Overcoming viral Infection in shrimp culture by RNA Inteference (RNAI)

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Shrimp farming and infections

Shrimp farming is an economically important industry, particularly in developing countries sustaining the livelihood of many coastal communities.Shrimp and shrimp products make up a large portion of the global fish market, with an annual trade value exceeding $11 billion or 15% of the total fish exports in 2018 (FAO, 2022). However, as shrimp farming becomes more intensive, the shrimp are exposed to greater stress, making them more vulnerable to diseases.

Viral infections have caused significant damage to the shrimp industry, leading to significant economic losses for farmers and the industry as a whole(Flegel, 2006).Since the early 1990s, viruses such as White Spot Syndrome Virus (WSSV), Tura Syndrome Virus (TSV), Hepatopancreatic Parvovirus (HPV), Monodon Baculovirus (MBV),Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), and Yellowhead Disease Virus (YHV) inflicted both wild and cultured penaeid shrimps worldwide, due to their ability to adapt and infect a wide range of hosts(Gong & Zhang, 2021). Among these viruses, WSSV infection (Fig 1) has been the most lethal, widespread, and still prevalent to present.

Despite the existence of several techniques that have been effective against shrimp viruses under laboratory conditions, they have not been successful in field trials(Dang et al., 2010). One of the reasons for this is that laboratory conditions do not fully replicate the complex environmental factors present in the shrimp farming industry, which can impact the effectiveness of potential treatments.

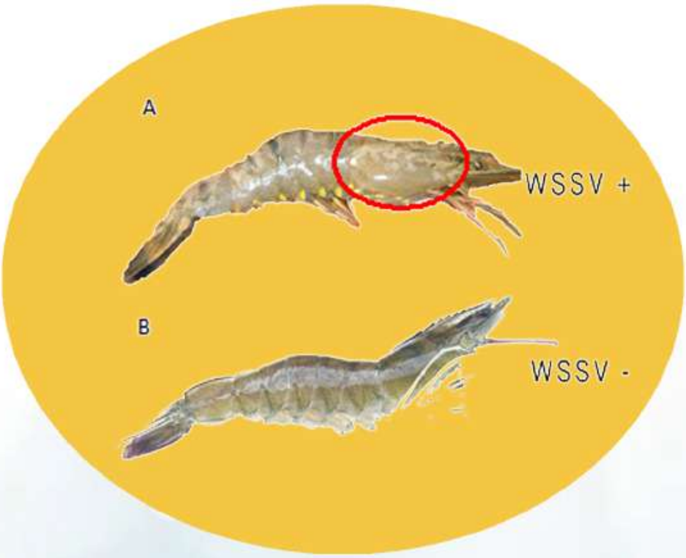


Fig. 1. WSSV-infected shrimp (A) and normal shrimp (B) collected from the Southern part of the Philippines

Moreover, unlike vertebrates, shrimps do not have an acquired immunity system that is required to respond to a specific infection and solely rely on their innate non-specific immunity against infections. The shrimp's innate immune response is a complex process involving various components that work together to recognize and eliminate invading pathogens, however, since it is non-specific, it may not provide long-lasting defense against specific pathogens, which is why it can be difficult to develop effective treatments for viral infections in shrimp. To address this challenge, it is crucial to further elucidate the host-pathogen interaction by investigating the functional genes involved in the viral infection.

RNA interference (RNAi)

Among the developed tool in gene functional studies, RNA interference (RNAi) was commonly utilized due to its reliability in gene silencing functions. In RNAi, small RNA molecules are used to target and degrade specific messenger RNA molecules, preventing the translation of genetic information into proteins (Nguyen et al., 2018). In aquaculture, RNAi can be used to inhibit the expression of genes that are responsible for certain traits or diseases in fish and Shellfish by introducing gene-specific double-stranded RNA (dsRNA) into a target organism (Nguyen et al., 2018).RNAi mechanism was first discovered in plants and nematodes and has since been found to be conserved across various organisms, including animals and humans(Wang & He, 2019). In shrimp, major proteins involved in the RNAi pathway, such as dicer and argonaut, have been identified for further applications. RNAi has been demonstrated to have a significant role in antiviral immunity in shrimp, and several studies have shown that injecting dsRNAs or siRNAs specific to viral genes can successfully inhibit viral infections. The successful activation and suppression of various viral proteins and immune-related genes confirms the critical role of RNAi in the antiviral immunity of shrimp and shed information on the therapeutic potential of this technique (Gong & Zhang, 2021).

