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Biocontrol of Luminous Vibriosis in Shrimp Aquaculture: A Review of Current Approaches and Future Perspectives

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ABSTRACT
Healthy shrimp culture system is always in harmony with the ecology of the pond environment. This can be manipulated by developing a dense heterotrophic bacterial community that takes care of waste generated in the system through in situ bioremediation. Considering the importance to reduce an occurrence of luminous vibriosis in shrimp aquaculture, countless studies have been carried out with an objective to screen anti-vibrio biological agents, which can be used as an alternative to antibiotics. In such studies, microalgae, bacteriophage, and probiotic bacteria have been found to have potential benefits in reducing vibriosis. Eco-based shrimp farming, green water technology, bio-floc technology, phage therapy, and integrated multi-trophic aquaculture (IMTA), since their inception, hold a promising alternative to antibiotics in the near future. This article seeks to secure all the available information on different biological agents, their involvement in lowering *Vibrio* load, and strategies to control *Vibrio* infection in shrimp aquaculture.

KEYWORDS
Bio-floc; green water technology; probiotics; microalgae; phage therapy

Introduction

Vibrio sp. are one of the primary disease-causing pathogenic agents in shellfish aquaculture, ubiquitous throughout the world. These gram-negative facultative anaerobic bacteria of the Vibrionaceae family often result in complete mortality in hatcheries and grow-out ponds. Intensification of shrimp culture has led the shrimp species toward exposure to stressful conditions, which open up a wide path for vibriosis infection. *Vibrio harveyi, V. parahaemolyticus, V. anguillarum, V. vulnificus*, and *V. splendidus* are the major pathogenic strains of *Vibrio*, which have been reported to cause disease outbreaks in shrimp. *V. harveyi* is one of the most important luminous marine bacteria, and luminescence produced by it is easily visible at night in the infected post-larvae, juveniles, and adults. Slow growths, lethargy, patches on the body, and muscle opacity are the signs of vibriosis (Karunasagar et al., 1994). In India, large-scale mortality of cultured *Litopenaeus vannamei* in grow-out ponds due to vibriosis has been observed during the latter half of 2014. That has been revealed in order to unravel the real fact of early mortality syndrome (EMS) occurrence (Kumar et al., 2014). The study confirmed the role of *V. parahaemolyticus*, an opportunistic pathogen for mass mortalities of cultured shrimp that can be triggered by stressful environment. In such scenario, various chemicals and antibiotics were being applied as an immediate resort for controlling bacterial infection. Indiscriminate use of antibiotics in aquaculture industry has caused severe economic loss and establishment of antibiotic resistant bacteria (Abraham et al., 1997; Tendencia and De la Pena, 2001). Thus currently, antibiotics cannot assure efficient control of luminescent vibriosis. During the last two decades, microbial intervention in aquaculture has been significantly evolved as an alternative measure to antibiotics. Probiotics, based on the principle of competitive exclusion and immune stimulants for improving defense mechanism are giving promising preventive result in protecting aquatic animals from luminescent vibriosis. Microbial-based systems, green water culture technique, and bio-floc technology have gradually gained acceptance for healthy shrimp farming and since few years appealed as a better technique in the shrimp industry. These culture systems represent one of the most viable strategies toward sustainable aquaculture practices based on the promotion of autotrophic or heterotrophic microbial proliferation (Martinez-Cordova et al., 2014). Besides, polyculture of shrimp with suitable species of fish, mollusk, and seaweed (Integrated Multi-Trophic Aquaculture, IMTA) also gives a healthy culture

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environment in addition to the extra profit. This article highlights the potential of biocontrol agents and new strategies to control *Vibrio* infection in shrimp aquaculture to make aquaculture industry more sustainable.

