CHAPTER V
CONCLUSIONS

1. The lymphoid organ cDNA libraries from the unchallenged and *Vibrio harveyi* challenged shrimp were constructed with the titers of 3.2×10^5 and 3.1×10^6 pfu, respectively.
2. One hundred and eighty (40.4%) clones out of 446 sequenced clones from the unchallenged library and 286 (44.5%) clones out of 642 sequenced clones from the *Vibrio harveyi* challenged library matched the previously deposited database in the GenBank, representing 283 different genes.
3. The matched EST clones were classified into 11 functional categories. The group of defense and homeostasis was the largest category whereas the group of cell division/DNA synthesis, repair and replication was the smallest group.
4. Twenty eight different putative immune genes represent in defense and homeostasis category. The subcategory of proteinases and proteinase inhibitors was the most abundant, comprising the cathepsin family and the serine proteases. Other immune genes are anti-lipopolysaccharide factor (ALF), lysozyme, proPO systems and heat shock proteins.
5. The cDNA microarray analysis indicates different gene expression profiling of shrimp in response to WSSV and *V. harveyi* challenge. The highest number of genes response to WSSV challenge was observed (135 genes) at 24 hpi whereas *V. harveyi* challenge were observed (156 genes) at 6 hpi.
6. The cDNA microarray showed that the expression of calmodulin (CaM), tubulin and asialoglycoprotein receptor (ASGPR) transcripts was increased upon viral and bacterial injection. The differentially expression of the genes were further confirmed by real time PCR analysis. The results suggested that calmodulin, tubulin, and ASGPR are possibly involved in the shrimp pathogen response.
7. Calcineurin, CDC like kinase 2 and protein phosphatase 1 genes,CaM binding and CaM candidate binding protein, were also examined for their expression,

and found to be differentially expressed in haemocytes upon pathogen challenge.

8. By using *in situ* hybridization, the percentages of the CaM positive cells of WSSV and *V. harveyi* challenged shrimp haemocytes were found to be higher than those of the control groups, indicating that the invading pathogens resulted in an increase of CaM transcript in shrimp haemocytes.
9. Immunohistochemistry analysis revealed the distribution of CaM expressing haemocytes throughout the shrimp cepharothorax, especially in gill and hepatopancreas. Upon bacterial invasion the CaM was also produced in epithelium cells of lymphoid organ, gill and hepatopancreas.