Implications of Quorum Sensing and Quorum Quenching in Aquaculture Health Management

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Abstract

The world human population is growing on an exponential phase and pace. Aquaculture, raising of aquatic animals in artificial or facilitated ecosystem, is evolving as the rapidly growing food production sector globally. The growth of aquaculture industry has been speculated to be inevitable that may certainly contribute toward meeting the food security of growing global population. India, with a vast coastline and enormous marine resources, is having greater potential to build up this industry as a productive economic sector. However, the bacterial infections in aquaculture hatcheries and farms cause a huge loss in productivity and remain a major challenge for the growth of this vital industry. Considering the ill effects to environment and public health, risk of development of antibiotic resistance, and persistence of antibiotic residues in aquaculture animal foods, it has necessitated the regulatory bodies across the globe to restrict the usage of antibiotics for aquaculture disease management. Hence, finding alternate measures for the aquaculture disease management in both hatcheries and forms is the current need. It has been well documented that exhibition of virulence factors and formation of biofilms are the major factors for the establishment of disease in aquaculture animals by the bacterial pathogens. Both these factors are being regulated by quorum sensing (QS), which is a population density-dependent expression of selected phenotypes in a coordinated manner through

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the production of autoinducers (AI). Quorum quenching (QQ) is a disruption of quorum sensing. Thus, QQ is considered as one of the most preferred preventive strategies for the ecofriendly management of aquaculture infections. The AI molecules involved in gram-positive and gram-negative QS system and also the enzymes and molecules involved in QQ are also widely studied in aquaculture systems. This chapter would provide an overview of QS and QQ systems being operated among aquaculture pathogens and other beneficial organisms in the aquaculture system with more emphasis on shrimp aquaculture. This chapter also emphasizes the recent developments on the impact of QS and QQ with special reference to the virulence of bacterial pathogens both in vivo and in vitro with a short focus on future perspectives of QQ and QS for the disease management in aquaculture systems.

Keywords

Aquaculture systems · Quorum sensing (QS) · Quorum quenching (QQ) · Marine bacteria · Disease management

18.1 Introduction

Aquaculture is one of the major emerging industries in the past few decades. The global population is growing on an uncontrollable speed and technologies are to be focused on the food security. Thus, aquaculture has emerged to complement the supply effect of fisheries sector. During 2018, it was estimated that all around the world, close to 800 million people are depending either directly or indirectly to the aquaculture and wild fisheries sector. Though the aquaculture sector is growing at an average growth rate of 6% per year, overfishing activities necessitate the increased production through aquaculture. A plethora of studies approximated that the aquaculture production needs to be increased by 20–30% to compensate the declining natural fishery wealth in our seas (Gupta 2018). Many review works emphasized to enlighten the unquestionable need for aquaculture with respect to food security. It was envisaged that rapid growth in human population along with prosperity in the economic status, nonetheless the need for seafood, especially fish foods, has increased manyfold in Africa. Few modeling studies have concluded that only the aquaculture sector could help in meeting the food security in Africa. Furthermore, it is also envisaged that more indigenous aquaculture techniques would reduce the reliance of imports (Chan et al. 2019). In low- and medium-income countries, fin fishes and their products contribute to food security and nutritional requirements. Aquaculture sector, in these countries, contributes not only for food security but also for the economic uplift of low-income people (Little and Bunting 2016). During 2016, Béné et al. (2016) have considered 202 research and review articles published during 2013–2014. They have concluded that the growth of aquaculture and sustained fishery activities would help in managing the food security issues. These developments could also improve the nutritional requirements and development of economy in the developing and upcoming nations. Crustaceans such as crabs and shrimps could also contribute for the nutrition and food security. Bondad-Reantaso et al. (2012) have also emphasized that aquaculture of shrimps, prawns, crabs, and mussels would be helpful in complementing the protein nutrition. Hence, their growth is very vital for the developing nations.

Humans have used fish and other aquatic animals as foods over many millennia. Though oceanic environment covers 70% of the surface of Earth, the overexploitation through wild catch fisheries may not offer viable resource to meet the global food security. Hence, it has necessitated the emergence of aquaculture (Ahmed and Thompson 2019; Stevens et al. 2018). Aquaculture has been done in both freshwater and marine ecosystems. In both the ecosystems, the technologies are almost same. They grow brood stocks of chosen animal, facilitate hatching of eggs, nurture the seedlings, acclimatize the juveniles into artificial ponds, and finally grow them until harvest. Technologies related to brood stock maintenance, hatchery techniques, feed formulation, pond management, etc. are found to be very crucial in getting aspired success in aquaculture. Indeed, these crucial technologies are also in one way named as challenges.

