Expression and refolding of Omp38 from *Burkholderia pseudomallei* and *Burkholderia thailandensis*, and its function as a diffusion porin

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In the present paper, we describe cloning and expression of two outer membrane proteins, *Bps*Omp38 (from *Burkholderia pseudomallei*) and *Bth*Omp38 (from *Burkholderia thailandensis*) lacking signal peptide sequences, using the pET23d(+) expression vector and *Escherichia coli* host strain Origami(DE3). The 38 kDa proteins, expressed as insoluble inclusion bodies, were purified, solubilized in 8 M urea, and then subjected to refolding experiments. As seen on SDS/PAGE, the 38 kDa band completely migrated to ~110 kDa when the purified monomeric proteins were refolded in a buffer system containing 10 % (w/v) Zwittergent® 3-14, together with a subsequent heating to 95 °C for 5 min. CD spectroscopy revealed that the 110 kDa proteins contained a predominant β-sheet structure, which corresponded completely to the structure of the Omp38 proteins isolated from *B. pseudomallei* and *B. thailandensis*. Immunoblot analysis using anti-*Bps*Omp38 polyclonal antibodies and peptide mass analysis by MALDI–TOF (matrix-assisted laser-desorption ionization–time-of-flight) MS confirmed that the expressed proteins were *Bps*Omp38 and *Bth*Omp38. The anti-*Bps*Omp38 antibodies considerably exhibited the inhibitory effects on the permeation of small sugars through the Omp38-reconstituted liposomes. A linear relation between relative permeability rates and M_r of neutral sugars and charged antibiotics suggested strongly that the *in vitro* re-assembled Omp38 functioned fully as a diffusion porin.

Key words: *Burkholderia*, cloning, diffusion pore, expression, outer membrane protein, refolding.