



Immunoprophylaxis: A Better Alternative Protective Measure against Shrimp Vibriosis - A Review

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Abstract – Aquaculture, especially shrimp production is the world's fastest growing food production industry, due to increased demands for seafood. Conceivably, disease outbreak is the major setback which brings about high mortality and reduction or loss of production. The conventional use of antibiotics both prophylactically and therapeutically had recently not only been ineffective but incriminated, in drug residue which pose danger to consumption by humans. Hence, a better alternative was importantly required. The use of Immunoprophylaxis has potential of being a safety measure in the prevention of outbreak of diseases or spread of already established disease in aquatic invertebrates, where the shrimps and prawns belong. Immunoprophylaxis includes the prevention of disease by production of passive immunity. The major infectious agents that are responsible for high morbidity and high mortality in shrimp industry are viruses and bacteria. Others include fungi, parasites and protozoans but less threatening than the two former. Among the referenced groups of bacteria in shrimp disease, gram negative bacteria are more virulent. *Vibrio*, a member of this gram negative groups has been implicated to be the most causative agent of bacterial infections in shrimps. This review examined the components of the cell membrane of these bacteria that have been reported to confer immunity on the host. They include outer membrane proteins, lipopolysaccharide and peptidoglycan. These components could elicit immune response and confer protection.

Keywords: Antibiotic, Aquaculture, Crustaceans, Immunoprophylaxis, Shrimp Vibriosis, *Vibrio* Species

Introduction

The UN FAO (Food and Agriculture Organization of the United Nations) forecasts that by 2020, aquaculture would meet more than half of the world's seafood demand, as there has been over exploitation and rapid decline in wild capture fisheries (Barman, Banerjee, Bandyopadhyay, Mondal, & Das Mohapatra, 2011) and increasing global population. The aquaculture industry is currently worth more than US\$10billion but the major setback that has been causing a decrease in output and loss of production, is mainly diseases due to bacteria (especially *Vibrio* species) and Viruses (Moriarty, 1999). Farmed shrimp has contributed more than 50 percent to the global total supply of shrimps and this contribution is on a progressive curve (Chowdhury, Talib, & Yahya, 2012). With

reference to the aquaculture Asia - pacific magazine, world production of farmed shrimps rose from 3.4 million tonnes in 2013 to 3.6 million tonnes in 2014. Asian producers were reported to have the giant share at 3 million tonnes whereas the estimated production in America was 671,000 tonnes (FAO, 2015). The greatest challenges in the global shrimp aquaculture are a disease particularly that of bacterial origin and chief among these is *Vibrio* species. Hence, a lot of shrimp famers loses several millions of US dollars each year. Many methods (including use of antibiotics and other related chemical compounds) that have been employed over the years to stem this menace have proven ineffective, resulting to either or both increase in virulence of pathogens and or antibiotic residue. That is not to say that antibiotic is exclusively useless or dangerous, but the incessant use and low technical know-how in its applications by farmers have posed more harms than good. The best known group of microorganisms that caused serious losses and devastating economic effect in shrimp culture were bacteria. Bacterial diseases caused mainly by *Vibrio* species have been incriminated in many cases and at least 14 species of *Vibrio* have been reported in penaeid shrimp culture systems (Lavilla-pitogo, 1998). In the research undertaken by (Jayasree, Janakiram, & Madhavi, 2006) on the diseases caused by *Vibrio* species in penaeid shrimps (*Penaeus monodon*), they identified five types of diseases including red disease, tail necrosis, shell disease, loose shell syndrome (LSS) and white gut disease (WGD). Six species of *Vibrio* such as *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, *V. vulnificus* and *V. splendidus* were found in the diseased penaeid shrimp. The uncontrollable use of antibiotics to curb this malady of economic and health importance at sub-therapeutic dosage may lead to development of resistant *Vibrio* strains. It has also been proven that the chances of antibiotic resistant bacteria in ponds where antimicrobial agent is barely used or controlled is very low (Thakur, Vaidya, & Suryawanshi, 2003). Bacteria isolates such as many species of *Vibrio* (*V. anguillarum*, *V. vulnificus*, *V. parahaemolyticus*, *V. alginolyticus*) are sensitive to antibiotics including streptomycin, erythromycin and chloramphenicol (Thakur *et al.*, 2003). In another circumstance, indiscriminate use of antibiotics had resulted in high resistance to *V. anguillarum*, *V. vulnificus* and *V. parahaemolyticus*. Oxytetracycline was known to be an effective antimicrobial agent against vibriosis in aquaculture, but some strains are still very resistant (Thakur *et al.*, 2003). Most of the isolated species of *Vibrio* were sensitive to Oxytetracycline, ciprofloxacin and norfloxacin. The luminous *V. harveyi* showed susceptibility to only three drugs but displayed high resistance to many other antimicrobial agents. With this obvious emergence of antibiotic resistant strains of *Vibrio*, it is pertinent for the consideration of replacements for antibiotics use in the control of bacterial diseases (Jayasree, Janakiram, & Madhavi, 2006). The need for a better alternative measure of bacterial disease prevention and control is therefore expedient. Use of probiotics (microorganisms which when ingested in adequate dose confers health benefits on the host) which is an important prophylactic measure in the prevention of diseases has been reported by many authors (Wang, Li, & Lin, 2008; Kesarcodi-Watson, Kaspar, Lategan, & Gibson, 2008; Nwachi, 2013), but some limitations of probiotics have also been identified (Dauda, Folorunso & Dasuki, 2013). This review takes into consideration the use of components of bacterial protective cell membrane for Immunoprophylaxis. The components such as outer membrane proteins (OMP) (Maftuch *et al.*, 2013), peptidoglycan (Purivirojkul, Areechon, & Srisapoome, 2006), lipopolysaccharide (Sakai, 1999) and beta- glucan (Cheng, Liu, Kuo, & Chen, 2005) are antigenic. Hence, confer passive immunity on the host penaeids.

