Role of Marine Metabolites in Shrimp Growth, Production and Disease Prevention

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Abstract

This review consolidates the recent information about the global aquaculture production status of fish and shellfish. Research reports on common and emerging microbial diseases of cultivable species of shrimp are narrated together with resultant reductions in productivity. The harmful effects antibiotics usage in shrimp aquaculture and consequent effects on the species as well as ecosystem are highlighted. The *in vitro* and *in vivo* tests of extracts containing metabolites isolated from marine macroalgae, sea grasses and invertebrates in restraining the proliferation of disease causing microbes are presented. The metabolite bearing marine species and methods of extraction of marine metabolites are concisely indicated. Studies on unique bacteria associated with the metabolite - bearing marine organisms, their identity and their significance in metabolite production are essential for future research and developmental activities in aquaculture and pharmaceutical applications. The administration of marine metabolites in the post larval and juvenile shrimps as feed additive for controlling the common microbial diseases such as vibriosis together with essential administration protocols are reported. Many of the tested marine metabolites are of immense use as they increased the survival of farm reared shrimps and their growth rates. The resultant specific growth rate attained together with survival contributed to the significant increase in production. Perusal of literature indicated that marine secondary metabolites isolated from marine macro algae and sponges exhibited immune enhancing activity in shrimps in a non specific manner when administered as a feed ingredient.

Key words: Disease management, growth promoter, immune enhancer, marine metabolites, shrimp aquaculture

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Introduction

As per the recent estimates of FAO (2016), the production of aquatic animals from aquaculture in 2014 amounted to 73.8 million tonnes, with an estimated first-sale value of US$160.2 billion. This total comprised 49.8 million tonnes of finfish; 16.1 million tonnes of molluscs; 6.9 million tonnes of crustaceans and 7.3 million tonnes of other aquatic animals including amphibians (US$3.7 billion). China accounted for 45.5 million tonnes in 2014, or more than 60 percent of global fish production from aquaculture. Other major producers included: India, Viet Nam, Bangladesh and Egypt. These statistics indicated that presently, the global aquaculture production has been increasing consistently. Feed is widely regarded as a major constraint factor to the growth of aquaculture production in many developing countries (FAO, 2016). The other constraint or problem for successful aquaculture is the disease outbreaks and consequent losses. In shrimp farming, intensive culture conditions increase the risks of waste accumulations, consequent stress for the stocks and proliferation of pathogens. The outbreak of viral diseases has increased the economic risks and slowed the shrimp farming entrepreneurship and industry development (Flegel, 2006). According to Stentiford *et al*. (2012) Disease in aquaculture will certainly limit future food supply from global crustacean fishery and aquaculture sectors. In a recent publication by Stentiford *et al.* (2017) new paradigms to help solve the global aquaculture disease crisis were stated with special reference to shrimp farming in Thailand.

It is well known that aquatic environments impose a constant and omnipresent risk of pathogen exposure to resident hosts, perhaps even more so than terrestrial systems (Oidtmann *et al*. 2013). Poor knowledge of background microbial diversity in farm systems leads to frequent emergence of previously unknown pathogens, surprising farmers and creating shock in the wider value chain (Lightner *et al*. 2012; Flegel, 2012 and Shinn *et al*. 2014). High throughput sequencing (HTS) applied to open aquatic systems is rapidly increasing our knowledge of prokaryotic and eukaryotic diversity and the complex symbiotic arena in which they exist (Karsenti *et al*. 2011). According to Bass *et al.* (2015), application 'environmental DNA' (eDNA) approaches to aquaculture pond systems with reference to the disease outbreak and non-outbreak systems will provide important

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information about by detecting specific pathogens of consequence to farmed hosts or those elements of the microbiome that facilitate their emergence as disease agents. Thus, the improved definition of a ‘pathobiome’ within hosts may be expected to supersede an historic focus on specific pathogens as sole perpetrators of yield-limiting disease (Gilbert, 2016). A shift from single-pathogen to pathobiome concepts may also expose a wider target to which pond management strategies can be applied (De Schryver, 2014). It becomes imperative to apply modern HTS approaches in order to accelerate the understanding of the complex trophic structures that exists within such systems and the health outcomes for farmed species stocked (De Schryver, 2014).