Taking advantage of microbes against pathogenic *Vibrios*

Probiotic conveniently defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Among other modes of actions, it acts as a non-specific immunomodulator, though the underlying molecular mechanisms are poorly understood. In aquaculture, the use of functional feeds with the incorporation of probiotics is being increasingly practiced. So far, a number of probiotic strains have been assessed and resulted in increased growth and survival of several species. The probionts, belong to Vibrionaceae, Pseudomonadaceae, and Bacillaceae, are found to give protection to carps, salmon, prawns, and oysters. With a view to limit the antibiotic use and emphasize the importance for optimizing innate immunity, probiotics are being used either singly or in mixtures providing enough evidence on immunomodulation as its dominant mode of action. Control of pathogenic Vibrios by using non-pathogenic and beneficial bacteria has received remarkable attention in the last decade (Newaz-Fyzul et al., 2014). There have been numerous studies on various strains of lactic acid bacteria (LAB), *Bacillus* sp., *Psuedomonas* sp. as potential probiotics in the aquaculture sector. Several studies reported that LAB may modulate various physiological proteins in normal as well as diseased conditions in a beneficial manner to improve the immune status of the host (Erickson et al., 2000; Isolauri et al., 2001). Use of *Bacillus* sp. resulted in improvement of growth, survival, water quality (Wang et al., 2005), and reduction of pathogenic Vibrios (Dalmin et al., 2001). *Bacillus subtilis* (strains L10 and G1) and *Pseudomonas* (strains MCCB 102, MCCB 103, and I-2) have been reported to confer higher resistance against shrimp pathogens, *Vibrio harveyi* and *V. parahaemolyticus* (Chythanya and Karunasagar, 2002; Balcazar and Tyrone, 2007; Chiu et al., 2007; Pai et al., 2010; Zokaeifer et al., 2012, 2014). *Vibrio gazogenes* NCIMB 2250 was also described to have an inhibitory effect against pathogenic Vibrios in *L. vannamei* (Thompson et al., 2010) and considered to bring good health to animals. *Lactobacilus plantarum* MR 03.12 when co-cultured with *V. harveyi* exhibited a complete reduction of the *Vibrio* at 24 hr and resulted in higher survival of *L. vannamei* (77%) after ten days of challenge study against control (67%) (Kongnum and Hongbpattarakere, 2012). Efficacy of probiotics on shrimp larval survival and resistance to *Vibrio* sp. has also been positively evaluated. *V. harveyi* infected *L. vannamei* larvae supplemented with B6 strain of lactic acid bacteria had significantly improved survival, post-larvae quality, and a decreased load of *Vibrio* sp. (Vieira et al., 2007).

Over the last two decades, probiotics have been applied in the aquaculture farms to control pathogenic bacteria, however, the quantum and closer frequency of application of probiotics have been considered to be its limitation factor (Vine et al., 2006). Also, with changes in environmental, culture condition, the efficacy of generally used probiotics in controlling the pathogenic bacteria is doubtful. Careful selection of licensed probiotics for human or animal nutrition (Defoirdt et al., 2007) from culture system with a different mode of action and application of mixed probiotics may help in increasing the chance of success. The successful acquisition of such novel probiotics might also depend on obtaining a better understanding of the microbial ecology of a cultured species as well as restricting the probiotic screens to the bacterial species that share the immediate environment with the cultured species (Kesarcodi-Watson et al., 2008).

Bio-floc technology (BFT): A sustainable approach promising disease prevention

Bio-floc technology approach provides a healthy and eco-friendly rearing system with the advantage of assimilation of pollutants from water, reduction of the need for water exchange, reutilization of feed, and also prevention of pathogenic load. Bacterial communities in bio-floc system play the key role in reducing total ammonia nitrogen and nitrite concentrations either by autotrophic nitrification or by heterotrophic assimilation. Floc composed of microalgae, detritus, zooplankton, bacteria contribute to shrimp nutrition resulting in a decrease in FCR and increase in yield (Brito et al., 2014). Autotrophic or heterotrophic micro-organism's proliferation in a BFT system is based on the C: N ratio. For the promotion of heterotrophic bacteria, C: N ratio is recommended to be approximately 20:1, however, for micro-algae and autotrophic organism development the ratio must be maintained lower (5:1 to 10:1) (Becerra-Dorame et al., 2011). One of the easy way to increase C: N ratio (>10) in aquaculture system is the addition of carbohydrates or organic carbon, i.e., molasses, tapioca, sucrose, wheat flour, which stimulates bacterial growth and subsequently nitrogen uptake through protein synthesis (Avnimelech, 1999). Bio-floc dominated with algae, detritus, suspended material, and autotrophic bacteria has been reported to be more beneficial for high-density zero exchange shrimp culture system (Xu et al., 2016). With the regular addition of carbon in water, polyhydroxyalkanoates (PHA) accumulating bacteria proliferate. These bacteria produce several biodegradable polymer as intracellular energy and carbon storage compound, like poly-β-hydroxy butyrate (PHB), a short chain fatty acid polymer having antibacterial or biocontrol properties (Sinha et al., 2008). PHA production varies depending on factors that include pH, temperature, and C: N ratio. Higher C: N ratio typically results in increased PHB accumulation (Magdouli et al., 2015). Crab et al. in 2010b has reported on the use of bio-floc as biocontrol agents against *V. harveyi* considering *Artemia franciscana* as a model system. Addition of bio-floc inhibited *V. harveyi* growth that might be due to the presence of extracellular compounds that disrupted quorum sensing activity. Acetate, glycerol, and glucose with and without *Bacillus subtilis* inoculum have been evaluated to promote bio-floc formation in farming of *Macrobrachium rosenbergii*, and that *Bacillus* spores improved the nutritional quality of bio-floc as well as inhibited proliferation of pathogens (Crab et al., 2010a). Barcenal et al. in 2015 reported *Vibrio* lytic and gut colonizing activity of *Micrococcus luteus* isolated from shrimp bio-floc rearing culture system. *Vibrio* cellular lytic activity of *Micrococcus luteus* exhibited in a wide range of salinity 0–30 ppt and pH range of 6–8 with optimum lytic activity at 20 ppt salinity and 8 pH. Colonization of the bacteria in shrimp gut and water with neutral or slightly acidic pH and utilization of *Vibrio* cells as nutrients by cell lysis significantly lower the *Vibrio harveyi* counts in shrimp hepatopancreas.

