CHAPTER I

INTRODUCTION

1.1 Background and Rationale

Yellow head virus (YHV) stands as one of the major virulent pathogens responsible for causing yellow head disease (YHD), which has resulted in extensive economic losses in shrimp aquaculture. YHV is characterized as a single-stranded RNA virus featuring a spiked envelope and a positive-sense genome. The YHV genome comprises approximately 27,000 nucleotides, placing the virus within the family *Roniviridae*, specifically in the genus *Okavirus* (Walker et al., 2005; Wongteerasupaya et al., 1995). Infection with YHV in Pacific white shrimp (*Litopenaeus vannamei*) can lead to mortality rates of up to 100% within 3–5 days following the initial appearance of gross YHD symptoms. Clinical manifestations of YHD encompass a pallid body complexion and a yellowish discoloration of the cephalothorax (Chantanachookin et al., 1993). Nevertheless, an effective vaccine for curing or preventing YHD remains elusive, given that shrimp lack adaptive immunity.

As invertebrates, shrimp rely solely on their innate immune system to defend against pathogen infections. While the innate immune system lacks specificity, it does boast a relatively rapid response time. Innate immunity swiftly identifies pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), peptidoglycans (PGN), β-glucans (GLU), or double-stranded RNA (dsRNA), through pattern-recognition receptors (PRRs). This recognition leads to the activation of pro-inflammatory cytokines (IL-1, IL-6, IL-8, and TNF-α), chemokines, and transcription factors (Kulkarni et al., 2021). Additionally, non-coding RNAs (ncRNAs) play pivotal roles in regulating the immune response. ncRNAs are RNA molecules that are not encoded for protein production and can be categorized into two types: housekeeping gene ncRNAs, including transfer RNA and ribosomal RNA, and regulatory ncRNAs, such as small interfering RNAs (siRNAs), microRNAs (miRNAs), and circular RNAs (circRNAs) (Zhang et al., 2019).

In shrimp, the expression level of numerous miRNAs are increased during white spot syndrome virus (WSSV) infections (Zhang et al., 2019). They exert their influence by downregulating immune response genes through binding to the 3′ untranslated region (3′UTR) of messenger RNA (mRNA) seed sequences, thereby interfering with the translation process. For instance, miR-589-5p was found to reduce the expression of *L. vannamei* hemocyanin (*Lv*HMC), resulting in high shrimp mortality following WSSV infection (Bao et al., 2020).

Circular RNAs (circRNAs) represent a category of non-coding RNAs (ncRNAs) that form a closed-loop structure, lacking both the 5′ and 3′ terminals (Chen et al., 2015; Qu et al., 2015). Typically, circRNAs arise from back-splicing events where the upstream splice acceptor connects with the downstream splice donor region. Three distinct types of circRNAs exist, categorized based on their formation location: exonic circRNAs, exonic-intronic circRNAs, and intronic circRNAs (Kristensen et al., 2019; Wang et al., 2017). Numerous studies have underscored the significant biological roles played by circRNAs. In human cells, distinct classes of circRNAs displayed up-regulation or down-regulation in hepatitis C virus (HCV)-infected cells. Notably, one up-regulated circRNA, circPSD3, exhibited a substantial impact on viral RNA levels in both HCV- and Dengue virus-infected cells (Chen et al., 2020). In *Drosophila melanogaster*, circRNA profiling revealed an age-related accumulation pattern from 10 to 40 days, extending the observations of Westholm et al. This provides evidence that global circRNA levels continue to rise from 20 to 40 days of age (Westholm et al., 2014). The white-spotted bamboo shark (*Chiloscyllium plagiosum*) employs at least two circRNAs, circ-38-1717 and circ-6-1096, which act as miRNA sponges (Zhang et al., 2020). Furthermore, circRNAs have been implicated in immune regulation in fish species, including grass carp (*Ctenopharyngodon idella*) (He et al., 2017), tilapia (*Oreochromis niloticus*) (Fan et al., 2019), Miiuy croaker (*Miichthys miiuy*) (Xin et al., 2022), crucian carp (*Carassis auratus gibelio*) (Hu et al., 2019), and blunt snout bream (*Megalobrama amblycephala*) (Wang et al., 2021). When fish encounter bacterial or viral infections, differentially expressed circRNAs (DECs) containing miRNA binding sites may interact with miRNAs, influencing the expression of immunomodulatory proteins and thereby enhancing the immune response.

In shrimp, the presence of circRNAs in WSSV-infected shrimp has been unveiled.

Among the 290 circRNAs, 160 DECs were up-regulated, while 130 DECs were down-regulated following WSSV infection. This discovery sheds light on the landscape of WSSV-responsive circRNAs and their potential functions (Limkul et al., 2023). However, the identification and characteristics of YHV-responsive circRNAs have not been reported.

In this study, we aimed to identify circRNAs associated with the response of *L. vannamei* to YHV infection. To achieve this, the expression profiles of circRNAs in the hemocytes of both control and YHV-infected shrimp were analyzed using the high-throughput next-generation sequencing technique on the Illumina HiSeq 2500 platform. The immune-related differentially expressed circRNAs (DECs) from six libraries were quantitatively identified and subsequently validated and confirmed them through quantitative reverse transcription-PCR (qRT-PCR), RNase R treatment, and Sanger sequencing. These findings lay the groundwork for future investigations into circRNAs in shrimp, elucidating their characteristic features and shedding light on their roles in shrimp immunity.

1.2 Research objectives

The objectives of this study are as below:

1.2.1 To identify YHV-responsive circRNAs in shrimp using omics technology

1.2.2 To analyze the expression of YHV-responsive circRNAs in shrimp using qRT-PCR

1.2.3 To verify the structural and chemical characteristics of YHV-responsive circRNAs in shrimp using PCR, Sanger sequencing, and RNase R treatment