18.2 Bacterial Pathogens in the Aquaculture Systems

Though the viral diseases contribute to the huge economic losses in aquaculture, there has been greater emphasis on viral diseases. However, less emphasis is being paid toward bacterial diseases of aquaculture animals. They are emerging as most serious microbial communities which affect the health and prosperity of aquaculture sector worldwide. Major diseases caused by various bacteria in aquaculture animals are summarized in Table 18.1 (Haenen 2017). Relatively, bacterial diseases of shrimps have attracted less attention in comparison with viral infections. However, the losses due to bacterial diseases are severe in terms of both capital and quality. The diseases can affect the shrimps both at hatchery and aquaculture farms. Among them, the diseases of shrimps at hatchery are more serious and cause huge economic losses, and at worse situations, they may entirely halt the industry. Luminescent bacteria, especially *Vibrio* spp., cause extensive damage in shrimp hatcheries and farms across the world. Vibrios are the proven sources of disease outbreaks in cultured tiger shrimp (Sung et al. 2001). However, strategies for the management of bacterial diseases are to be explored more extensively and comprehensively. People have tried to breed penaeid shrimps for disease resistance which has been reviewed by Cock et al. (2009). However, it is observable that bacterial diseases of aquaculture animals have been paid less attention.

Table 18.1 List of major bacterial diseases of aquaculture animals (summarized from Haenen 2017)

Sl. no.	Bacterial disease	Causative agent
1	Vibriosis	1. *Vibrio* spp. including *V. anguillarum, V. harveyi clade, V. parahaemolyticus, Aliivibrio salmonicida (V. salmonicida), V. vulnificus*
		2. *Photobacterium damselae*
2	Aeromonasis	*Aeromonas* spp. including *Aeromonas caviae, A. hydrophila, A. sobria, A. veronii, A. jandaei, A. salmonicida*
3	Edwardsiellosis	*Edwardsiella anguillarum, E. ictaluri, E. piscicida, E. tarda, Yersinia ruckeri*
4	Pseudomonasis	*Pseudomonas anguilliseptica, P. fluorescens*
5	Flavobacteriosis	*Flavobacterium branchiophilum, F. columnare, F. psychrophilum, Tenacibaculum maritinum*
6	Mycobacteriosis	Caused by *Mycobacterium* spp. and other filamentous actinobacteria:
		1. *Mycobacterium fortuitum, M. marinum*
		2. *Nocardia asteroides, N. crassostreae (ostreae), N. seriolae*
7	Streptococcosis	*Streptococcus agalactiae, S. iniae, Lactococcus garvieae, Aerococcus viridans*
8	Other bacterial diseases	1. *Renibacterium salmoninarum*
		2. Anaerobic bacteria such as *Clostridium botulinum, Enterobacterium catenabacterium*
		3. Intracellular infections by *Piscirickettsia salmonis, Hepatobacter penaei, Francisella noatunensis, Chlamydia* spp.

18.3 Various Disease Control Strategies in Practice

Both prevention and control strategies are being followed in aquaculture disease management practices. Aquaculture is a very rapidly developing food industry all over the world. It is one of the most needed industrial development for managing the food security issues in the developing countries. It is expected to meet the demands of overexploding human population in both Asia and Africa. India, with a vast coastline and enormous marine resources, is having greater potential to build up this industry as a productive economic sector. Shrimp culture is the most voluminous and important sector in the aquaculture industry of our country. Like agriculture, diseases caused by many organisms are the main challenge for shrimp aquaculture industry. Recently, analyses have shown that the economic losses because of infectious diseases in the shrimp aquaculture alone would come around three billion US dollars (Lundin 1996; Karunasagar et al. 2004). Assefa and Abunna (2018) emphasized that not a single disease control or disease prevention strategy could provide complete disease management. These researchers depicted to adapt integrated disease management practices by combining different strategies that would be more productive. Figure 18.1 summarizes the various strategies being followed for the management of aquaculture diseases. Each of the disease management strategies has its own merits and demerits. Though these methods are following two strategies,