Kim et al. in 2014 studied the bio-floc effect on immune activity of Pacific white shrimp, *L. vannamei* post-larvae and suggested that heterotrophic microbial population in bio-floc induces a permanent trigger toward the development of immune system thus build up a defense mechanism. The finding clearly indicated the role of bio-floc associated microbes in the enhancement of immune related genes. Similarly, Aguilera et al. in 2014 investigated the advantage of bio-floc technique for *L. vannamei* rearing over clear water technique. In this study, histopathology reveals the better immune status of floc reared shrimp than clear water, and a unique group of vibrios was found out in floc rearing system. Enhancement of cellular immune response and antioxidant status has also been observed in cultured *L. vannamei* in bio-floc-based system with two different C: N ratio of 15:1 and 20:1. Total hemocyte count and phagocytic activity were significantly higher in shrimp reared in bio-floc system (Xu and Pan, 2015). Panigrahi in 2015 mentioned regarding the natural probiotic effect of bio-floc against *Vibrio* sp. and his study reflected bio-floc grown *L. vannamei* showed improved protective response than conventional system when challenged against *V. parahaemolytics*. These studies have revealed that maintaining the autotrophic or heterotrophic bacterial load would possibly help in controlling the pathogenic bacteria load by imparting improved immune response.

Phage therapy: A biotechnological approach against *Vibrio* pathogen

The viruses that infect and kill bacteria are known as bacteriophages. About 10–20% of the heterotrophic bacteria in coastal ecosystem are lost per day due to this viral infection (Suttle, 2005). Phages offer potential scope as an alternative to antibiotics in controlling bacterial infections in aquaculture sector. Although, phages were discovered in the early 1915, but phage therapy for control of disease in fish and shrimp is not an age old practice. Phages as therapeutic agents against fish bacterial pathogen were used in the 1990s for inhibiting *Lactococcus garveyi* in Yellow tail fish. Successful attempts at the use of phages to control luminous vibrios in shrimp aquaculture have been reported since past ten years (Table 1). Most of the studies related to phages are concentrated upon isolation and characterization of bacteriophage and their in vitro evaluation for inhibition of shrimp pathogen. Electron microscopy observation of phage attachment to *Vibrio parahaemolyticus* cell wall and cell lysis throughout the infection cycle shown by Alagappan et al., 2010 gave a clear picture of the killing mechanism of phage (Figure 1). Direct supply of phages to culture water could be a potentially efficient and inexpensive method for controlling vibriosis. It is also reported that phages did not impact on nitrifying bacterial consortia and probiotic bacteria; *Bacillus cereus* (MCCB 101) and *Micrococcus* sp. (MCCB 104) in shrimp culture system (Surekha Mol, 2012). The reason is due to high specificity of phages even to particular bacterial strain. Lomeli-Ortega et al. (2014) evaluated two phages, A3S and Vpms1 against *V. parahaemolyticus* infections of *L. vannamei* larvae and found effective result. Shivu et al. (2007) isolated seven phages from hatchery and creek water lytic against 183 *V. harveyi* strain but found out none of them was able to infect other *Vibrio* species. Consequently, in a culture system that involves different species and strains of the pathogen, it is hard to control Vibrio infection, however, a mixture of phages may help in killing different strains of vibrios. Vinod et al. (2006) found an increase in survival (86% from 17 %) of *P. monodon* larvae during hatchery trial with the supply of a mixture of phages (lytic to *V. harveyi*). Karunasagar et al. (2007) reported about the possible inhibition of *Vibrio harveyi* biofilm in tanks and pipe

Table 1. Effect of phages on *Vibrio* associated with shrimp culture system.

