Published in final edited form as:
Trans R Soc Trop Med Hyg. 2008 December ; 102(0 1): S58–S60. doi:10.1016/S0035-9203(08)70016-6.

Sero-characterization of lipopolysaccharide from *Burkholderia thailandensis*

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Summary

We report the successful purification of lipopolysaccharide (LPS) from *Burkholderia thailandesis*, a Gram-negative bacterium, closely related to the highly pathogenic organisms *Burkholderia pseudomallei* and *Burkholderia mallei. B. thailandensis* LPS is shown to cross-react with rabbit and mouse sera obtained from inoculation with *B. pseudomallei* or *B. mallei*, respectively. These data suggest that *B. thailandensis* LPS shares similar structural features with LPS molecules from highly pathogenic Burkholderia species. This information may prove useful in ongoing efforts to develop novel vaccines and/or diagnostic reagents.

Keywords

lipopolysaccharide; immunogenic; Burkholderia

1. Introduction

Burkholderia thailandensis is a Gram-negative saprophyte found in the environment in Southeast Asia. It is closely related to the highly virulent animal and human pathogens *Burkholderia pseudomallei* and *Burkholderia mallei*, but demonstrates low levels of virulence in comparison. Similarities and differences between the genomes of *B. thailandensis* and highly pathogenic Burkholderia species have the potential to aid

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Conflicts of Interest: None declared.

Ethical Approval: None required.

Authors' contributions

OQ, JLP, and KAB conceived and designed the study. OQ and JLP purified LPS and conducted Western blots. BMJ and GCW dosed animals and prepared immune serum. OQ, JLP and KAB analysed and interpreted the data. GBK provided laboratory support. DME, AGT and KAB obtained financial support. OQ and KAB prepared and revised the manuscript. KAB is the guarantor of the paper.

identification of virulence factors. Currently, cell surface molecules such as lipopolysaccharide (LPS) and capsular polysaccharide (CPS) are amoung the few virulence factors identified in *B. pseudomallei* and *B. mallei* (Brett et al., 2007; Nelson et al., 2004). In contrast, little is known regarding the surface of *B. thailandensis*, although LPS isolated from this organism has been used to detect antibodies against LPS from *B. pseudomallei* in an indirect immunofluorescent assay (Iihara et al., 2007). To further extend these studies, we have isolated LPS from *B. thailandensis* and examined its immunoreactivity with rabbit or mouse sera obtained following inoculation with *B. pseudomallei* or *B. mallei*, respectively.

2. Materials and Methods

LPS was extracted from *B. thailandensis* E264 and *B. pseudomallei* K96243 according to previously published procedures (Nelson et al., 2004). Purified LPS was separated by SDS-PAGE and visualized by silver staining. LPS was also transferred to nitrocellulose and immunoblotted with serum from mice or rabbits immunised with heat-killed *B. mallei* 23344 (+ IL12) or *B. pseudomallei* K96243, respectively. Serum-binding was detected by horseradish peroxidase-conjugated antibodies together with an enhanced chemiluminescence substrate and photographic film.

3. Results and Discussion

With no vaccines currently available against *B. pseudomallei* and *B. mallei*, the surface-exposed molecules of these organisms are the focus of much scientific interest. The identification of distinct surface structures beween Burkholderia species and strains would also prove beneficial for diagnostic purposes. Although lipopolysaccharide (LPS) is known to be highly immunogenic, relatively little is known regarding the structural variety of LPS across the Burkholderia genus. Indeed, genomic comparisons show that the genes predicted to encode the biosynthetic pathway for LPS biosynthesis identified in *B. pseudomallei* and *B. mallei*, are also present in *B. thailandensis* (Kim et al., 2005), however LPS from *B. thailandensis* remains largely uncharacterized.