In shrimp aquaculture, infectious diseases are causing particularly devastating economic and social impacts, with total losses exceeding 40% of global capacity (Israngkura and Sae-Hae, 2002). Emergent diseases, often with cryptic or syndromic aetiology (such as early mortality syndrome in shrimp), have collapsed production in nations across Asia (Lee C-T *et al*. 2015), confirming disease as the major constricting factor for expansion of the aquaculture industry to 2050 (Stentiford *et al*. 2012) Increasingly globalised trading of seafood between net exporting and importing nations expands the geographical range over which these effects are felt (Jennings *et al*. 2016). In this context, 50 early-career scientists from the United Kingdom and Thailand met with industry professionals and policymakers in March 2016 to consider the future challenge of managing disease in global aquaculture and to discuss new paradigms for mitigating their negative effects. This Opinion summarises major outcomes of those discussions and proposes a need to refocus strategic scientific and policy priorities relating to aquatic animal health in support of an expanding and sustainable industry to 2050.

Impacts of antibiotics

Application of antibiotics and other chemicals in shrimp aquaculture has its own intricate problems. For example, regular use of antibiotics in shrimp hatchery or grow out system may lead to development of not only antibiotic resistant fish/shrimp bacteria, but also human bacteria. The presence of antimicrobial residues in products of aquaculture is a threat to human health. Information is still lacking on the absorption and distribution of antibiotics in fish and shrimp and persistence of residues or effects of them in the environment. Hence, promoting the holistic systems approach in managing shrimp health problems needs special attention (Selvin *et al.* 2009).

Considering the potential threat of diseases on the one hand and the environmental issues on the other hand, disease management aspects should concentrate on environment friendly strategies. Proactive disease managing techniques are to be evolved as they are essential to protect the aquacultured shrimps from emerging and virulent strains of microbes. Researchers have reviewed the potential benefits of probiotics, which could be a promising alternative for antibiotics in aquaculture, demonstrating beneficial effects to host by combating diseases, improving growth and also stimulating immune responses of host toward infections (Newaj-Fyzul *et al*. 2014; Hai, 2015).

Common shrimp diseases

Shrimp aquaculture mostly concentrated on species such as *Penaeus monodon* and *P. vannamei*. Both these species are known to be vulnerable to a wide range of microbial diseases including viral, bacterial, fungal and protozoan diseases. Among viruses, IHHNV, YHV, TSV, WSSV, and IMNV are considered as major ones affecting shrimps. Their distribution pattern, pathology, morphology, and genomic organization, diagnostic methods and common intervention practices were reported by Seibert and Pinto (2012). Mass mortalities and failure of the culture system have also been recorded. Of late, *P. vannamei* has been cultivated throughout the world. The common disease of economic importance reported to affect *P. vannamei* include: WSD, infectious hypodermal and hematopoietic necrosis (IHHN), Taura syndrome (TS), yellow head disease (YHD) and infectious myo necrosis (IMN).

WSD caused US$6billion loss. IHHNV has caused about US$0.5-1 Billion loss in America. The impact of TS on the shrimp-farming industry in the America was estimated to be US$ 1-2 billion up to 2001 while in Asia it is of $0.5-1 billion. An unpublished data form Brazilian shrimp farmers' association has estimated that the loss due to infectious myo necrosis IMN from 2002 to 2006 in Brazil exceeded $100 million. The new unique disease, Acute Hepato Pancreatic Necrosis disease or AHPND earlier known as early mortality syndrome (EMS) has been devastating *P. vannamei* farms in China since 2009, Vietnam since 2010, Malaysia since 2010, and Thailand since 2012, where in 100% mortalities have been reported during the first 20-30 days after stocking. The AHPND has caused about 60% drop in shrimp production in the affected region compared with 2012 and the global estimate of the loss per year is about US$1 billion.

In Indonesia this IMNV caused significant losses exceeding $1 billion by 2010. A total of $100-200 million loss has occurred mainly because of the disease in America, but in Asia this disease has emerged in the year 2006, and the estimated loss is about $1 billion. Significant loss from YHD amounts to $1 billion per year in Asia. Since 2012, Thai shrimp farmers have suffered major economic losses owing to Early Mortality Syndrome (EMS). The disease reduced shrimp production of Thailand from 5,40,000 tons in 2012 to 2,56,000 tons in 2013 and 2,10,000 tons in 2014, respectively (Chuchird *et al*. 2015). The pathogenic *Vibrio parahaemolyticus* was suspected to be associated causing mass mortality as it induced 100 % mortality with typical EMS pathology to experimental shrimp (Tran *et al*. 2013).