Target pathogen	Phages isolated	Morphological characteristic	Effects	Reference
V. harveyi	VHML	Family: Myoviridae icosahedral head of 40–50 nm diameter, and a sheathed rigid tail of 150–200 nm length	Temperate bacteriophage with narrow host range specifically lysogenic to *V. harveyi*	Okey and Owens, 2000
V. harveyi	VHS1	Family: Siphoviridae icosahedral head of 66 nm diameter and an unornamented, flexible tail of 153 nm length	When *Vibrio harveyi* 1114GL is lysogenized with VHS1, its virulence for shrimp, *Penaeus monodon* increases by more than 100 times, with production of a toxin(s) associated with shrimp hemocyte agglutination	Pasharawipas et al., 2005
V. harveyi	Vi ha 10 & Vi ha 8	Family: Siphoviridae Vi ha 10: head 40–45 nm in dia. with hexagonal outline and a non-contractile tail of diameter 7 nm and length 60 nm	Vi ha 10 isolated from Oyster & Vi ha 8 from P. monodon hatchery lysed 70% and 68% of Vibrio strain, respectively, and a cocktail of these two lysed 94% of *V. harveyi* strain	Karunasagar et al., 2007
V. harveyi V. parahaemolyticus & V.alginolyticus	Vi ha 19	Family: Myoviridae Isometric head with contractile tail. Total length: 186.1 nm Head diameter: 94.4 nm	70% lytic efficiency to *V. harveyi* and also lytic to *V. parahaemolyticus* and *V. alginolyticus*	Surekha Mol, 2012
V. harveyi	PW 2	Family: Siphoviridae icosahedral head of 50 ± 3.8 nm with nanocontractile tail of 136 ± 6.2 nm length	Phage absorption rate was 80% in first 15 min of vibrio infection and then 90% within 30 min	Phumkhachorn and Rattanachaikunsopon, 2010
V. harveyi	VHP6b	Family: Siphoviridae	Pathogenic to 27 *V. harveyi* strains and phage treatment improved survival of post-larvae by 40 to 60%	Patil et al., 2014
V. parahaemolyticus	Vp1	Family: Myoviridae icosahedral head (50–60) nm in dia. and contractile tail of 7 nm dia. and length 100 nm	lysed 8 isolated strains of *V. parahaemolyticus*	Alagappan et al., 2010

by using a cocktail of two Vibriophages. Phage-derived bacterial lysates have been used as vaccines for immunization against bacterial diseases (Alagappan et al., 2010).

Important issues regarding the use of bacteriophages are that the virulence of other *Vibrio* pathogens of shrimp may be influenced, and there is potential for transfer of virulence (Flegel et al., 2005). The phages selected should be lytic and should not revert to lysogenic, and it should not carry any toxic gene that would make the host more virulent to shrimp. Unlike lytic phages, lysogenic ones can integrate their DNA into host's chromosomes also known as lysogenization. These phages can transfer horizontal transfer of bacterial genes from one bacterium to another (Surekha Mol, 2012). Hence, a careful selection of phage before use in therapy has to be given utmost importance.

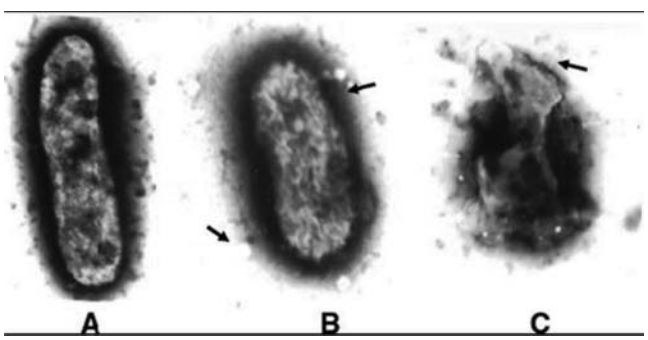

Figure 1. Infection stages of phage Vp1 in *V. parahaemolyticus*. Electron microscopic observation (TEM) at 930,000 magnification. (A) Bacterial cell without infection; (B) phage (P) attached to bacterial cell wall (arrows); (C) lysed bacterial cell due to phage infection. Bar 0.1 lm (obtained from Alagappan et al., 2010). © Springer. Reproduced by permission of Springer. Permission to reuse must be obtained from the rightsholder.

Microalgae-based culture: A strategy for disease prevention

Microalgae exhibit an extensive and underexplored resource of metabolic versatility and flexibility. They are the natural resources of valuable protein, polyunsaturated fatty acid, carotenoids, and phycocolloids (Guedes et al., 2011). Additionally, they indeed proved to retain antimicrobial, antioxidant, and anti-inflammatory properties. *Porphyridium, Spirulina, Haematococcus* species are the illustrative examples for the microalgae constituting all these characteristics (Table 2). Considering its efficacy as antimicrobial agent several algae such as *Chlorella, Chaetoceros, Skeletonema, Nitzchia*, and *Leptolyngbia* have

Table 2. Microalgae constituting antibacterial, anti-inflammatory, and anti-oxidant properties.