RNA interference (RNAi) has been recognized for its high degree of specificity and efficacy, making it a promising platform for developing therapeutics. However, the development of RNAi-based agents has been hindered by several challenges. For instance, small interfering RNAs (siRNAs) are unstable in serum and are rapidly degraded, limiting their therapeutic potential (Nguyen et al., 2008). Additionally, delivering siRNAs across the cell membrane has proven to be highly inefficient. These challenges have prompted extensive research efforts aimed at developing effective delivery strategies for RNAi-based therapeutics (Dong et al., 2019; Nguyen et al., 2008).Several studies have explored RNAi delivery methods in shrimps for controlling viral diseases. Charoonnart et al. (2021) showed the effectiveness of dsRNA-encapsulated liposomes in delivering RNAi against white spot syndrome virus (WSSV). Chen et al. (2020) investigated exosome-mediated delivery of RNAi against WSSV and observed a significant reduction in viral replication.

Liu et al. (2018) developed a chitosan-based nanoparticle system for oral delivery of RNAi against WSSV, resulting in a significant reduction in viral load and Zhang et al. (2017) reported the use of microRNA-mediated RNAi delivery in shrimp against WSSV, which effectively inhibited viral replication and improved shrimp survival.

Fig. 2. dsRNA injection of shrimp in SEAFDEC Tigbaoan station

The most effective way to induce RNAi in crustaceans is through dsRNA injections (Fig 2). Although this injection-based approach was successful on the laboratory scale, new delivery methods have been continuously developed to allow for quick, large-scale RNAi research (Sagi et al., 2013). A more practical delivery method would be oral administration (feeding), which is also feasible for larvae and post-larvae in shrimp hatcheries (Itsathitphaisarn et al., 2017). A comparative study on the effect of in vivo and in vitro produced dsRNA in *Penaeus vannamei* indicated that both are effective in mitigating viral infection. However, prolonged feeding might have sustained a more beneficial protective effect during the challenge test (Fig 3) (Gumatay et al, 2017 (unpublished)).

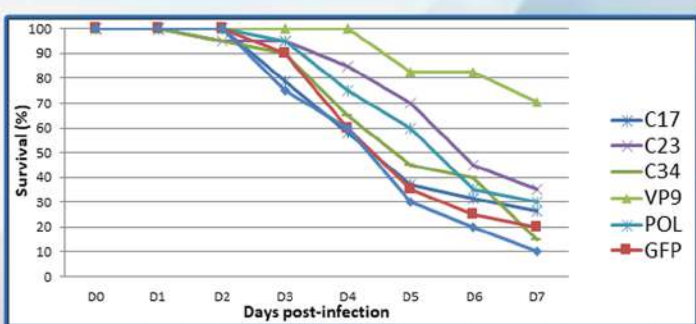


Fig. 3. Cummulative survival percentage

The dsRNA production through this system is also more cost-effective compared to in vivo production (Fig 4). Other dsRNA oral delivery methods reported to be effective in controlling shrimp viral diseases include the use of dsRNA-expressing bacteria and microalgae mixed with shrimp feeds, the use of living brine shrimp pre-fed with dsRNA or dsRNA enclosed in nanocontainers such as chitosan and liposomes and added in shrimp pellets (Itsathitphaisarn et al., 2017; Dekham et al., 2022; Charoonnart et al., 2021; Theerawanitchpan et al., 2012).

These methods have been used to control viral diseases in shrimp. However, continuous optimization of the delivery methods is essential for large-scale use and to further understand the potential risks and unintended effects of RNAi-based therapeutics on non-target organisms and the environment.

In conclusion, RNAi delivery has made significant promising progress in recent years in aquaculture, particularly in controlling viral diseases in shrimp and other aquaculture species. Several studies have demonstrated the efficacy of various RNAi delivery methods, including dsRNA injection, feeding with dsRNA-producing bacteria or microalgae, and oral administration of dsRNA-encapsulated liposomes, and chitosan-based nanoparticles

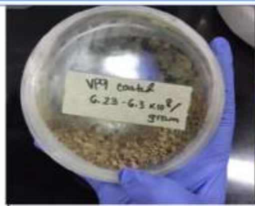
MODE OF PRODUCTION		
YIELD	2L culture of recombinant bacteria = 4 mg of dsRNA	1 kit = 4 mg of dsRNA
COST	**USD 270**	**USD 997**

Fig. 4. The cost of producing dsRNA using a bacterial system compared to in vitro production

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