Incidence and residence of Vibriophages in the White Shrimp *Litopennaeus vanammei*

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Introduction

Bacteria of *Vibrio* genus occurs as normal residents during shrimp culture. While several species had been described as opportunistic pathogens many of them might be a part of natural shrimp microbiota. Also, a number of vibriophages occur during shrimp production (Vinod *et al*., 2005; Okano *et al*., 2007; Crothers-Stomps *et al*., 2009; Alagappan *et al*., 2010). Although, their role was not completely elucidated it is generally assumed that they act as a regulator of the Vibrio abundance or mediating changes in the target bacteria, altering their role. Since bacterial resistance became a major problem for human health, phage therapy has been suggested as an alternative to control pathogenic vibrio populations. However, there are still many doubts about direct release of phages in shrimp culture ponds and its ecological implication. This study is aimed to evaluate the Vibrio and vibriophage incidence during white shrimp culture and the effect on shrimp juvenile dynamics.

Material and Methods

Vibrio and vibriophage incidence during shrimp farming were evaluate using standard isolation procedures. During intensive larvae production, water and larvae samples were collected and examined; juvenile tissue samples from external ponds cultures were also processed. Briefly, the samples were processed in a tissue grinder, decimally diluted in sterile sea water and inoculated in TCBS media. Part of the samples was frozen at -20^{o} C and used to evaluate the presence of phages for each isolated bacteria. The grown bacteria in TCBS were purified and used in enrichment technique for phage isolation according to Carlson (2005). Molecular identification was also performed by partial sequentiation of the 16s rDNA. Each isolate was induced to lysogenic expression according to Raya and Hébert (2009). Phage residence in spawning females and juvenile shrimps was evaluated during 15 days after ventral sinus inoculation with two phage suspensions.

Results and Discussion

During larval production, fluctuations in the abundance of Vibrio were observed. Apparently, vibrio populations are sensitive to normal culture operations including the use of some chemicals. These populations are different between tanks and have a different behavior, two contrasting cases were detected. Also, changes in the occurrence of lytic vibriophages were recorded, 15 lytic phages were isolated from water, shrimp larvae and haemolymph samples of juvenile shrimp. *V. alginolyticus* and *V. proteolyticus* and their respective phages were the most common isolated microorganisms. 60% of isolated vibrios were lysogens and the associated phages were successfully induced. However these lysogens resulted to be susceptible to infection by lytic phages.

Vpms1 and PL14D phages were inoculated in healthy broodstock females, and they remained in broodstock females haemolymph for at least 15 days post-inoculation. In juvenile shrimps, phage concentration tends to decline rapidly during the first 72 hours, but remains stable during all experiment. There is a selective mechanism from host to concentrate vibriophages, most of them tend to reside on haemolymph (~12,088,079 PFU´s), but there are many of them that reside within hepatopancreatic (~2,739,623.27 PFU´s) and muscular tissue (~2,212,613.07 PFU´s).

Conclusion

Vibriophages may occur as natural biota during shrimp culture, most of them occur as lysogenic Vibrios. Lytic phages are less common than temperate phages and shown a residence period in culture shrimps tissues and can remain selectively by relatively long periods.

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