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TRIWIT RATTANAROJPONG : THE EXPRESSION OF A *BURKHOLDERIA PSEUDOMALLEI* ANTIGEN IN MAMMALIAN CELL. THESIS ADVISORS : TARARAJ DHARAKUL, M.D., Ph.D., SIRIRURG SONGSIVILAI, M.D., Ph.D., PRASERT AUEWARAKUL, M.D., Dr. Med. 94 P.

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Burkholderia pseudomallei is the causative agent of melioidosis which is a fatal disease of human and animals. This pathogen is an intracellular bacterium, which can survive in both phagocytic and non-phagocytic cells. Similar to other intracellular bacteria, the effective immunity that confers protection depends on the role of cytotoxic T lymphocyte (CTL). To study the role of cytotoxic T lymphocyte response in melioidosis, the assay requires a target cell that expresses the antigen derived from a pathogen. Therefore, the preparation of a target cell expressing *B. pseudomallei* antigen is the initial step for the study of CTL response against this pathogen.

In this study, an antigenic gene, pBps-1, of *B. pseudomallei* was successfully cloned into pcDNA 3.1(+). The pBps-1/pcDNA 3.1(+) recombinant plasmid could express a 18.7 kDa pBps-1 protein in *E. coli*, as detected by SDS-PAGE and Western blot analysis. The recombinant plasmid was subsequently transfected into J774A.1 cell by electroporation. The electroporation condition used for transfection was established by using the expression of reporter gene, green fluorescent protein (GFP), before transfecting this cell line with the obtained recombinant plasmid. However, the expression of pBps-1/pcDNA 3.1(+) in mammalian cell could not be detected by staining with Coomassie brilliant blue or Western blot analysis. Factors that may affect these results were discussed.