In Mexico, Escobedo-Bonilla (2016) had narrated about the emerging microbial diseases. Several bacterial viral agents were recorded to reduce production in shrimp farms. Bacterial diseases include vibriosis caused by different Vibrio species, a rickettsia-like bacteria cause necrotizing hepatopancreatitis (NHP-B), filamentous bacteria contribute to surface and gill fouling of shrimp as well as chitin-degrading bacteria provoke cuticle necrosis. Bacterial pathogens causing this injury include *Vibrio* sp., *Aeromonas* sp., *Spirillum* sp. and *Flavobacterium* sp. In hatcheries, a bacterial disease probably caused by *Vibrio harveyi* induced high mortalities to larval shrimp in zoea II stage (Escobedo-Bonilla, 2016). In Mexico, major viral pathogens which caused high mortalities in shrimp included Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV or Penstyldensovirus), Taura Syndrome Virus (TSV), Yellow-Head Virus (YHV) and White Spot Syndrome Virus (WSSV) (Escobedo-Bonilla, 2016).

In Latin America, presumptive systemic streptococcal infections were detected histologically in farmed *P. vannamei* juveniles. Differing Streptococcus identifications were obtained using API 20 Strep and Biolog systems, the former identifying the isolate as *Streptococcus uberis* and the latter as *S. parauberis*. Injection of specific pathogen-free (SPF) *P. vannamei* with the bacteria resulted in 100% mortality by 3 d post-injection with successful recovery of the agent from moribund test shrimp hemolymph samples. The recovered isolate was used in per os and waterborne exposure studies of SPF *P. vannamei* with mortalities ranging from 40 to 100% and 80 to 100%, respectively. These findings were described as the first reported case of streptococcosis in marine penaeid shrimp in the West by Hasson *et al.* (2009).

From India, Selvin *et al.* (2009) gave a comprehensive account about the shrimp diseases and their management options. Most of their studies were on *Penaeus monodon*. In the hatcheries, occurrence of white tail disease (WTD) caused by *Mr*NV and XSV in hatchery-reared post-larvae of *Penaeus indicus* and *P. monodon* were reported by Ravi *et al*. (2009). Recently, Gunalan *et al*. (2014) reviewed the disease occurrence among *Litopenaeus vannamei* in the shrimp culture systems from different geographical regions of India. Their observation indicated minor regional differences in the six major disease outbreaks in the *L. vannamei* culture systems. Most of the Black gill disease occurrences were in Andhra Pradesh; IHHNV in Andhra Pradesh and Orissa (north eastern coastal areas); WMD in Tamil Nadu and Andhra Pradesh; White gut disease in Tamil Nadu and Andhra Pradesh; Muscle cramp disease in Tamil Nadu and Andhra Pradesh.

Marine Metabolites in Shrimp Disease Control Strategies:

Marine Macro algae:

Lipton and Jean Jose (2006) initially reported about the immune enhancing activity of marine macro algae and their applications in shrimp farming as a non specific immune enhancer. Lipton *et al.* (2009) evaluated the macroalgal feeding strategies in improving the expulsion of pathogens from the hemolymph of shrimps administered with medicated feed and subsequent experimental infection by pathogenic vibrios. Their studies clearly indicated that the incorporation of macroalgal extract in feed to shrimp has successfully cleared the pathogenic bacteria faster from the hemolymph of *Penaeus monodon*. Impact of marine secondary metabolites (MSM's) from *Hypnea musciformis* as an immunostimulant on haemogram count and V*ibrio alginolyticus* infection in *Penaeus monodon* at different salinities were elaborated by Jose, Lipton and Subhash (2008). Shrimp pathogenic vibrios like *Vibrio alginolyticus* and *V. fischeri* were inhibited by the methanolic extract of H. musciformis. Simultaneous studies by Huxley and Lipton (2009) inferred that the marine macroalgae *Sargassum wightii* also provided immunomodulatory effect in *P. monodon*. Hemolymph parameters such as phagocytosis, agglutinin and bactericidins in the hemolymph were evaluated to substantiate the effect.

