3836900 SIMI/M : MAJOR : MICROBIOLOGY ; M.Sc. (MICROBIOLOGY)

KEY WORDS : *BURKHOLDERIA PSEUDOMALLEI* / PHOSPHOLIPASE C / VIRULENCE FACTOR

NUTTIKA SUWANNASAI : MOLECULAR CLONING OF PHOSPHOLIPASE C GENE FROM *BURKHOLDERIA PSEUDOMALLEI*. THESIS ADVISORS : SUNEE KORBSRISATE, Ph.D., SUTTIPANT SARASOMBATH, M.D., DIP. AMER. BOARD OF PATHOLOGY AND DIP. AMER. BOARD OF NUCLEAR MEDICINE, AMORNRUT LEELAPORN, Ph.D. 120 p. ISBN 974-662-021-5

Burkholderia pseudomallei is the causative agent of melioidosis. This bacterium is believed to produce several virulence factors such as hemolysin, protease and phospholipase C (PLC). However, roles of these factors in pathogenesis of this organism have not been studied intensively. By using a gene-specific fragment from the hemolytic PLC gene of *Pseudomonas aeruginosa* as a probe and data from Southern hybridization, we cloned a 4.4 kb *Eco*RI restriction fragment from *B. pseudomallei* which expressed phosphatidylcholine-hydrolysing phospholipase C (PC-PLC) activity in *Escherichia coli* under its own promoter. The expressed PC-PLC, which can be detected in both cell lysate and culture supernatant of *E. coli* harbouring the plasmid, is heat-stable and non-hemolytic to sheep erythrocytes. The partial amino acid sequence of this gene showed 46% homology with the sequence of *P. aeruginosa* non-hemolytic PLC. Expression of PC-PLC protein was confirmed by Western blot analysis with serum from melioidosis patients. It has been revealed that the protein was reactive with both IgM and IgG antibodies from the patients. This result suggested the potential usefulness of the protein in serodiagnosis of melioidosis. Furthermore, the result obtained in this study will provide an initial step for further study the role of PLC in pathogenesis of *B. pseudomallei* infection.