

# CHAPTER 1

## INTRODUCTION

### 1.1 Background

MicroRNAs are small non-coding, single stranded of 18-25 nucleotides RNAs (Shivdasani, 2006). MicroRNA genes comprise about 1-3% of genes in the human genome (Bartel, 2004; Zhao and Srivastava, 2007). Some reported that miRNA genes are regulators, estimating to be 1-5% of the animal genes (Berezikov, 2005; Stark *et al.*, 2005; Giraldez *et al.*, 2006). MicroRNA genes have been predicted to regulate more than 30% of protein coding sequences (Berezikov *et al.*, 2005; Lewis *et al.*, 2005; Nilsen, 2007). Miranda *et al.* (2006) even speculated that miRNA genes regulated more than 90% of human genes. Up to date, a lot of miRNAs genes either conserved or tissue-specific have been identified in various organisms especially those with completed genome sequences. The microRNA Registry Release 11.0, contains 6396 entries of precursor miRNAs, expressing 6211 mature miRNAs in dicot plants (15 species), monocot plants (5 species), animals (38 species), mycetozoa (1 species), protistae (1 species) and viruses (12 species) (miRBase, 2008). The number of entries is expected to be increasing.

However, miRNA genes within marine invertebrates (shrimp, prawn, sea cucumber, abalone, lobster) and other vertebrates (tilapia, groupers, trouts, salmon and tuna) which are of aquaculture interests still remain largely unknown. The main obstacle towards miRNA study is the unavailability of sequence data for these organisms. Fortunately, recent efforts have been made to develop the tilapia genome and tiger prawn expressed sequence tags (EST) libraries. In National Centre for Biotechnology Information (NCBI) database, the

number of *Litopenaeus vannamei* EST has reached 155558 sequences whereas for *Fenneropenaeus chinensis* (Chinese shrimp) has reached 10500 sequences. In addition to that, the *Euphausia superba* (antartic krill-a shrimp like marine invertebrate) EST database has been established. It can be expected that more aquatic animal genome databases will be established.

*Litopenaeus vannamei* is known as the white shrimp, whiteleg shrimp or pacific whiteleg shrimp (Gulf State Marine Fisheries Commission, 2005; FAO, 2008). It is a brackishwater shrimp which has high economic value in the aquaculture industry. Due to its advantages over *Penaeus monodon* (tiger prawn) and other marine prawns, such as lower protein requirement, rapid growth rate, resistance to certain diseases; and tolerance to low salinity, temperature and stress, it was introduced into Asian countries in the late 1990s (Lungren *et al.*, 2006). According to the Food and Agriculture Organization (FAO) Fishery Statistics, 2006 global production of *Litopenaeus vannamei* reached 2,133,381 metric tonnes with a value of US\$7,774,098,000 (FAO, 2008). Lungren *et al.* (2006) reported that the production of white leg shrimp in the Asia-Pacific region has exceeded a million tons in 2006 where most contributions came from China (735,055 tonnes), Thailand (276,600 tonnes) and other South East Asian countries. In Malaysia, this species has been intensively cultured in many prawn farms. In order to revitalize the black tiger prawn (*Penaeus monodon*) culture, Malaysia have imported 11,502 metric tonnes of *L. vannamei* broodstock during 2006 whereas in 2007 (January to June), another 13,542 metric tonnes of *L. vannamei* broodstock were imported (5<sup>th</sup> NaFiS 2008). Malaysia aims at meeting the national shrimp production of 180,000 metric tonnes (contributing RM4.3 billion) by 2010 year (5<sup>th</sup> NaFiS 2008).



**Figure 1.1**  
**Adult Stage of *Litopenaeus vannamei***

## **1.2 Importance of the Study**

Although a large number of ESTs are available for *Litopenaeus vannamei* deposited in NCBI database, no miRNA genes have been reported in this species. Both computational and molecular methods were used in miRNA gene prediction in this study.

This study is essential and important in identifying the potential miRNA genes and their targets in *Litopenaeus vannamei*. Besides that, this may also help researchers to understand the functions of miRNA genes in regulating gene expression in pacific whiteleg shrimp. All these works are useful for aquatic animal monitoring programs.

## **1.3 Research Objectives**

- (i) To identify microRNA genes in pacific whiteleg shrimp (*Litopenaeus vannamei*) using computational methods.
- (ii) To validate candidate microRNA genes in pacific whiteleg shrimp (*Litopenaeus vannamei*) using molecular methods.

- (iii) To study the function and regulation of the candidate microRNA genes found in *Litopenaeus vannamei*.