Shrimp disease management using macro alga *Ulva* diet was proven to be an effective eco-friendly management strategy for sustainable shrimp farming (Selvin *et al.* 2011). The Ulva diet was found to be a potent immunomodulator and therefore it was considered as a proactive drug. As per their earlier finding, 88% of viable *V. fischeri* cells were cleared-off from the haemolymph within 1 h in the Ulva treated group. These findings suggest the quick production of bactericidins in the haemolymph of Ulva treated group. Thus, the rapid bacterial clearance rate of shrimp haemocytes was stimulated by feeding with Ulva. Therefore, it was conjectured that bacteridins found in shrimp plasma might be induced released from haemocytes by Ulva diet. Huang *et al*. (2006) reported the effect of seaweed *Sargassum fusiforme* polysaccharide extracts on vibriosis resistance and immune activity of the treated shrimp. Literature also evidenced the *in vivo* antiviral (WSSV) potency of seaweed-based medicated feed in *Penaeus monodon* (Manilal *et al*. 2009). Selvin *et al.* (2011) mentioned about the easy preparation of the ulva diet for shrimps from the raw extract of the macroalgae. They also reiterated that purification strategies and consequent synthetic analogue development process need not be undertaken by any shrimp farmer for preparing the diets. As the extract was prepared from the dried material, such source material can easily be stored for 12 months. To sustain the host-defense system and relative protection against pathogenic invaders, the Ulva medication can be used as a prophylactic agent for the entire culture period at minimal expenditures. Based on the findings, it was concluded by them that the secondary metabolites of *U. fasciata* form an excellent source for developing potent formulations as a package of proactive management practice for sustainable shrimp farming.

As the ulva medicated feed was proved to be one of the major non specific immune modulator and inhibiting vibriosis in shrimps, several studies followed in marine macro algal extracts. Chakraborthy, Lipton and team (in 2011) fractionated the raw organic extracts of *U. fasciata* extracts using standard methods. Interestingly, their reports revealed that the Guaiane sesquiterpenes isolated and purified from the *Ulva fasciata* extract is a major compound exerting antibacterial properties.

Further studies by a team of researchers evaluated the antimicrobial potential of marine organisms collected from southwest coast of India against multi-resistant human and shrimp pathogens (Manilal *et al.* 2010). (Wulfen) Lamouroux and *Hypnea valentiae* (Turner) Montagne with specific activity Pramitha and Lipton (2013) evaluated the antibiotic potentials of red macroalgae *Hypnea musciformis* against fish and shrimp pathogenic bacteria. It is interesting to note that the aqueous extract of many of the common tropical macroalgae had antibiotic activities (Chritobel & Lipton, 2011). In vivo studies on the therapeutic values of marine macroalgae against the fish pathogen *Aeromonas hydrophila* (Celia & Lipton, 2012).

Cells responsible for removing foreign material include circulating hemocytes and fixed phagocytes, primarily in the gills and digestive gland (Lipton *et al.* 2011). Humoral factors such as non-self recognition proteins, prophenoloxidase, and antimicrobial peptides are produced, stored, and released from hemocytes. Hemocytes can adhere to a pathogen, triggering phagocytosis and thereby producing highly toxic reactive oxygen species which help eliminate foreign particles. The humoral and cellular defense mechanisms of shrimp can be assessed by bacterial clearance assays. The bacterial clearance capability of shrimp was steady in the control and experimental groups from day 40 onwards, indicating an age-dependent pattern and that shrimp hemolymph attains a high capability for removing invading pathogens. The bacteria were ultimately cleared in all treatments including the control, but the rate of clearance was improved by the medicated feed. These data support the idea that rapid clearance of live bacteria, whether by bactericidal mechanisms in the hemolymph or by physical trapping and removal to peripheral sites, contributes to disease resistance in shrimps by limiting the spread of free pathogens to other tissues The use of marine secondary metabolites from H. musciformis during the juvenile stage of *P. monodon* can assist in such clearance (Lipton *et al*. 2011).

Dashtiannasb *et al.* (2016) and Dashtiannasb & Yeganeh (2017) evaluated the effect of ethanol extract of a macroalgae *Laurencia snyderia* on growth parameters and vibriosis resistance in shrimp *Litopenaeus vannamei* in Iran. Their growth and survival data indicated that the mean survival was maximum (95.5±3.4%) for those shrimps that received enriched Artemia at 0.6 mg mL-1 concentration. The survival shrimps fed enriched Artemia at 0.2 and 0.4 mg mL-1 concentration were 92.22±3.2% and 92.22±5.2% respectively. In the control set, a minimum survival of 91.33% was recorded. The weight gain of 333.2 ±3.3 mg was noted in the experimental group fed with enriched artemia with 400 mg mL-1. But the minimum weight gain of 237.4 ± 4.6 mg was displayed in control group that was significantly different. The specific growth rate (SGR) of the *L. vannamei* juveniles after 30 days culture in different treatments and control varied with a statistically significant increase in the experimental treated group. The group also showed high resistance towards induced vibriosis with *Vibrio harveyi* challenge.

