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EXPRESSION AND PURIFICATION OF SOLUBLE RECOMBINANT GST-TAGGED BPSL2774 PROTEIN FROM BURKHOLDERIA PSEUDOMALLEI K96243

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Introduction: *Burkholderia pseudomallei* is the causative agent of melioidosis, an infectious disease endemic in Southeast Asia and northern Australia. Cases have been reported in Pahang, Johor Bahru and Kedah. The disease is difficult to combat as *B. pseudomallei* has shown resistance to various antibiotics and much is still not understood about its pathogenicity. It is suggested that investigating the bacterium hypothetical proteins may provide potential new targets for the development of antimicrobials. The gene of interest in this study, BPSL2774, encoding BPSL2774 hypothetical protein, is a target gene that was predicted as essential using transposon-directed insertion site sequencing technique (TraDIS). We aimed to express and purify soluble GST-tagged BPSL2774 protein at sufficient concentration for future functional assays.

Materials and method: The BPSL2774 gene has previously been amplified from genomic DNA of *B. pseudomallei* K96243 and cloned into pDEST15 (GST-tag) plasmid vector. In this work, the clone was transformed into *E. coli* BL21(DE3) expression strain cells for up-scaled protein preparations in 0.5 L and 1 L cultures. The auto-induction method was adopted for protein expression. GST-tag affinity chromatography was performed for protein purification and the fractions obtained were analyzed using SDS-PAGE.

Results: The target protein was successfully expressed in soluble form and its highest concentration from a 0.8 mL elution fraction was at 1.38 mg/mL. Mass spectrometry analysis of 60 kDa coomassie-stained gel band cut confirmed the presence of the soluble expressed target protein, co-purified with *E. coli* chaperonin proteins, possibly due to their interaction with the target protein. Higher purity can be achieved through further purification steps following initial GST-tag affinity chromatography.

Conclusion: The purified protein was at an acceptable purity and at sufficient concentration for use as samples in a glycosyltransferase bioluminescence assay in the near future.