Shrimp Vibriosis

Vibriosis is a prominent bacterial disease in the culture of shell fish and fin fish and has global high record of mortality rate. The distribution of *Vibrio species* is worldwide and infections due to *Vibriosis* are frequently encountered in the hatcheries but outbreaks are very common in pond reared shrimps (Lavilla-pitogo, 1998). The ubiquity of the causative agents of this disease has made it most popular

bacterial disease in shrimp and prawn industry. Vibriosis is caused by a group of gram negative bacteria, members of family *Vibrionaceae*. Epizootics may be due to either environmental influences which favour the growth and exponential multiplication of existing tolerated bacteria at low level within the haemolymph of the shrimp or penetration of bacteria through the protective chitin or exoskeleton (i.e. barriers between the internal and external surface) of the host crustaceans. Hence *Vibrio* spp are chitinoclastic bacteria implicated in shell disease (Vogan, Costa-Ramos, & Rowley, 2002). Pores or wounds on the exoskeleton may also be a good source of entry of *Vibrio* into the host system (Alday-Sanz, Roque, & Turnbull, 2002). Bacterial penetration through the gills is common because they are covered by thin exoskeleton (Taylor, 1992). The intestines which are not lined by exoskeleton also predispose the shrimp to *Vibrio* attack. The bacteria which are present in food, water and environment can easily penetrate the system (Jayabalan, Chandran, Sivakumar, & Ramamoorthi, 1982). Serious economic loss and high mortalities have been reported in *Penaeus monodon* due to infection by *Vibrio harveyi*, a luminous gram negative bacterium. Over 280 shrimp hatcheries along the coastline of Thailand suffered setback from seed production due to luminescent bacterial disease caused by *V. harveyi*. There are molecular and genetic variations in the group of *V. harveyi*. This is evident in their varying degree of virulence (Lavilla-pitogo, 1998). Vibriosis in penaeid shrimps has been reported by many researchers and at least 14 species of *Vibrio* have been implicated as the cause of the bacteria diseases. They include *Vibrio harveyi*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio splendidus*, *Vibrio mimicus*, *Vibrio vulnificus*, *Vibrio anguillarum*, *Vibrio damsella*, *Vibrio cambelli*, *Vibrio fischeri*, *Vibrio ordalli*, *Vibrio orientalis*, *Vibrio logei* and *Vibrio mediterrani* (Eaves and Ketterer, 1994; Lavilla-pitogo, 1998).

