1. INTRODUCTION

Fisheries and aquaculture remain important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world. In 2014, fish harvested from aquaculture amounted to 73.8 million tones, with an estimated first-sale value of US$160.2 billion that included 6.9 million tones of crustaceans, valued at US$36.2 billion (FAO, 2016). Thus shrimp farming forms an important source of food and revenue in the coastal regions that fulfills the huge demand in the global market. Unfortunately, the increase in production is also accompanied by a steady rise in the number of new diseases affecting the development and sustainability of the sector. Diseases occur mainly due to viruses, bacteria, protozoa and fungi. The diseases caused by other organisms except virus are now managed using improved culture practices, routine sanitation and use of probiotics and chemotherapeutics. Diseases caused by viruses are more vulnerable and cause severe economic loss to the farmers (Lightner, 2011; Kalaimani et al., 2013). Shrimp are affected by viral disease due to the following reasons. (1) Lack of innate and adaptive immunity in the shrimp which is the key component for the viral defense mechanism. (2) Shrimp pathogens are transmitted vertically resulting in the massive viral increase. (3) Shrimp are affected by different strains of the same virus or sequentially with multiple viruses (Walker and Winton, 2010).

According to the current report more than 20 viral diseases are now recognized in cultured penaeid shrimp (Hernandez-Rodriguez et al., 2001). Among the lethal diseases, White spot syndrome disease causes 100% mortality in the shrimp aquaculture. It is
caused by White Spot Syndrome Virus (WSSV), a rapidly replicating virus with high mortality rate in the shrimp farming (Sanchez-Martinez et al., 2007). Apart from shrimp, this virus affects other natural marine species including salt and brackishwater penaeids, crabs, lobsters, freshwater shrimp and crayfish (Alla et al., 2013). WSSV virus was first described in China and Taiwan in *P. japonicus* during 1993. It became pandemic in the year 1994 with rapid spread in the shrimp farming areas throughout Asia (Rout et al., 2005). Disease and mortality are mainly observed in juvenile shrimp (1-5g). Shrimp affected by this virus, develop white spots rapidly, a calcium deposit (0.5-2.0mm in diameter) observed on the exoskeleton, appendages inside the epidermis but this initial symptom is also observed in bacterial disease and alkalinity in the shrimp pond water (Sanchez-Martinez et al., 2007). However, the actual mechanisms for the white spots are not known and it may be due to dysfunction of the integument that sequentially ends with accumulation of calcium salts within the cuticle. Affected animal shows some extended chromatophores with a colour of pink-red to reddish-brown in the cephalothorax cuticle. These infected shrimp also show reduced feed intake, cuticle loss, accumulation of fluid resulting in branchiostegites swelling, thinning and delayed clotting of hemolymph and increased lethargy (Alla et al., 2013). The infected shrimp swim slowly near the pond surface and subsequently sink to the bottom and die (Sanchez-Martinez et al., 2007). The hypertrophied nuclei of infected cells will change from eosinophilic to progressively more basophilic inclusion bodies (Karunasagar et al., 1997). Later stage of infection shows karyorrhexis, cellular disintegration and results in formation of necrosis characterized by vacuolization (Alla et al., 2013).
1.1 Morphology of virus

WSSV virus is placed in a newly created virus family, *Nimaviridae* and it belongs to the genus *Whispovirus*. This virus is an enveloped ellipsoid to bacilliform- double strand DNA virus with distinctive tail like appendages projected at one end and found throughout the body of infected shrimp (Sanchez-Martinez et al., 2007). The thickness of the viral envelope is 6-7nm, composed of lipid and a tri-laminar membranous structure with two electron transparent layers separated by an electron opaque layer. Inside the envelope, a striated shaped nucleocapsid of 182-250nm long and 60-80nm wide and 6nm thick external wall is present which contains five major proteins and 39 structural proteins (Alla et al., 2013; Sanchez-Martinez et al., 2007; Rajendran et al., 1999). The size of the virion is approximately 350nm in length and the size of the genome is 292,967 bp. The WSSV genome is a circular double stranded DNA and it is the largest animal virus genome that has been entirely sequenced. The size of the genome was found to vary in WSSV strains from different geographical locations viz. 292,967 bp, 305,107 bp, 307,287 bp for the Thailand (WSSV-TH), China (WSSV-CN) and Taiwan (WSSV-TW) isolates respectively (Marks et al., 2004). Among these three types, WSSV-TH is more virulent with 50% mortality occurring within 2-3 days of infection in *P. monodon* (Poulose, 2011). The genome of this virus is classified into four groups according to their functions. (1) Structural genes, (2) Functional genes, (3) Latency and sequester genes and (4) Regulatory genes (Sanchez-Martinez et al., 2007).
1.1.1 Structural genes