Microalgae	Properties	Reference
Porphyridium	Anti-microbial	Chen et al., 2005
	Anti-inflammatory	Matsui et al., 2003
	Antioxidant	Tannin-Spitz et al., 2005; Sun et al., 2009
Spirulina	Anti-bacterial	Ozdemir et al., 2004; Kaushik and Chauhan, 2008; Rao et al., 2010
	Antioxidant and Anti-inflammatory	Deng and Chou, 2010
Haematococcus	Antioxidant, Anti-inflammatory	Guerin et al., 2003
	Anti-bacterial	Rao et al., 2010

Table 3. Study carried out on anti-luminous *Vibrio* factors associated with green water culture system of shrimp.

Micro-algae	Efficacy in reduction of luminous *Vibrios*	Reference
Leptolyngbia sp	94–100% reduction from 10^4 to 10^1 CFU/mL 24 hr post exposure	Lio-Po et al., 2002
Chaetocerouscalcitrans and *Nitzchia*	10^4 to 10^0 CFU/mL at 24 and 28 hr post exposure	Lio-Po et al., 2002
Skeletonema costatum	Bacteriostatic effect during seven days exposure period	Lio-Po et al., 2002
Chlorella sp	Not sufficient enough for Vibrio reduction when alone in green water system	Tendencia et al., 2005

been assessed for inhibition of pathogenic *Vibrio* in aquaculture sector (Table 3). Dietary inclusion of green algae *Tetraselmis suecia* to *Penaeus indicus* maturation diet significantly reduced *Vibrio* count in the gut content of broodstock as well as in rearing water of maturation and spawning tank. Similarly, use of these algae as partial live larval feed showed reduction in *Vibrio* count in rearing water and larvae sample (Regunathan and Wesley, 2004). Introduction of immobilized microalgae *Oocystis borgei* and *Nannochlorosis occulata* into *L. vannamei* culture system shows improvement in water quality, immune response, and undetectable *Vibrio* count after nine and 15 days of experimental culture (Huang et al., 2002). Nowadays, private hatcheries of South East region of India are considering *Thalassiosira sp.* for *Vibrio* control in larval rearing tanks (from local communication). Though in vitro antimicrobial activities of *Thalassiosira rotula* against *V. harveyi* has been well reported (Qin et al., 2013), however, a comparative evaluation between *Chaetoceros* sp. and *Thalassiosira* sp. for *V. harveyi* inhibition in *Penaeus monodon* larval rearing showed infected larvae fed with *Chaetoceros* exhibited better survival rate (58.67%) than the other (Taupik, 1997). Inhibitory mechanism of microalgae against bacteria is due to the production of free fatty acid as a defense strategy or due to competitive exclusion effect of the bacteria associated with algae. Microalgae release free fatty acid by binding of fatty acid with glycerol and sugar (Desbois and Smith, 2010). The unsaturated or saturated long chain fatty acid and their derivatives have shown bactreiocidal activity against a wide range of micro-organisms and reported to induce lysis in bacterial protoplast (Naviner et al., 1999). The antibacterial property of microalgae is well supported by a study defining production of high content of fatty acid (>82%) by *Isochrysis galbana* when cultured along with *Vibrio* sp. Mixed culture of *Isochrysis galbana* with *Vibrio* showed inhibition of *V. alginolyticus*, *Vibrio campbelli*, and *Vibrio harveyi* counts to undetectable levels in two, four, and seven days (< 0.01 Vibrio sp/mL). Interestingly, the density of *Isochrysis galbana* increased from 58 to 95% in mixed culture with vibrios versus the control with no *Vibrio*. The microalgae produced high content of polyunsaturated fatty acids (48%) in stationary phase of growth, which has been attributed due to production and accumulation of lipids that provide alternate source of carbon and energy for algae cells under stress (Molina-Cardenas, 2014). Inhibition of *Vibrio* sp. growth was also found to be strain specific, i.e., unlike other *Vibrio* sp. *Isochrysis galbana* did not inhibit *V. parahaemolyticus*.

Inhibition of pathogenic vibrios by specific bacteria inoculation to micro-algae-based larvae culture system might be a viable alternative to eliminate the use of high volume of probiotics. This presumption has been well supported by a study resulting in inhibition of *Vibrio alginolyticus* by inoculation of a bacterial strain (SK −05) to *Skeletonema* culture medium. The finding also clearly stated that inhibition of *Vibrio alginolyticus* was not due to bacteriostasis or antibiosis of SK −05 but due to an effect of competitive exclusion mechanism produced by *Skeletonema costatum*. The microalgae by consumption of organic exudates maintain a poor organic medium unsuitable for vibrios growth (Rico Mora et al., 1998). The mechanism might be the concept behind the mixotrophic method of aquaculture invented by Blue Aqua International Pte Ltd. By proper balance and maintenance of phytoplankton and bacterial communities throughout the production cycle, this culture method has been claimed to increase productivity with enhanced water and soil quality in intensive culture system.