Clinical signs

Having established that occurrence of shrimp vibriosis cannot be exonerated from environmental factors such as high or too low water temperature, pH instability, low dissolved oxygen (DO), salinity, improper handling, poor water quality etc. (Brock, and Lightner, 1990; Lightner, and Lewis, 1975), it is therefore important to note the clinical manifestation of vibriosis in shrimps. Mortalities have been reported both in larvae and adult shrimps (Lavilla-Pitogo, Baticados, Cruz-Lacierda, & Leobert, 1990). Vibriosis in adult shrimps may manifest as hypoxia, reddened body, red to brown gills, anorexia, lethargy, abnormal swimming behaviour (shrimps observed swimming at the edges and surface of ponds) (Anderson, Shamsudin, Shariff, & Nash, 1988). Luminescence vibriosis can be caused by six *Vibrio* spp. (*V. harveyi* and *V. splendidus* are the major) and is readily discernible at night in all ages of infected penaeid shrimps, whereby the areas of lesion found conspicuously shining in the night (Lightner *et al.*, 1992; Lavilla-pitogo *et al.*, 1990). Black or brownish spot or erosion on the carapace (Plate 1), rostrum, gills, abdominal segments, tail and appendages (Lavilla-Pitogo *et al.*, 2000) red discolouration, loss of appendages and black discolouration of the body and gills (Khuntia, Das, Samantaray, Samal, & Mishra, 2008). *Vibrio* infections may be systemic other than lethargy. There are no external pathognomonic lesions (Eaves and Ketterer, 1994). In other words, there are other bacterial infections caused by *Aeromonas* spp. and *Pseudomonas* spp. which external clinical signs can occur as in the case of *Vibrio* spp. This will make the other organisms to be differentials to many suspected cases of vibriosis before confirmatory diagnosis. Grossly, apart from the localised lesions of the cuticle typical of bacterial shell disease, the muscles are cloudy (Lightner, 1993). The hepatopancreas may also be cloudy, it is characterised by atrophy with multifocal necrosis and inflammation of the haemocytes. Detachment of the epithelial cells may also be seen (Chen *et al.*, 2000). Histo-pathologically, vibriotic septicemia typically results in the septic haemocytic nodules formation in the lymphoid organ, heart muscles and connective tissues of the gills, hepatopancreas and many other tissues and organs (Jiravanichpaisal, Miyazaki, & Limsuwan, 1994).



Figure 1: Freshwater prawns showing multi-focal black spots (arrow) on the carapace and tail rot (rough edged) lesion (arrow head). Source: molecular pathology laboratory, faculty of veterinary medicine,

Diagnosis

According to Lavilla-pitogo (1998), vibriosis can be diagnosed based on clinical signs; gross lesions and histological demonstration of rod-shaped gram negative *Vibrio* bacteria in lesions, nodules or haemolymph. Post-larvae or small sized shrimps can be wholly crushed and then streak on thiosulfate citrate bile sucrose (TCBS) agar (figure 2), a selective media for *Vibrio* spp. Haemolymph and organs excised from adult shrimp may also be streaked onto the agar plate or general marine agar plate. *Vibrio* isolates may also be identified by various methods including gram stain and many conventional biochemical tests, rapid identification can be obtained in the field by using the API-20 NPT kits system (Lightner, 1996) or BIOLOG (an alternative to the API system) (Lavilla-pitogo, 1998) PCR (polymerase chain reactions) may also be used to identify *Vibrio* to species level with the correct primers.



Figure 2: Vibrio spp isolated from diseased shrimp turned green TCBS agar to yellow.

Source: molecular pathology laboratory, faculty of veterinary medicine, UPM

Treatment

The best form of treatment is to prevent the occurrence of the infections. Stressing the cultured shrimps should be avoided as much as possible, because stress is a major factor that predisposes shrimps to diseases. Sanitation and efficient water management can be used to prevent the entry of *Vibrio*(s) in cultured shrimp water (Lavilla-Pitogo, Baticados, Cruz-Lacierda, & de la Pena, 1990). Many preventive methods have been reported (Lightner, 1993; Anderson, 1988; Jiravanichpaisal *et al.*, 1997).

