CHAPTER V
SUMMARY AND CONCLUSION

Two approaches of immunoscreening experiments were performed to detect *in vivo* expressed gene in bacteremic melioidosis patients. The first experiment, *In Vivo* Induced Antigen Technology(IVIAT), the pooled melioidosis sera were extensively absorbed with whole cell, whole cell lysate and denature whole cell lysate of *B.pseudomallei*. The absorbed sera were used to screen the pET30a expressed genomic library. From immunoscreening of 13,000 recombinant clones, thirty one positive clones were isolated and characterized. Although the well known virulence genes could not be isolated and all of the tested positive clones reacted with the normal serum in western blot experiment, IVIAT approach may be an alternative method for isolating *in vivo* expressed gene of melioidosis if the weak point of the protocol was improved.

The second approach employed a sensitive new protocol of chemiluminescence detection by using proteinA-CDP star instead of anti-human gamma globulin-ECL chemiluminescence. The pooled serum was absobed with only the *E.coli* host cell lysate in stead of the *B.pseudomallei* whole cell lysate. In addition, the genomic library was constructed in λZAPII vector to increase the yield of recombinant clones. At least 6 suspected in vivo expressed genes were identified by this new approach. Two were well known virulent genes, *gmhA* (a capsule bisynthetic gene) and *bipD* (type III secretion protein). Another two genes code for conserved hypothetical proteins. The last two isolated genes were *groEL* (a chaperonine protein) and a gene encoding transmembrane protein. All of the isolated genes are potentially useful as diagnostic antigens for melioidosis. The results from this new approach

demonstrate that the developed protocol work very well. However, our experimental data indicated that some protocols need to be reviewed to get more suspected *in vivo* expressed genes efficiently through a process such as follows : the convalescent serum should be included in the experiment; the ZAPII expressed genomic library need to be reconstructed according to many isolated positive clones that contained the same genes.

The results from this immunoscreening experiment are only the preliminary study to search for *in vivo* expressed gene of *B.pseudomallei* in bacteremic melioidosis patient. The suspected *in vivo* expressed genes could be amplified from the *B.pseudomallei* genome and the expressed protein is tested with the melioidosis serum in western blot experiment as demonstrated in this approach. Finally, the genes isolated from this approach could be confirmed to be expressed *in vivo* with mRNA transcirpt detection by RT-PCR in both clinical specimens such as lung, liver spleen and animal infection model. This study creates a basic knowledge for further study not only in melioidosis pathogenesis but also other bacterial pathogens.