Crude extract prepared from the red seaweed *Laurencia snyderiae* obtained from the Persian Gulf was evaluated for shrimp growth performance and to determine *in vivo* efficacy of this seaweed in the prevention of shrimp vibriosis. The ethanol extract from *L. snyderiae* (EELS) that was fed to the *Artemia* instar I for their enrichment was found to be non toxic to them. Subsequently, juvenile shrimps of *Litopenaeus vannamei* were fed with these enriched *Artemia* at 0 mg mL_{-1} (Control group), 200 mg mL_{-1}, 400 mg mL_{-1} and 600 mg mL_{-1} for 30 days. The results obtained showed a significant increase (p<0.05) in survival rate in treatment groups compared with that in the control group. Shrimps fed with enriched *Artemia* showed a significant improvement in growth parameters when compared to those in the control group. When these juvenile shrimps were exposed to *Vibrio harveyi* (after 30 days) they showed notably lower mortality than the control. These results indicate that EELS has a good potential

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in promoting growth and antibacterial activities against *V. harveyi* that is useful in shrimp aquaculture (Dashtiannasb *et al.* 2016 and Dashtiannasb & Yeganeh, 2017).

Sea grasses:

Apart from macroalgae, sea grasses and their associated bacteria were also reported to exhibit antibiotic activities, including Vibrios from aquaculture sites (Merina and Lipton, 2010). Incorporating their cell free cultures or exo cellular products in the feed will be of immense use in the aquaculture industry and further studies are required in this direction.

Marine Sponge metabolites and sponge - associated bacterial metabolites:

Secondary metabolites from five sponge species inhibited the growth of eight virulent marine fish pathogens (Annie *et al*. 2008, Annie & Lipton, 2012). Antimicrobial Potential of the Marine Sponge *Sigmadocia pumila* from the South Eastern Region of India by Dhinakaran and Lipton (2012a) provided clues for developing therapeutics against common bacterial pathogens of shrimps and fishes. In addition to sponge extracts, the culturable bacteria from sponges were isolated and evaluated for bioactivity. Several initial studies on the sponges and their associated bacteria towards controlling infectious microbes of fish and shellfish were accomplished by Lipton and his team (Lipton *et al.* 2014 a, b). Bacteria were isolated from different sponges collected off Kanyakumari (Lat: 8º4’60N; Long: 77º34’0 E) and Vizhinjam (Lat: 8º22’45N; Long: 76º59’0 E) coasts of Southern India. A new media incorporating each specific sponge extract was employed to retrieve maximum groups as well as numbers of bacteria from *Callyspongia diffusa, C. subarmigera, Clathria gorgonoides, Echinodictyum gorgonoides, Ircinia fasciculate, Phloeodictyon sp., Sigmadocia carnosa, Spongia officinalis, Thalysias procera* and *T. vulpina, Zygomycale angulosa*. In the sponge extracted media, a higher bacterial numbers of $4.8x10^6$CFU/ml with 12 distinct colonies were retrieved from *C. subarmigera* compared to $6.4x10^5$CFU/ml with 5 distinct colonies in normal media. *Bacillus rubidae* (GenBank: JN873082) and *Serratia amyloliquefaciens* (GenBank: JN873083), and *Arthrobacter* sp (GenBank: JN 873081) retrieved from *C. subarmigera* tissue exhibited high antibiotic activities than the conventional antibiotic discs. Pathogenic fish and shellfish isolates such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Vibrio* *alginolyticus* and *V. harveyi* were inhibited by the cell free culture extract of the sponge-associated *Bacillus* and *Serratia*. In another series of tests, the sponge tissues of *Echinodictyum gorgonoides* yielded 3.2X10^6CFU/g of bacteria with 5 distinct genera at the pH optimum of 8.0. The cell free culture filtrate of the isolates has maximum activity towards *Bacillus subtilis*, *S. aureus* and *P. aeruginosa*. The culture filtrates of the bacteria exhibited similar activities as that noted from the organic extracts of the whole host sponge tissue extracts (Dhinakaran & Lipton, 2012b).

A recent study of the cell free supernatant of sponge *Callyspongia diffusa* -associated bacteria showed significant antibacterial activity against most of the fish and shellfish pathogenic isolates. Molecular characterization of one of the potent isolate showed a lineage with *Shewanella algae* (Rachanamol *et al.* 2014). Almost all the sponge isolates and cell free supernatants also showed activity against *V. harveyii*, *V. fluvialis*, *V. anguillarum*, *Proteus vulgaris* and *L. lactis*. Such vibriostatic efficacy of *Shewanella algae* isolated from *Penaeus monodon* against *V. parahaemolyticus* and *V. alginolyticus* was reported by Shakibazadel *et al*. (2008) and this was statistically proved.

