Carbohydrate Research 381 (2013) 6–11

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Note

Revised structures for the predominant O-polysaccharides expressed by *Burkholderia pseudomallei* and *Burkholderia mallei*

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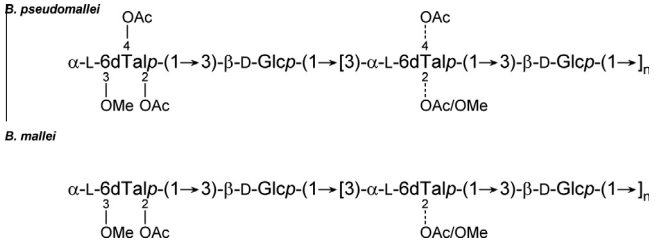
ARTICLE INFO

Article history:
Received 20 June 2013
Received in revised form 4 August 2013
Accepted 14 August 2013
Available online 24 August 2013

Keywords:
Burkholderia pseudomallei
Burkholderia mallei
Burkholderia thailandensis
Lipopolysaccharide
O-polysaccharide
Structure

ABSTRACT

O-Polysaccharides (OPS) were isolated from purified *Burkholderia pseudomallei* and *Burkholderia mallei* lipopolysaccharides by mild-acid hydrolysis and gel-permeation chromatography. 1-D and 2-D ^{1}H and ^{13}C NMR spectroscopy experiments revealed that the OPS antigens were unbranched heteropolymers with the following structures:



Collectively, our results demonstrate that the predominant OPS antigens expressed by *B. pseudomallei* and *B. mallei* isolates are structurally more complex than previously described and provide evidence that different capping residues are used by these closely related pathogens to terminate chain elongation. Additionally, they confirm that *Burkholderia thailandensis* and *B. pseudomallei* express OPS antigens that are essentially identical to one another.

Burkholderia pseudomallei and *Burkholderia mallei*, the etiologic agents of melioidosis and glanders, respectively, are both CDC Tier 1 select agents.[1–3] These facultative intracellular, Gram-negative pathogens are highly infectious via the respiratory route, and can cause severe diseases in humans and animals.[4–7] Diagnosis and treatment of these diseases can be challenging, and in the absence of optimal chemotherapeutic intervention, acute human disease is frequently fatal.[8–10] Melioidosis and glanders are emerging/re-emerging infectious diseases for which no licensed vaccines currently exist.[11–13] Due to the potential misuse of *B. pseudomallei* and *B. mallei* as agents of biological warfare and terrorism, as well as their impact on public health in endemic regions, there is significant interest in developing vaccines for immunization against melioidosis and glanders.[12,14,15] Because of this, one of the long term objectives of our research is to identify and characterize protective antigens expressed by these pathogens and use them to develop efficacious vaccine candidates.

Several studies have demonstrated that *B. pseudomallei* and *B. mallei* express a number of important virulence determinants that are required for survival in animal models of infection. Included among these are the Bsa type III secretion system, the VirAG two-component regulatory system, the cluster 1 type VI secretion system, and a capsular polysaccharide.[16–21] Importantly, studies in our lab and others have also shown that the O-polysaccharide (OPS) components of *B. pseudomallei* and *B. mallei* lipopolysaccharides (LPS) are both virulence determinants and protective antigens.[22–26] Consequently, these carbohydrate moieties have become important components of the various glycoconjugate vaccines that we are currently developing for immunization against melioidosis and glanders.

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0008-6215/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carres.2013.08.013

Unlike other Gram-negative pathogens, *B. pseudomallei* and *B. mallei* isolates appear to express only a limited repertoire of structurally diverse OPS antigens.[27–29] At present, the significance of these observations with regard to virulence and evasion of host immune responses remains to be defined. Nevertheless, this phenomenon certainly bodes well from a vaccine development standpoint. Previous studies have shown that the predominant OPS serotype expressed by *B. pseudomallei* is an unbranched polymer consisting of disaccharide repeats having the structure →3)-β-D-glucopyranose-(1→3)-6-deoxy-α-L-talopyranose-(1→ in which the 6-deoxy-α-L-talopyranose (6dTal) residues possess 2-*O*-acetyl (2-*O*-Ac) or 2-*O*-methyl (2-*O*-Me) and 4-*O*-acetyl (4-*O*-Ac) modifications.[30] Interestingly, studies have also suggested that *B. mallei* expresses an OPS antigen that is structurally similar to that expressed by *B. pseudomallei* except that the 6dTal residues lack acetyl modifications at the *O*-4 position.[31]

Recently, we demonstrated that the predominant OPS serotype expressed by the closely related, but non-pathogenic species, *Burkholderia thailandensis*, was structurally more complex than initially reported.[32,33] Based upon these findings, we initiated the present study to reinvestigate the structural characteristics of the predominant OPS species expressed by *B. pseudomallei* and *B. mallei*.

