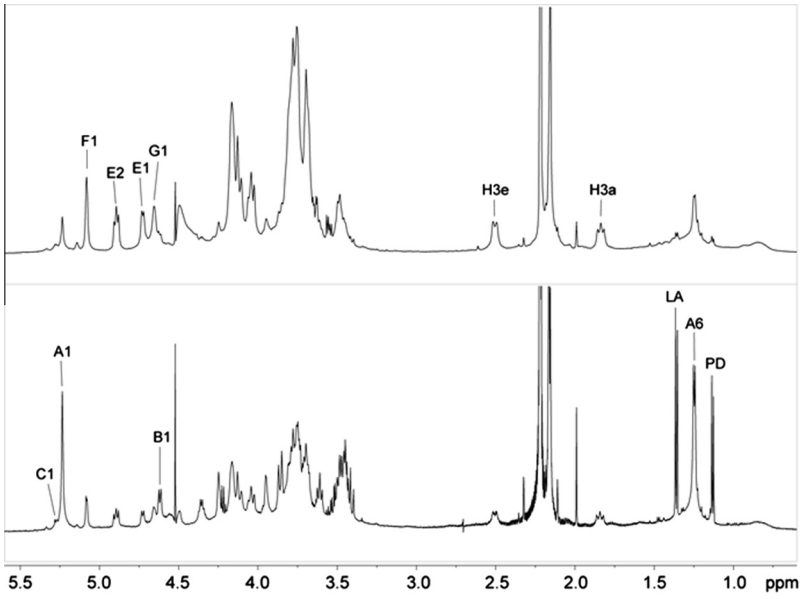

Figure 1. 1D ^{1}H NMR spectra of two preparations of the expressed *B. oklahomensis* E0147 polysaccharides. The upper panel shows a higher abundance of EPS and the lower panel shows a higher abundance of LPS. The characteristic peaks of the EPS and the LPS are labeled in the top and bottom spectra, respectively. Labels correspond to the numbering system in Tables 1 and 2.

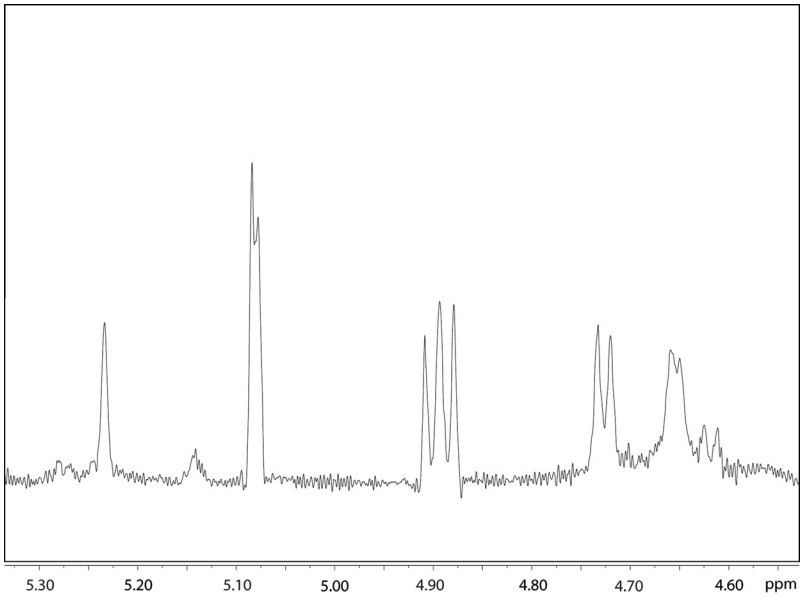

Figure 2. Anomeric region of the resolution-enhanced 1D ^{1}H NMR spectrum of *B. oklahomensis* E0147 O-polysaccharide.