Of late there are supply problems and the utilization of metabolite producing sponge specific bacteria will be of immense use in drug developments. In addition, Lipton and Shine (2010) developed mariculture techniques to grow the explants tissues of sponges for easy harvesting and extraction of metabolites. Sponges such as *Callyspongia subarmigera* (Ridley) and *Echinodictyum gorgonoides* (Dendy) were cultured and the extracts of cultured sponge exhibited antagonistic effects towards fish and shellfish pathogenic bacteria as noted in the natural habitat collected sponges.

The results of the cell free supernatant of associated bacteria showed that all the bacterial isolates of *Callyspongia diffusa* showed a significant antibacterial activity against most of the fish and shellfish. The isolate The BLAST search of the 16S rRNA gene sequence the isolate showed that the one of the potent isolate showed a lineage with *Shewanella algae* (Rachanamol *et al.* 2014). Almost all the sponge isolates and cell free supernatants also showed activity against *V. harveyii*, *V. fluvialis*, *V. anguillarum*, *Proteus* *vulgaris* and *L. lactis*. Such vibriostatic efficacy of *Shewanella algae* isolated from *Penaeus monodon* against *V. parahaemolyticus* and *V. alginolyticus* by Shakibazadel *et al.* (2008) was statistically proved.

Probiotics

Probiotics have been proven to be positive promoters of aquatic animal growth, survival and health. In aquaculture, intestines, gills, the skin mucus of aquatic animals, and habitats or even culture collections and commercial products, can be sources for acquiring appropriate probiotics, which have been identified as bacteria (Gram-positive and Gram-negative) and nonbacteria (bacteriophages, microalgae and yeasts). While a bacterium is a pathogen to one aquatic animal, it can bring benefits to another fish species; a screening process plays a significant role in making a probiotic species specific (Hai, 2015).

Potential probiotics may be commonly obtained from various sources viz. the GI tracts of aquatic animals (Del'duca *et al*. 2013, Beck *et al*. 2015) and fish mucus (Tapia-Paniagua *et al*. 2012). Particularly they are the collected cultures (Thompson *et al*. 2010) and commercial products (Suzer *et al*. 2008). In marine bivalve hatchery, Subhash *et al.* (2007) studied about the role of probiotics in pearl oyster hatcheries. Their studies revealed the influence of probiotic bacterium *Lactobacillus acidophilus* on the enhanced survival and growth of pearl oyster *Pinctada fucata* spats.

Exclusion of *Vibrio* spp. by an antagonistic marine actinomycete *Streptomyces rubrolavendulae* M56 was reported by Augustine *et al* (2015) from India. Subsequent review by Tan *et al.* (2016) gave an insight in to the use of the genus *Streptomyces* bacteria as an alternative to antibiotics, being a probiotic in controlling diseases and improving the health and quality of aquaculture production. The prospects and limitations of *Streptomyces* species as a probiotic in aquaculture were also discussed by Tan *et al.* (2016).

In white shrimp *Litopenaeus vannamei* and *Fenneropenaeus indicus* vast strains of *Bacillus*have been tested as probiotics in order to improve dry matter digestibility, phosphorus, and crude protein. Consequences of *Bacillus* administration with a dose of 50 g kg^{-1} feed revealed higher growth sizes (Cook *et al.* 2006). Other research has suggested the importance of managing the probiotic in all ontogenetic stages of the shrimp to generate a constant effect on the production of digestive enzymes (Strozzi, L. Mogna, 2008). In order to develop a potent endogenous probiotic from shrimp, screening of digestive canal bacteria of health *Litopenaeus vannamei* resulted in four species, they were identified as *Bacillus megaterium* BM1, *Bacillus firmus* BM2, *Actinobacillus* spp. BM3 and *Pseudomonas stutzeri* BM4. *B. megaterium* BM1 was the ideal probiotic candidate for enhancing growth on *L. vannamei*, it resulted in production of digestive extra cellular enzymes and a premium value of steady growth rate. Concentration of 10^6 cells g^{-1} diet from *B. megaterium* BM1 in an *in vivo* study resulted in beneficial effects for the growth and feed utilization of *L. vannamei* (Yuniarti *et al*. 2013).

