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Manuscript entitled

"Functional reconstitution, gene isolation and topology modelling of porins from *Burkholderia pseudomallei* and *B. thailandensis*"

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by

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Abstract

The intracellular pathogen *B. pseudomallei* is the causative agent of tropical melioidosis, and *B. thailandensis* is a closely-related gram negative bacterium that does not cause serious disease. Like other bacteria, their major outer membrane (OM) porins, *Bps*Omp38 and *Bth*Omp38, respectively, may have roles in antibiotic resistance and immunity. We purified both proteins and found them to be immunologically-related, SDS-resistant, heat-sensitive trimers of M_r ~110,000. In functional liposome swelling assays, both proteins showed similar permeabilities for small sugar molecules, compatible with a pore diameter of between 1.2 and 1.6 nm. Secondary structure analysis by FTIR revealed almost identical spectra with predominantly β-sheet structures, typical of bacterial porins. MALDI-TOF and ESI/MS analysis of each protein showed extensive sequence similarities to the OpcP1 porin from *B. cepacia* (later found to be 76.5% identical). Based on information from the incomplete *B. pseudomallei* genome sequencing project, the genes encoding Omp38 were identified and amplified by PCR from *B. pseudomallei* and *B. thailandensis* genomic DNA. The nucleotide sequences are 99.7% identical, and the predicted processed proteins are 100% identical. Topology prediction and molecular modelling suggest that this newly-isolated and cloned porin is a 16-stranded beta-barrel, and the external loops of the protein could be important determinants of the immune response to infection.