Results

The *B. pseudomallei* RR2808 OPS sample examined in this study is essentially identical to the *B. thailandensis* E264 OPS antigen recently characterized by our laboratories.[33] Similar to previous reports, the RR2808 OPS consists of a →3)-β-D-glucopyranose-(1→3)-6-deoxy-α-L-talopyranose-(1→ disaccharide repeat. Earlier investigations by Perry et al. indicated that the 6dTal residue can be 2-O-acetylated (~67%) or 2-O-methylated and 4-O-acetylated (~33%).[30] The 1-D and 2-D spectra obtained in the present study confirmed these two residues, but indicated the presence of additional substitution patterns that have not been previously detected in *B. pseudomallei* OPS (Figs. 1–3 and Table 1). Thus, *O*-2 can be unsubstituted or substituted with acetyl or methyl, *O*-3 can be glycosylated with β-glucose (in all the internal residues) or methylated (in the non-reducing end residue), and *O*-4 can be unsubstituted or acetylated. We found all possible combinations of these substitutions, except residues with two methyl groups and residues that were unsubstituted on *O*-2 and acetylated on *O*-4 (Table 1). Table 2 details the exact percentages of the differently substituted 6dTal residues.

The *B. mallei* BM2308 OPS sample examined in this study gave simpler spectra compared to the RR2808 OPS. This was due to lack of any 4-O-acetylated 6dTal residues as intimated in the 1-D proton spectrum by the absence of signals at 5.33, 4.45, and 1.09 ppm (H-4, H-5, and H-6 of 2-*O*-Me-4-*O*-Ac-6dTal), and 1.13 ppm (H-6 of 2,4-di-*O*-Ac-6dTal and 3-*O*-Me-2,4-di-*O*-Ac-6dTal).[34] Analysis of the 2-D COSY, TOCSY, and HSQC spectra confirmed the absence of these residues and revealed the 3-O-methylated non-reducing end residue without a 4-*O*-acetyl group (Figs. 1–3 and Table 1).

The 6dTal residues F, G, and H have not previously been described in the *Burkholderia* literature (Tables 1 and 2). F is 2-O-methylated and like A is characterized by an upfield H-2 and a downfield C-2, but in contrast to A its H-4 is not shifted downfield, indicating the lack of a 4-*O*-acetyl substituent. G is a non-reducing end terminal residue with an *O*-methyl group in its 3-position, analogous to E, but lacking 4-O-acetylation, as evidenced by its upfield H-4 signal. G was detected in *B. mallei* OPS, but not in *B. pseudomallei* OPS. Residue H has the same constitution and nearly identical carbon chemical shifts as Residue C, but showed upfield displacement of its H-1 and H-2 relative to C. The 1-bond C-H coupling constant of its anomeric position is 173 Hz, clearly identifying it as having α-configuration. Methylation in the anomeric position could account for an upfield shift of H-1 and H-2, but there is no indication from HMBC or NOESY that this residue is a methyl glycoside. Conversely, NOESY and HMBC show correlations of

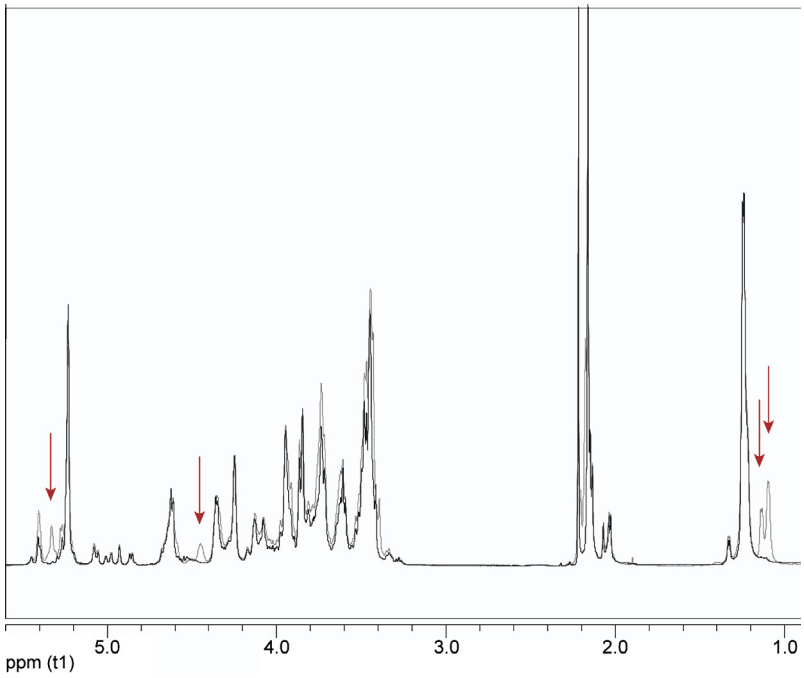

Figure 1. Overlaid 1-D proton spectra of *B. pseudomallei* RR2808 (gray) and *B. mallei* BM2308 (black) OPS. The red arrows emphasize peaks present only in *B. pseudomallei* OPS (all of which belong to the 4-*O*-acetylated 6dTal residues).