Apart from products from bacteria, fungal metabolites were also evaluated for shrimp growth and disease control. Wahjuningrum *et al.* (2016) reported better survival rate, daily growth rate and feed conversion ratio in shrimps treated with Nodulisporium sp. KT29. The fungal *Nodulisporium* sp. KT29 metabolites contained bioactive compounds including 0.54% β-(1,3) glucan and phytochemical compounds (121 ppm of phytosterol, 23 ppm of saponin and 31 ppm of polyphenol Saputra *et al.* (2016). The presence of phytochemical substances, β-glucans and polyphenols were inferred for the immunostimulants and antioxidants activities and negative effects of stress, increase diseases resistance, and improve various physiological performances (Shelby *et al.* 2007; Welker *et al.* 2007, Soltanian *et al.* 2009; Kiron 2012). According to Hai & Fotedar (2009), the administration of β-glucan on shrimp caused the structure of intestinal surface becoming wider, so that the nutrients absorption became better. The improvement on the digestion and the nutrients absorption will cause the enhanced feed efficiency and protein absorption, which will generate a higher growth performance (Dawood *et al*. 2015).

In general, the organic acids are among the most promising substances as they have been reported to possess anti-Vibrio spp. activities (Mine and Boopathy 2011; Adams and Boopathy 2013; da Silva *et al*. 2013), and increased survival rate of shrimps (Walla *et al*. 2012; Su *et al*. 2014; Romano *et al*. 2015; Ng *et al*. 2015). Astaxanthin, a type of carotenoid, can also improve shrimp survival rate and enhance resistance to several stress conditions, such as low dissolved oxygen, low salinity, low temperature, and ammonia stress (Flores *et al*. 2007; Niu *et al*. 2009). Therefore, both organic acids and astaxanthin have the potential to be used in shrimp farming as feed additives. The objectives of this study were to evaluate the effect of dietary supplementation of formic acid and astaxanthin on growth, survival and tolerance to *V. parahaemolyticus* infection in Pacific white shrimp under laboratory conditions.

Marine Immunostimulats

Immunostimulants are substances, which elicit non-specific defense mechanisms and enhance the barrier of infections against pathogens. They are isolated from natural sources and then synthesised chemically (Example: cell wall preparations from bacteria, fungi, mushroom). Most of the research on immunostimulants has been directed towards treatment of cancer in humans. Immunostimulating compounds induced production of cytokine proteins like interleukins, interferon, tumor necrosis factor and colony stimulating factors.

Wang *et al.* (2017) have recently reviewed the current knowledge and future perspectives about the application of immunostimulants in aquaculture.The active principles of immunostimulatory cell wall preparations are various muramylpeptide fragments, lipopolysaccharides, lipopeptides, acyloligopeptides and specific ides composed of glucose units which are linked through b-1, 3 and b-1, 6 bonds. These glucans can exist in various structural forms, water soluble oligomers; water insoluble macromolecules and particulate matters. Mary Jane *et al.* (2015) gave an updated account about the uses of immunostimulants in shrimp culture. In shrimps so far, 11 types of pattern recognition receptors (PRRs) have been identified viz., -1,3-glucanase-related proteins, -1,3-glucan-binding proteins, c-type lectins, scavenger receptors, galectins, fibrinogen-related proteins, thioester-containing Down syndrome cell adhesion molecules, serine protease homologs, trans-activation response RNA-binding protein, and Toll-like receptors. Aside from pattern recognition, these PRRs have different binding specificities and effector functions (Wang & Wang 2013).

In fishes, the killed mycobacteria and muramy1 dipeptide enchanced resistane of coho salmon, *Oncorhynchus kisutch* against several bacterial pathogens. Injection of the synthetic lactolyl tetra peptide FK-565, increased the phagocytic activity and non-specific resistance of rainbow trout against *Aeromonas salmonicida* infections. Resistance of carp to infections by *Edwardsiella tarda* by activating the non-specific was achieved by administration of schizophyllan, scleroglucan and lentinar. The non-specific disease resistance in Atlantic salmon was enhanced by glucan preparation from *Saccharomyces cerevisiae*. Since then several researchers have suggested the possible use of glucans against viral infections in fish and shrimps.

In crustaceans, immunostimulants can increase the phagocytosis of pathogens by activating phagocytic cells in the hemolymph, increase the antibacterial and antiseptic properties of hemolymph, activate the prophenoloxidase system and mediate signal recognition and phagocytosis (Wang *et al*. 2017). In shrimps, the Wheatgerm Agglutinin (WGA), a lectin, administered as feed additive has promoted the bacterial resistance of *Penaeus orientalis*. M-Glucan (a mixture of insoluble *b*-1, 3 and b-1, 6 poly glucoses) was found as a short-term immunostimulant for the shrimp, *Penaeus monodon*. Immersion treatment with yeast beta-glucan was demonstrated to enhance growth and vibriosis resistance in tiger shrimp *P. monodon*. In the treated shrimps, the disease resistance could be correlated with enhanced phenoloxidase activity and intrahemocytic production of superoxide anion. In shrimps the prophenoloxidase ('Propo'), the defense enzyme system, is activated by immunostimulants. The activation of 'Propo' results in recognising pathogens and providing resistance.

