# VI. SUMMARY

In this thesis, two cDNA clones of *P. marneffei*, *P6* and *P23*, were selected for subcloning into an expression vector, pGEX-4T-1. These clones encoded a hypothetic protein and putative 30-kDa heat shock protein, respectively. The recombinant plasmids were screened and transformed into the expression host, *E coli* BL21. The transformants were induced by the lactose analog, IPTG to express the GST fusion proteins under the control of the *tac* promoter. The GST fusion proteins were analysed by SDS-PAGE and used as an antigen to detect specific antibody against GST fusion proteins by Western blot assay. The results were as follows:

1. The *P6* and *P23* cDNA fragments of *P. marneffei* were ligated into the expression vector, pGEX-4T-1. Agarose gel electrophoresis of the recombinant plasmids showed an increased size of the bands compared to that of no insert DNA.

2. The pGEX-4T-1 containing DNA fragment of *P6* or *P23* was determined for the size of the inserts by restriction endonuclease analysis with *Xho* I and *Bam* HI. Agarose gel electrophoresis showed two bands of 0.56 and 4.96 kb of the plasmid containing a DNA insert.

3. The pGEX-4T-1 containing DNA fragment of *P6* or *P23* was transformed into the expression host, *E. coli* BL21. The *E. coli* BL21 was induced to express the GST fusion proteins by addition of IPTG. The optimal induction conditions are incubation with continuous shaking at 200 rpm at 25 °C and 2 h of the induction time with 0.1 mM of IPTG. SDS-PAGE analysis of the crude proteins revealed that the pGEX-4T-1 containing *P6* cDNA fragment showed high expression level of the GST-P6p protein of 58, 34-32 kDa. The pGEX-4T-1 containing *P23* cDNA fragment showed high expression level of the GST-P23p protein of 53-kDa, while the pGEX-4T-1 without insert DNA expressed a high level of the GST protein of 27 kDa.

4. The GST fusion proteins were purified from crude proteins by an affinity chromatography. The purified GST and GST fusion proteins were detected by SDS-PAGE and Western blot assays. The SDS-PAGE showed the protein band of the expected size. SDS-PAGE of the purified GST-P6p showed the protein band of 58-, 34-32 kDa. SDS-PAGE of the purified GST-P23p showed the protein band of 53-kDa. The Western blot assay of the GST-P6p or GST-P23p fusion proteins with antisera from the rabbit immunized with *C. neoformans* F109 or *H. capsulatum* H760 showed negative reaction. The Western blot assay of the GST-P6p fusion protein with antisera from a rabbit immunized with *P. marneffei* F4 and 20% (2 out of 10 cases) of AIDS patients infected with *P. marneffei* showed positive reaction. The Western blot assay of the GST-P23p fusion protein to antisera from the rabbit immunized with *P. marneffei* F4, *C. neoformans* F109 or *H. capsulatum* H760 showed negative reaction. The Western blot assay of the GST-P23p fusion protein with antisera from 20% (other 2 out of 10 cases) of AIDS patients infected with *P. marneffei* showed positive reaction.

5. The results of this study revealed that the recombinant proteins which were expressed from two cDNA clones of *P. marneffei*, *P6* and *P23* can react to specific antibody in serum from the AIDS patients infected with *P. marneffei*. However, a large number of serum samples from *P. marneffei*-infected patients have to be tested and the specificity of both recombinant antigenic proteins requires extensive investigation.