Conventional Use of Antibiotic

Antimicrobial agents have played and are still playing essential roles in many aspects of living organisms. Both terrestrial and aquatic animals are beneficiaries of bactericidal activities of these agents. Some pathogens have been eradicated by the use of broad spectrum antibiotics and farms have been economically rescued due to increased survival rate of the animals (Eshraghi, You, & Barbetti, 2005). Some pathogens are either strongly sensitive, sensitive or strongly resistant to antimicrobial agents and their use in diagnosis is quintessential (Liu, Cheng, Hsu, & Chen, 2004). However, their indiscriminate use and abuse have resulted in more harm than good. The issue of drug residue has affected many exported shrimp products and thus causes serious economic loss. According to shrimp news international on the 15th of September, 2015, an online news update on shrimp matters reported that in the month of August, 2015, a total of 207 seafood entry lines were refused by the USA Food and Drug Administration (USFDA), where a new record was set. Of the above figure, 72 (35%) were of shrimp entry lines. The refusal was a result of detection of banned-antibiotics residue in the shrimp products. The report also had it that it was the highest amount of refusal reported by FDA in any month since 2002 (Rosenberry, 2015). Antibiotics have been considered in the control of vibriosis but there was poor efficiency in the result and this had led to many shrimps farms closed down due to poor survival (Moriarty, 1999). In the Philippine, vibriosis contributed to major loss in the production of shrimp in 1996. In Thailand, a farmer who considered the use of colloidal silver in all feeds recorded a very huge mortality resulting from vibriosis in 1999. The resistance of *Vibrio* spp. to every antibiotic used such as furazolidone, chloramphenicol, streptomycin and oxytetracycline was very significant (Moriarty, 1999). Antibiotics are chemotherapeutically or prophylactically administered to

farmed shrimps against bacterial infections. Oxytetracycline, chloramphenicol, oxolinic acid, and furazolidone were integrated in commercial feeds as treatment against luminescent vibriosis in grow-out ponds (Baticados *et al.*, 1990). Abraham *et al.* (1997) was reported to have isolated *V. harveyi* strains from diseased shrimps that showed resistance to chlorotetracycline, ampicillin, ciprofloxacin, erythromycin, furazolidone, nalidixic acid, gentamycin, neomycin, nitrofurazole, nitrofuratoin, ofloxacin, oxytetracycline, polymyxin B, sulphasomidine, streptomycin, rifampicin, sulphafurazole and sulphamethazole. Many *Vibrio* spp. showed a consistent increased percentage resistance to antibiotics from ponds that have not used any antibiotics to those from ponds that have used antimicrobial agents before or currently using it (Tendencia & De La Peña, 2001). This implies that there is less risk of bacteria resistance to antimicrobial agents in farms that have not previously used antibiotics. Hence, less risk of drug residue and transfer of pathogenic strains. With these serious challenges of antibiotics, there is need for a better alternative to disease prevention and control in shrimp aquaculture.

Immunostimulants

The defence mechanisms in all living organisms are generally common, but there are still some dissimilarities between vertebrates and invertebrates. The former has a more complex mechanism. The latter are generally believed to lack adaptive immunity and exclusively rely on their innate immunity. It is pertinent therefore to inculcate products that can enhance host immunity and resistance to infectious diseases in shrimps disease prevention and control (Farzanfar, 2006). Cellular and humoral immunities occur both in vertebrates and invertebrates. Immunostimulants in crustaceans are alternatives to increased resistance to infectious diseases, not by enhancement of specific humoral and cellular immune responses, but by enhancing non-specific defence mechanisms. Immunostimulants have played an effective role in increasing the immunocompetence and disease resistance in aquatic animals (Sakai, 1999). Many immunostimulants in fish and shellfish have been researched upon, these include chemical agents, polysaccharide, extracts of plant and animal origins, components of bacteria, cytokines and nutritional agents (Sakai, 1999). This review takes into cognisance the bacterial components such as outer membrane proteins (OMPs), peptidoglycan and lipopolysaccharides that are immunogenic and have played crucial roles in shrimp protection against bacterial infection especially vibriosis. In crustaceans, three types of circulating haemocytes are generally identified: hyaline cells, semi-granular cells and large granular cells. These cells formed the basis for cellular immune responses which include phagocytosis and elimination of foreign bodies or microbial agents (Hose, Martin, & Gerard, 1990). Haemocytes form an integral part of the crustaceans' immune system. They are associated with enzymes prophenoloxidase (proPO) and phenoloxidase (PO) in prophenoloxidase activating system which are involved in encapsulation, melanin formation and function as non-self-recognition systems (Johansson & Soderhall, 1989). Phenoloxidase (PO) is activated from prophenoloxidase by a serine proteinase in the presence of small amount of microbial components such as peptidoglycan, lipopolysaccharides or β -1,3-glucan through the recognised receptors. PO catalyses the step wise oxygenation and reactions of monophenols through O-diphenols to O-quinones which subsequently leads to the formation of melanin (Johansson & Soderhall, 1989). Phagocytosis is an important mechanism in crustaceans to get rid of microorganisms or foreign bodies. Reactive oxygen intermediates such as superoxides, hydroxide radicals and peroxides are produced during the process of phagocytosis. This activity is simply known as respiratory burst and these products have microbicidal activities (Song & Hsieh, 1994). Many of these parameters such as total haemocyte count (THC), phenoloxidase activities, superoxide dismutase (SOD) an enzyme that catalyses the two steps of rapid dismutation of the toxic superoxide anion to non-toxic molecular oxygen and hydrogen peroxide etc. have been used to evaluate the immune status of shrimps (Maftuch *et al.*, 2013).