Finfish-based biological control: An effective disease prevention strategy

Fish constitute antibacterial property in its mucus that helps in preventing microbial colonization by inhibiting bacterial growth. Inhibitory activity of skin mucus against some bacteria could be due to protein moiety (Ebran et al., 1999). Mucus layer of fish skin and gills contains epidermal cells like goblet cells and club cells, which produce many immunological molecules likes lysozyme, lectins, complement, immunoglobulin. They have antimicrobial properties against many infectious agents like bacteria and viruses (Esteban and Cerezuela, 2015). Mucus extracts of snakehead, eel, and rainbow trout have been reported with bactericidal property against *Pseudomonas aeruginosa* and *Aeromonas hydrophila* (Austin and McIntosh, 1988; Wei et al., 2010). Several commercially valued marine food fish species such as tilapia, groupers, sea bass, siganids, snappers, and milkfish have been cultured with shrimp to attain sustainability in shrimp farming. Since 1996, tilapia integration in shrimp aquaculture for lowering luminous *Vibrio* load has been very well recognized. Green water technique that cultures shrimps in fish (i.e., *Tilapia*) grown water, abundant in *Chlorella* has been reported to control *V. harveyi* load in cultured pond preventing an onset of infections. The effectiveness of this technique has been attributed due to the presence of anti-vibrio factors in microbiota and skin mucus of tilapia in culture system (Lio-Po et al., 2005). Aside from lowering bacterial count, tilapia culture in shrimp pond is also recommended for improvement in soil and water quality (Tendencia et al., 2015). Tendencia et al. in 2004 reported that stocking of tilapia at a biomass more than 300 g/m^3 efficiently inhibited the growth of luminous *Vibrio* in shrimp (biomass = 80 g/m^3) rearing water. In a different setup, an in vitro study was conducted to determine the efficacy of GIFT tilapia, grouper, and milk fish on growth of luminous bacteria in polyculture system. *Tilapia hornorum* (Jewel tilapia), *Oreochromis niloticus* (GIFT), and *Epinephalus coioides* (grouper) at a biomass of 500 g/m^3 has also shown inhibitory effect on luminous bacteria growth in shrimp (biomass = 80 g/m^3) rearing water and positively affected shrimp survival. Decreasing luminous bacterial count was observed in all culture system; however luminous bacteria count was lower in tanks cultured with GIFT tilapia followed by grouper and milkfish. Milkfish shows promising inhibitory activity against luminous bacteria in in vitro study but with shrimp rearing system there was little antibacterial effect.

Further, a pond-based study suggested polyculture of shrimp with fish; *Liza parsia* and *Mystus gulio* for reduction of vibriosis (Abraham et al., 2014). Water and sediment samples from polyculture ponds recorded comparatively low luminescent bacterial count than in monoculture ponds. Snapper *Lutjanus argentimaculatus*, seabass *Lates calcarifer*, and siganid *Siganus guttatus* also show promising luminous bacterial activity in shrimp culture water. In vitro antibacterial assays by Tendencia et al. in 2006 showed that bacteria isolated from the rearing water of siganid, snapper, and sea bass have inhibitory effect against luminous bacteria. Similarly, Lio-Po et al. in 2005 reported that skin mucus of siganid could significantly reduce luminous bacteria count after three hours. These studies clearly suggest that finfish integrated shrimp culture system is effective in control of luminous bacteria in water; however, fish biomass to be used for efficient control of bacteria has to be standardized. Scaling up to pond level study could have better implications in determining the possible involvement of environmental factors in *Vibrio* proliferation.

Integration of bivalves with shrimp: Potential for controlling luminous *Vibrios*

Bivalves are efficient filter feeder of organic material that increases its efficacy in maintaining good water quality and healthy culture system. Several researchers have highlighted the effectiveness of oysters in the reduction of suspended organic and inorganic matter and a total number of bacteria in pond culture system. In a small scale study, effluent water from shrimp pond was treated through three stage system; sedimentation in the first phase, filtration by Sydney rock oyster; *Saccostrea commercialis* in the second stage and dissolved organic nutrient absorption by macroalgae; *Gracilaria edulis* in the final stage. In addition to reduced particulate load bacteria concentration was also reduced from 19×10^{10} L^{-1} to 6×10^{10} L^{-1} in oyster treatment. Further in a different setup, the presence of oyster also affects phytoplankton species composition and sedimentation (Jones et al., 2001). *Nitzschia straiatum* was dominated by beneficial microalgae *Skeletonema costatum* in the culture system (Lio-Po et al., 2005).