In a previous study, *B. pseudomallei* immune sera was shown to react with *B. thailandensis* LPS by Western blot (Anuntagool et al., 1998). More recently, LPS from different Burkholderia species was conjugated to Luminex microspheres and assayed for reactivity with antisera raised against whole bacterial cells (Iihara et al., 2007). Interestingly, *B. pseudomallei* antiserum recognized beads coupled to LPS from *B. pseudomallei*, *B. mallei* and *B. thailandensis*. Here, we report the purification of LPS from *B. thailandensis* strain E264 and *B. pseudomallei* K96243 bacterial pellets using a modified hot phenol extraction method. Dilutions of purified *B. thailandensis* LPS were separated on a 12% SDS-PAGE gel and visualized by silver staining (Figure 1A). A typical “ladder” banding profile between approximately 30 and 60 kDa was observed, indicative of LPS. However, a large band of approximately 100 kDa, which did not stain with Coomassie blue dye (data not shown) was also present. The identity of this additional band remains unknown, though its presence in *B. thailandensis* LPS extractions has been reported previously (Anuntagool et al., 1998). *B. thailandensis* has no capsule, so this band is unlikely to be CPS and may instead represent an unidentified carbohydrate component of the bacterial cell surface, or simply contaminating cellulose from dialysis tubing. After transfer to nitrocellulose, purified *B. thailandensis* E264 LPS was probed with serum from mice or rabbits immunized with either heat-killed *B. mallei* 23344 together with IL12 (Figure 1B), or *B. pseudomallei* K96243 (Figure 1C). In both cases the immune serum reacted with the 30–60 kDa LPS ladder from *B. thailandensis*, suggesting that the LPS from *B. thailandensis* shares structural similarities with LPS from both *B. mallei* and *B. pseudomallei*. Interestingly, the 100 kDa band did not react, suggesting that it is *B. thailandensis*-specific or not Burkholderia-derived. As a positive

control, purified *B. pseudomallei* K96243 LPS was included and shown to react with serum from rabbits immunized with heat-killed *B. pseudomallei* K96243 (Figure 1C). LPS from *B. pseudomallei* strain 576 did not react with this serum. *B. pseudomallei* strain 576 displays a serologically distinct, atypical LPS and served as a negative control in these experiments. Moreover, serum from naïve mice (Sigma) did not react with *B. thailandensis* LPS (data not shown), demonstrating that the observed cross-reactivity is Burkholderia specific. Unlike *B. pseudomallei* which expresses lipid A, *B. mallei* expresses a heterogeneous mixture of acylated lipid A species (Brett et al., 2007), which may prevent monoclonal antibodies directed against *B. pseudomallei* LPS from reacting with LPS from some *B. mallei* strains. However, the LPS molecules investigated here are clearly similar enough to warrant further analysis. This study represents the first use of immune serum derived from *B. mallei* to probe *B. thailandensis* LPS and further investigation of the structure of LPS from *B. thailandensis* should yield valuable information for use in vaccine and diagnostic development against *B. pseudomallei* and *B. mallei*.

Acknowledgments

Funding: This study was supported by the Western Research Center of Excellence (NIH/NIAID grant U54 AI 057156), NIH/NIAID grant N01-AI-30065, the Welch Foundation (TI-3D grant from the University of Texas at Austin), and the Defence Science and Technology Laboratory, Porton Down, UK.

Abbreviations

LPS	lipopolysaccharide
CPS	capular polysaccharide

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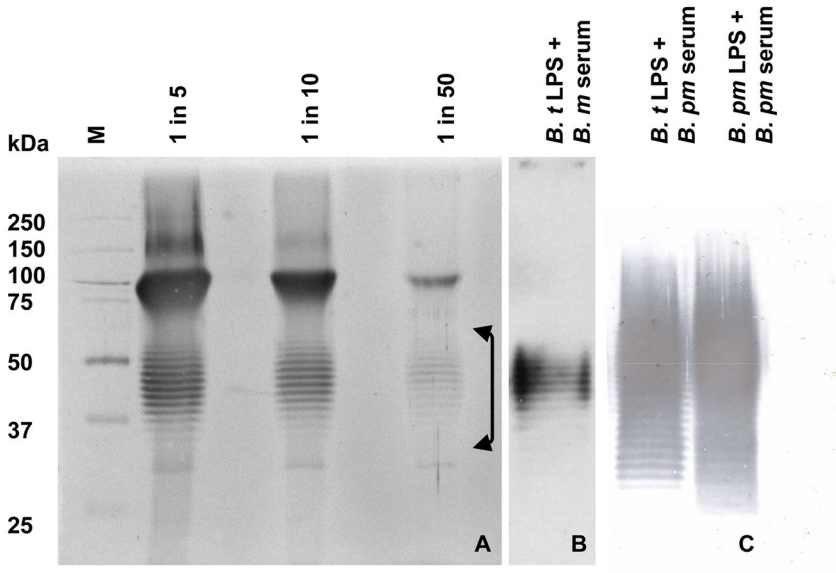

Figure 1.

Identification and seroreactivities of Burkholderia LPS molecules. (A) Silver-stained 12% SDS PAGE gel of *B. thailandensis* E264 LPS dilutions, (B) Western blots of either *B. thailandensis* E264 LPS probed with serum from mice immunized with heat-killed *B. mallei* 23344or *B. thailandensis* E264, (C) *B. pseudomallei* K96243 LPSs probed with serum from rabbits immunized with heat-killed *B. pseudomallei* K96243. M; molecular weight markers. The characteristic ladder banding pattern of LPS is indicated by a bracket.