In fish, the non-specific defense system is activated by the immunostimulants. The first

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line of defense - i.e., non specific humoral defense or proteases, lysins and agglutinins in mucous cell secretion; The second line of defense provided by the mucosal lining cells and the third line of defense achieved by blood cells, especially granulocytes and monocytes which destroy microbes present in the circulation are activated. Endocytically active cells such as endothelial cells, macrophages and granulocytes in organs and tissues, which degrade microbes or microbial products, take up the final defence. The final endocytic and degradation process strongly depend on the effectiveness of reticulo endothelial system, which consist of endothelial cells, and macrophages, which line the small blood vessels (sinusoids and ellipsoids). The central cells in the production of antimicrobial substances are macrophages and granulocytes, which are activated by the immune enhancers.

Hemocytes are also activated by immunostimulants. In addition, they enhance the clotting activities and produce bactericidins. In tiger shrimp *Penaeus monodon*, increased bacterial clearance was noted after injection with glucan. The bacterial clearance ability of haemolymph drawn from the tiger shrimp *Penaeus monodon* immersed in a viable cell suspension of *Vibrio vulnificus* showed that *Vibrio* cells were largely eliminated from shrimp haemolymph within 12 h following invasion and completely undetectable at 24 h. The anti-*E. coli* activity of plasma, phenoloxidase (PO) activity, as well as the production of superoxide anion (O^2) were significantly enhanced due to administration of glucan and zymosan. Immunostimulants can promote recovery from the status of immunosuppression caused by stress. The peptidoglycan- fed black tiger shrimp exhibited a higher tolerance to dissolved oxygen, salinity and stress than those fed with the controlled diet.

The immunostimulants have several advantages:

1. Being natural products, there is no environmental hazard.
2. Unlike vaccines, which give protection to a specific pathogen, immunostimulants provide a wide range of protection against several pathogens.
3. Most of the immunostimulants can be synthesized and the problem of residual effect on shrimps or fish is not encountered.
4. Shrimps depend more heavily on non-specific defense mechanisms than mammals and therefore immunostimulants have a significant role in health management

strategies in aquaculture.

5. When glucans were administered along with *Aeromonas hydrophila* vaccine, the response was even more enhanced, suggesting that yeast glucans have important role in disease management in warm water aquaculture.

In shrimps, three main types of circulating haemocytes have been identified and isolated by isopycnic cetrifugation on Percoll gradient. Semi granular cells respond to microbial polysaccharides such as lipopolysaccharides and B-1,3-glucans by degranulation. Since the degranulated cells attach and spread on foreign surfaces, they have an important role in encapsulation. Granular haemocytes with large granules are a repository for the prophenoloxidase (pro-PO) activating system. In crustaceans, clotting is mediated by coagulogens present in the plasma and also compartmentalized within circulating cells. The plasma factor is converted to covalently linked polymers of coafulin by Ca^{2+}-dependent transaminase whereas the cell factor is converted to a gel by a serine protease proclotting enzyme, which may be triggered, by microbial molecules such as lipopolysaccharide (LPS) and -1,3-glucans.

Table Marine organisms with promising immunostimulatory effect

Source organism	Experimental organism	Assay/inhibitory activity
Porphyra yezoensis (Macroalga)	Murine	Phagocytic assay
Undaria pinnaftifida (Macroalga)	NS	Phagocytic assay
Ecteinascida turbinata (Tunicate)	Eel	Phagocytic assay
Haliotis discus hannai (Abalone)	Trout	NK cell assay
Hyrtiss erecta (Sponge)	NS	Immunomodulatory
Briareum exavatum (Gorgonid)	NS	Immunostimulatory
Ulva fasciata (Seaweed)	Shrimp and Fish	Immunostimulatory

NS – Not Specified

Shrimp aquaculture though lucrative, has its own threats. The sustainability of shrimp farming is achieved by a comprehensive and holistic way of managing the adverse conditions with better feeding and disease management strategies. In this context, certainly, the marine metabolites are of immense use and they are to be explored further.

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