Chemical compounds; peptides, terpenes, polypropionate, polypeptides, fatty acid derivatives, sterol identified and characterized from hemolymph of several molluscan species (oysters, sea slug, and mussel) possess broad spectrum antibacterial activities. Anti *Bacillus megaterium* activity has been detected from plasma samples of *Crassostrea virginica* (Eastern oyster) and *Mytilus edulis* (Blue mussel). 40% and 80 % Solid phase extraction (SPE) fractions from the acid extract of *Cerastoderma edule, Ruditapes philippinarum*, and *Credidula furnicata* have shown inhibitory activity against *Vibrio alginolyticus*, and fractions of *Cerastoderma edule* have shown a significant broad range of antibacterial activity against marine aquaculture pathogen (Defer et al., 2009).

A study on polyculture of the green mussel, brown mussel, and oyster with shrimp in culture tanks resulted in the reduction of luminous bacteria (Tendencia, 2007). Shrimp survival was highest when cultured with the green mussel culture tank (84%), followed by brown mussel (72 %) and oyster (71%). Luminous bacteria count was reduced to 10^1 CFU/mL within five days in green mussel culture tank while 16 days in brown mussel and 17 days in oyster culture tank. Nevertheless, an in vitro study on ethanol, methanol, hexane, butane, and acetone extracts of Green mussel; *Perna viridis* and edible oyster; *Crassostrea madrasensis* exhibited very limited activity against *Vibrio* sp. (Annamalai et al., 2007).

Marine mollusk can produce anti-microbial peptides (AMPs) as part of their innate immune system. AMPs have been isolated and extensively studied in *Mytilus edulis, M. galloprovincialis, Crassostrea virginica, Crassostrea gigas*, and abalone, *Haliotis discus.* AMPs have potent activity against bacteria, fungus, and virus, which leads this promising study for the treatment of bacterial disease. For example, Mytilin B, a synthetic antibacterial peptide from mussel *Mytilus galloprovincialis* and

Myticusin-1, an 11 K Da anti- microbial peptide from *M. coruscus* hemolymph have exhibited remarkable antibacterial properties against gram positive and gram negative bacteria (Liao et al., 2013). These findings clearly reveal the possible involvement of AMPs in the host immune response of mollusk against bacterial infection.

Integration of seaweeds with shrimp: Potential for controlling luminous *Vibrio*

Seaweeds have their exclusive microbial community on the thallus surface, which helps in inhibiting the growth of pathogenic bacteria (Moore et al., 2002). Seaweeds are reported to have a broad range of biological activities such as antibiotics, antiviral, anti-tumor, and anti-inflammatory. Since active compounds from seaweed have been documented to have antibacterial and immunostimulant activity, many studies have been conducted for prevention and treatment of vibriosis in shrimp culture system. Chemical compounds like fatty acids (Rosell and Srivastava, 1987), phenols (Thirunavukkarasu et al., 2014) have been identified as inhibitory compounds for bacteria pathogen.

Ethanol extract of *Gracilaria fisheri* has shown increased immunostimulant and antimicrobial activity against *Vibrio harveyi* infected *P. monodon* juveniles. Administration of *Gracilaria fisheri* extract at a minimal inhibitory concentration *range* of 90–190 μg/mL stimulated cellular and humoral defense parameters associated with increased total hemocyte count, granulocyte count, polyphenol oxidase, SOD activities, and superoxide anion production (Kanjana et al., 2011). Similarly, *P. monodon* postlarvae fed with the metabolites of red seaweed *Hypnea musciformis* positively affected immune factors and showed higher survival against *Vibrio alginolyticus* infection (Jean Jose et al., 2008). Hot water extract of *Gelidium amansii* (Fu et al., 2007) and *Gracilaria tenuistipitata* (Hou and Chen, 2005; Yeh and Chen, 2009) increased total hemocyte counts, respiratory burst activity, polyphenol oxidase activity of *Litopenaeus vannamei* and showed improved resistance when challenged against *V. alginolyticus*. The bioactive compound of *Sargassum wightii* exhibited potential anti-bacterial activity against shrimp *Vibrio* pathogen out of which maximum zone of inhibition (32 mm) was exhibited by methanol extract against *V. parahaemolyticus*. Acetone extract of *S. wightii* produced a maximum zone of inhibition (26 mm) against *Vibrio anguillarum*, and ethyl acetate extract showed inhibition of 24.66 mm against *V. harveyi*. The results clearly indicated that seaweed *S. wightii* is an interesting source for biologically active compounds that may be utilized for prophylaxis and therapy of bacterial fish diseases (Thirunavukkarasu et al., 2014).