These genes mainly encode for the envelope and nucleocapsid. The external cover is called as envelope and it mainly protects the virus from degradation during infection (Sanchez-Martinez et al., 2007). Among the genes of structural protein, VP28 is essential for infection process in attachment and internalization of the virus in the shrimp cells. This protein has multiple glycosylation sites to recognize the shrimp surface area (Yi et al., 2004). The nucleocapsid is an internal structure that protects and packages the genome of WSSV. VP35 was the first identified nucleocapsid protein and plays an important role in targeting host nucleus (Chen et al., 2002). Another important structural protein is VP26 which has actin-binding motif that promotes the attachment of virus to shrimp cell membrane (Xie and Yang, 2005). VP664, a major and largest structural protein is important for the assembly and morphogenesis of the virion and formation of ring subunit (Leu et al., 2005).

1.1.2 Functional genes

These genes are mainly involved in the virus proliferation, life cycle functions including replication, phosphorylation of host proteins and nuclease activity (Sanchez-Martinez et al., 2007). Few functional genes have been identified and one such gene is ORF \textit{wsv112} that encodes for dUTP pyrophosphatase (dUTPase EC3.6.1.23). This dUTPase has vital role during infection, replication and nucleotide biosynthesis (Liu and Yang, 2005).
1.1.3 Latency and sequester genes

These genes have maximal activity during transcription and are involved in the persistence of virus within host cell. Major functions of these genes are to maintain a low number of viruses, inactivate host genes until it gets optimal conditions such as pH, salinity and temperature for the virus to replicate. These genes are studied to be involved in WSSV genome latency (Sanchez-Martinez et al., 2007).

1.1.4 Temporal regulatory genes

Temporal regulatory genes participate at specific times during infection. These temporal regulatory genes activate the expression of early and late genes of the virus (Liu et al., 2005). Three immediate early genes of WSSV-TW, ie1, ie2 and ie3 from ORF126, ORF242 and ORF418 respectively have been characterized (Sanchez-Martinez et al., 2007). Ie gene expression depends solely on the host proteins and encodes the proteins essential for viral DNA synthesis (Arturo, 2010).

In spite of these findings, there is no cure for WSSV infection. To address this problem crustacean cell culture system has to be developed for improving modern diagnostic tool and probe for penaeid shrimp, crayfish, lobster, crab and other crustaceans (Toullec, 1999). Cell culture system is vital for studying basic cell biology interaction between disease causing agents and host cells, effect of drug in cells, processes of nutritional values, toxicity studies, effect of new drug on particular disease, basic fundamental biological research and therapeutic treatments (Jose et al., 2010; Nathiga Nambi et al., 2012). The development of primary cell line is very important to combat
any emerging viral disease (George and Dhar, 2010). Permanent cell line for the crustacean species is not yet developed and the success rate for primary tissue culture is very low (Rinkevich, 2005). The first successful record of primary cell culture for penaeid shrimp was done by Chen et al., (1986) where in the chemical and physical condition was optimized for the growth of *P. monodon*. Toullec, (1996) established the first long term primary cell culture method for crustaceans and described the replication and survival rate of cultured cells. Even though they have made some success in crustacean cell culture, permanent crustacean or penaeid shrimp cell line is not yet established (Toullec, 1999). Recently, Jose et al., (2012) reported the development of primary cell culture system from lymphoid organ and studied the susceptibility of WSSV. Presence of WSSV in primary culture of lymphoid organ was confirmed by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and immunofluorescence assay using monoclonal antibody against 28 kDa protein of WSSV. This work formed the platform for research in virus-cell interaction, virus morphogenesis, up and down regulation of shrimp immune-related genes, discovery of novel drug for WSSV in penaeid shrimp culture and crustacean.