Outer Membrane Proteins

Bacterial outer membrane proteins (OMPs) comprise the outer surface which exposes the bacteria to the receptive region of the host cells and immune defence factors. They play an important function in organisms' virulence (Ebanks, Goguen, McKinnon, Pinto, & Ross, 2005). According to Glauert *et al.*, (1969) as Cited by Ebanks *et al.*, (2005) the cell wall of gram negative, the group which *Vibrio*, *Aeromonas* and *Pseudomonas* spp. belong is morphologically composed of four (4) defined structures: cytoplasmic membrane, peptidoglycan layer, the outer membrane and an outermost layer. The outermost layer is composed of proteins which have been reported to play a crucial role in the defence mechanism of bacteria against the bactericidal effects of immune and non-immune serum. They enhance the adhesion to different host cell surfaces (Trust, Kostrzynska, Emödy, & Wadström, 1993). They also enhance the protection of bacteria from devastating environmental agents such as proteases (Chu *et al.*, 1991). With reference to immune responses in the host and as targets for therapeutic agents, the outer membrane proteins (OMPs) of bacteria have been of considerable interest due to their location at the host-bacteria border. The OMPs in addition to their role in bacterial defence against host factors, is also involved in virulence-related functions such as invasion, adhesion and host cell modulation (Ebanks *et al.*, 2005). OMPs are good biological components as vaccine candidates (Khushiramani, Girisha, Bhowmick, Karunasagar, & Karunasagar, 2008). Intramuscular administration of OMP *V. alginolyticus* enhances immune responses in *Penaeus monodon* (tiger shrimp) as well as the resistance to *V. harveyi* (Maftuch *et al.*, 2013). OMP combined with a herbal adjuvant from *Asparagus racemosus* is effective in promoting immunity in goldfish against bacterial infections (Thangaviji, Michaelbabu, Anand, & Gunasekaran, 2012).. In other terrestrial animals, immunogenic effect of OMPs has also been proven. The outcome of vaccinated animals challenged with pathogenic *Pasteurella multocida* showed that, all the buffalo calves administered OMP vaccine survived but only 67 percentages in the vaccinated group of whole cell of *P. multocida* survived. However, none of the animals in the control group survived (Pati, Srivastava, Roy, & More, 1996).

Peptidoglycan

Peptidoglycan (PG) that was extracted from gram positive bacterial cell wall was reported to enhance immunity in shrimp against pathogens and thus increased their survival rate. It therefore became a potential immunostimulant agent (Purivirojkul *et al.*, 2006). Peptidoglycans and other microbial polysaccharides can elicit the activation of phenoloxidase (PO) from its inactive form of prophenoloxidase (proPO). PO is a terminal enzyme that catalyses the reactions in the haemocytes which result into the microbicidal effect (Li, Yeh, & Chen, 2010). Purivirojkul *et al.*, (2006) also reported that penaeid shrimps fed with peptidoglycan mixed with feed at the appropriate dose for 7 days developed competent immunity including superoxide anion, phenoloxidase activity, bactericidal effect and clearance ability. Boonyaratpalin *et al.*, (1995) reported that *Penaeus monodon* (black tiger shrimp) fed with peptidoglycan showed a higher tolerance to dissolved oxygen (DO), salinity and stress than those not fed with peptidoglycan. It was also reported that better growth and feed conversion rate was observed with feed supplemented with peptidoglycan than with normal feed (without peptidoglycan) This is an indication that the protection potential of peptidoglycan goes beyond biological and extends to adverse effect of environmental factors and growth promoter. That does not suffice to use peptidoglycan as a hormone or abuse its use. Long duration of immunostimulants administration has been reported to be ineffective and waste of resources. High dose of immunostimulant may not enhance and may inhibit immune responses as the effects are not directly dose-dependent (Sakai, 1999).