Seaweeds have been considered to be an environmentally clean way of converting inorganic nutrients as effective biofilter and also a rich source of bioactive compound characterized by a broad spectrum of biological activities. Many aspects of shrimp-seaweed integrated system have been delineated regarding nutrient up taking efficacy of seaweed and growth performance of shrimp and seaweed, however, the impact of live seaweeds in an integrated system on the microbiological property of water is not very well documented. Pang et al. in 2006 documented changes in total bacteria and *Vibrio* count in an integrated recirculatory seaweed abalone culture system experiment. Instead of monoculture experiment

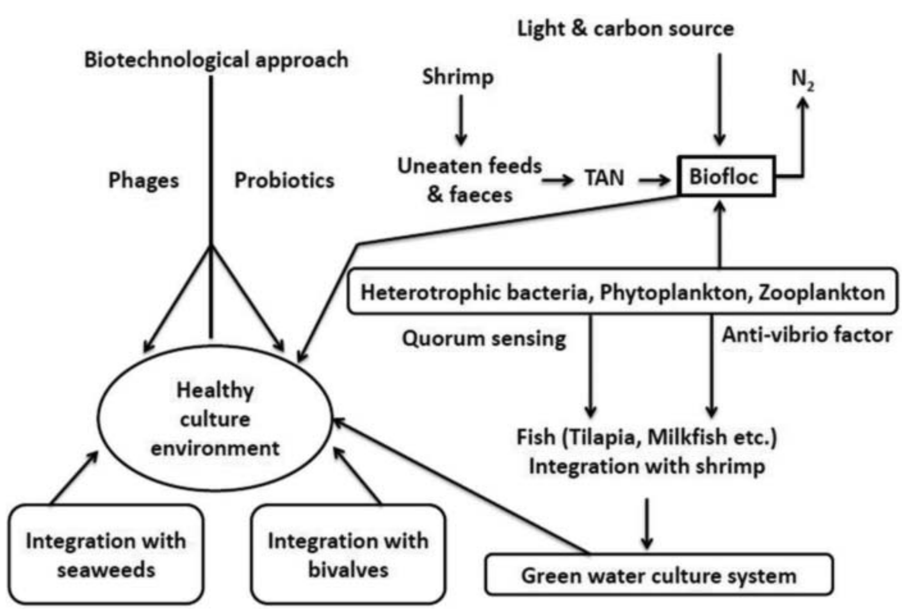

Figure 2. Schematic of integrated and biotechnological approaches to sustain healthy shrimp culture environment.

of abalone, polyculture with *Gracillaria textorii* showed a lower level of total *Vibrio* count and higher level of total bacteria count. Further, a change in *Vibrio* community had also been observed in polyculture experiment. Other seaweed species such as *Grateloupia filicina, Ulva lactuta, Chondrus crispus* have been tried but did not show any significant result in the study. Similarly, *Gracillaria birdie* has been reported to maintain lower *Vibrio* density in addition to lower NO_3-N, an increase in crude protein content and growth in *L. vannamei* when cultured in an integrated bio-floc system. The presence of this seaweed @ 2.5 and 5 kg wet weight/m^3 in shrimp bio-floc system reduced *Vibrio* density by 54–83% (Brito et al., 2014). Further, these few studies indicate live seaweeds integration may acquire the sustainability of the shrimp culture system by controlling the composition of microbial community. Further study needs to be addressed in the selection of suitable seaweed species to control vibriosis in shrimp ponds.

Conclusion and future perspectives

Polyculture and integrated multitrophic aquaculture are gaining popularity in brackish water aquaculture because of economic viability, environmental sustainability as well as its potential in maintaining healthy culture environment. Promising results have been obtained for monitoring luminous vibriosis in shrimp culture operations through polyculture trials with fin fish, specifically with tilapia. Though other fin fishes, bivalves, seaweed have also tended to reduce luminous *Vibrio* load, most of the studies are limited to tank culture system. Integration trials of these hydrobionts in pond culture system should be prioritized to lower luminous *Vibrio* load and to improve pond environmental condition (Figure 2). Ability to produce an inhibitory material does not necessarily mean that it plays an ecological role (Sieburth, 1968). Manipulation of pond environment which favors beneficial micro-organisms proliferation is very much necessary to sustain a healthy culture system. Green water culture technique was reported to prevent disease outbreaks attributed to luminescent *Vibrio*, however, as far we know, to date, to protect cultured animals against luminous vibriosis, it is not entirely satisfactory. There has been ample of evidence that bio-floc system provides protection to cultured shrimp against pathogenic bacteria. Further, technology refinement for maintenance of a novel group of heterotrophic bacteria community in the bio-floc system by addition of probiotics would be necessary to make a disease free culture system. Preparation of anti-infective peptide from bacteriophage and its use in synthetic form also give future research direction in therapeutics to control vibriosis.

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