CHAPTER 5:

CONCLUSION

Homology-based searching against an EST database with known miRNA gene sequences can be used as a method to identify miRNA candidates. In this study, 11 miRNA candidates were computationally identified from a *Litopenaeus vannamei* EST database, composed of sequences extracted from the GenBank database. Candidate gene sequences lva-miR-256, lva-miR-272 and lva-miR-1476-3p were selected to be validated for their presence in 6 different target tissues. Besides that, potential mRNA targets have been identified for each miRNA candidate. The 11 miRNA candidates were predicted to target 20 mRNA and 27 EST sequences. Through this, the function of the miRNA candidates can be predicted. These miRNA candidates are found to correspond to a wide range of targets with functions, including; defense-related proteins, enzymes, transcription factors, cell death, metabolic and developmental processes. The validated lva-miR-256 is possibly involved in cellular, developmental and metabolic process; lva-miR-272 regulates cellular processes and lva-miR-1476-3p regulates mostly cellular and developmental processes.

MicroRNA genes play an important role in cell biology. Therefore, the *Litopenaeus vannamei* miRNA expression profiles can be established to help improve the quality of this aquaculture species and for use in broodstock and breeding programs. For example, in the late developmental stage, the molting process can be accelerated by suppressing the miRNA that regulates molting hormone or ecdysteroid-regulated protein. The infectious diseases and stress responses due to low oxygen demand and high number density can be improved by inducing miRNAs that control the heat shock proteins. Besides that, the miRNA can serve as a potential treatment to viral diseases in aquatic animals. Recent

researches have been conducted on serum miRNAs and this could serve as a potential novel biomarker for detecting various cancer and diseases (Chen *et al*., 2008; Gilad *et al*., 2008). This can help to reduce the losses suffered by the aquaculture industry. In addition to that, in future, the identified miRNA genes may serve as model to identify homologous or tissue-specific miRNA in other sea-water invertebrates and vertebrates. This discovery will allow further improvement in aquatic animal aquaculture management.

The number of miRNA candidates that can be identified using a purely computational method is limited. So, for identifying novel miRNA candidates, next generation deep sequencing method will greatly assist. As for the study of miRNA expression profiling, this can be conducted using deep sequencing and microarray approaches (Beuvink *et al*, 2007; Chen *et al*., 2008). This will be helpful particularly in identifying developmental-specific, cell death, metabolic regulation, neuronal patterning, and tissue-specific or diseases-associated miRNA genes. This study has layered the fundamental for miRNA discovery and functional analysis in *Litopenaeus vannamei*.