Lipopolysaccharide

Lipopolysaccharide (LPS) is a component of cell wall of gram-negative bacteria. It was included in the list of immunostimulants reported by (Sakai, 1999) used in fish and shrimp. Just as mentioned of peptidoglycan in its role in the activation of phenoloxidase, so also lipopolysaccharide in their role in microbicidal activities (Liu & Chen, 2004). In the study of Cheng *et al.*, (2005), *L. vannamei* fed with

diets containing peptidoglycan and lipopolysaccharide exhibited high level of protection against *Vibrio*, white spot syndrome virus (WSSV) and penaeid rod shaped DNA virus (PRDV) infections. Lipopolysaccharides are very potent substances even in significantly low doses. The potency of lipopolysaccharides is very high that even at very low doses, LPS can stimulate proliferation of haemocytes, enhance phagocytic and microbicidal activities of shrimps (Barman, Nen, Mandal, & Kumar, 2013). The addition of LPS and other components of bacteria cell wall to shrimp diets is an effective immunostimulant in order to increase penaeid shrimps resistance to *Vibrio* infections (Cheng *et al.*, 2005).

Beta-glucan

The use of beta-glucan and its derivatives in immune enhancement and disease resistance have recorded tremendous success rates in aquaculture. A polymer of glucose known as glucan, which is present in the cell walls of fungi, plants and bacteria is a potent immunostimulant and seemingly the most promising in immune enhancement in fish and shrimps (Barman *et al.*, 2013). Beta-glucan can be used to effectively control vibriosis (Itami *et al.*, 1998). High dose can result in negative effect on survival or failure of protection of the animals against the pathogens, thus optimal dose is recommended (Scholz *et al.*, 1999). Shrimps' immunity can be kept at increased level by beta-glucan for up to four weeks and its protection against white spot syndrome virus (WSSV) has been efficiently proven (Bai, Gu, Zhang, Xu, & Mai, 2014). The number of haemocytes which is a marker for increased immune systems in crustaceans rose in a group of shrimps administered with beta-glucan (Song & Hsieh, 1994). Tremendous successes have been recorded in the use of glucans (essential structural components of fungal cell walls), in order to enhance the non-specific immune response in crustaceans.

Bacterin

The use of bacterial whole cells is a common practice in vaccination either as dead or live attenuated for diseases prevention and control. However, it has been successfully inculcated into crustaceans immunostimulation. The examination of methods for vaccine preparation and route of administration has revealed the protective effect of *V. anguillarum* bacterins against vibriosis (Alippi, 1999). High survival rate (92.3-100%) and increased phagocytic activity were recorded seven days post vaccination when *Litopenaeus vannamei* (pacific white shrimp) was injected with formalin-inactivated *V. harveyi* (Pope *et al.*, 2011). A commercial anti-vibrio polyvalent vaccine has been tested to produce antimicrobial activity against certain species of *Vibrio* which is contained in its constituents (Powell *et al.*, 2011). Post-larvae of *Penaeus monodon* primed and boosted with bacterial suspensions (*V. anguillarum*) was reported to significantly increase in weight and survival rate when compared with the untreated group, upon subsequent bacterial challenge (Azad *et al.*, 2005).

Others

Several other non-microbial agents have been successfully used as immunostimulants and protective agents against vibriosis in shrimps. Synthetic chemicals such as levamisole (anthelmintic) have been reportedly used against shrimps vibriosis (Omama A. Abboud, 2011), sodium alginate from brown algae (Cheng *et al.*, 2005), heightened resistance to *V. anguillarum* and stimulation of activities of macrophages have been reported in rainbow trout injected with chitin (Sakai, Kamiya, Ishii, Atsuta, & Kobayashi, 1992), survival rate was increased in *L. vannamei* (Wang, & Chen, 2005), cytokines and hormones (Barman *et al.*, 2013), extracts of plants and animals and other nutritional factors such as vitamin C and E (Sakai, 1999).

Conclusion

Immunostimulants appear to be an inestimable and most promising tool for prophylaxis in farmed shrimps and fish. It has a wider margin of safety than chemotherapeutics and their spectral of efficacy are more than vaccination. Perhaps this phenomenon is not a replacement for good management practices, proper hygiene and adequate nutrition. Optimal dose is of essence in the administration of these substances. It is expedient to understand fully the immunodynamics and immunokinetics of these compounds in order to ensure maximum efficiency and effectiveness in their applications. More studies about other compounds or natural substances with beneficial effects should also be explored. Sustainable aquaculture may be assured with the exploration of this modest microbicidal technique. The challenges of drug residues and resistant strains would be surmounted and an eco-friendly and cost effective practice of aquaculture is imminent. Farmers can breathe a sigh of relief through increased production and profitable outcome and consumers can be sure of healthy sea foods (drug residue free).

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