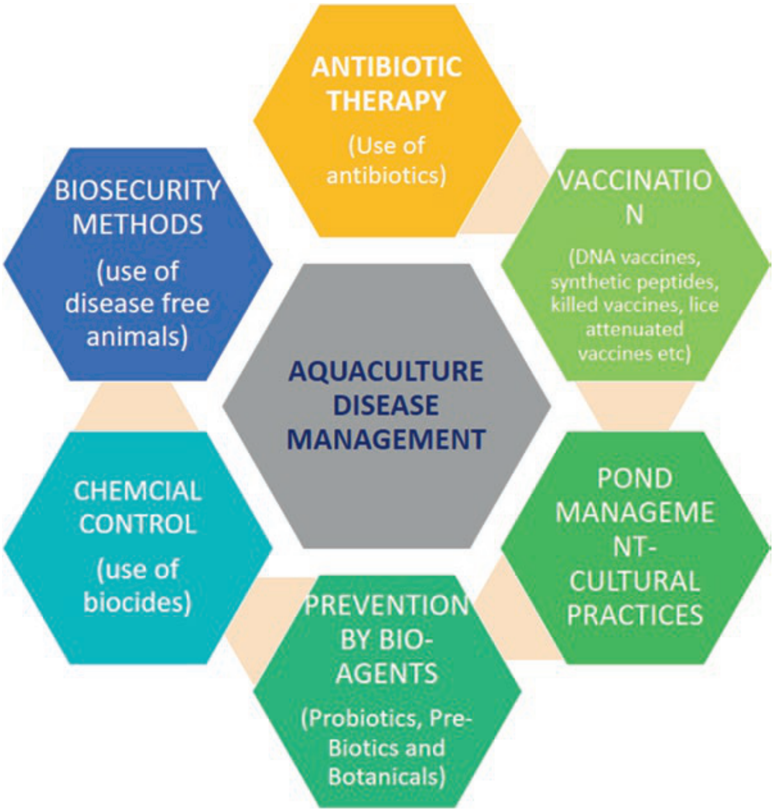

Fig. 18.1 Summary of aquaculture disease management practices

i.e., prevention and control, the preventive strategies are most preferred due to avoidance of risk factors. Table 18.2 also summarizes various aspects of these disease management practices in aquaculture.

Among various disease control strategies, use of antibiotics has been studied quite extensively since it has been widely used by aquaculture farmers. Roque et al. (2001) have demonstrated the control of vibriosis in penaeid shrimps by using 15 antibiotics with different levels of microbial susceptibility in Mexico. Heuer et al. (2009) extensively reviewed about environmental and human health hazards, resulting in response to overuse of antibiotics in aquaculture. There is increasing interest in using probiotics for the control of shrimp disease in both farm and hatcheries (Gatesoupe 1999; Jayaprakashvel et al. 2014). Additionally, Das et al. (2010) have isolated and characterized three *Streptomyces* spp. from oceanic environment for their effective use as aquaculture probiotic organisms. They have also demonstrated satisfactory control in the population strength of vibrios and reduced the disease severity. Though probiotics are very good preventive agents, their curative effect is very poor (Wang et al. 2008). In this scenario, research may be focused to develop a novel disease control strategy by utilizing bacterial intercellular communication systems.

Table 18.2 Summary of disease management practices in aquaculture hatcheries and farms

Sl. no.	Strategy	Advantage	Disadvantage
1	Antibiotic therapy: use of antibiotics	1. It is a control strategy 2. It could produce disease control in shorter period 3. It can provide curative effect as well 4. It could save the harvest which is already infected	1. Leads to development of antibiotic resistance in aquaculture pathogens 2. Leads to pose risks of resistance spread through other organisms 3. The use of antibiotics is restricted to save environment and public health
2	Vaccination: use of immune boosting methods	1. Use of: i. Heat-killed vaccine ii. Live attenuated vaccines iii. DNA vaccines iv. Recombinant vaccines v. Synthetic peptide vaccines 2. It is a preventive strategy	It cannot provide curative effect The immune system of aquaculture animals is comparatively poorly developed
3	Pond management and cultural practices	(a) Preventive strategy (b) Less expensive (c) Uses improved husbandry/management practices, movement restrictions, genetically resistant stock, dietary supplements	(a) Time consuming (b) Labour intensive (c) Cannot provide curative effect
4	Prevention by bioagents	(a) It can provide effective prevention (b) Uses probiotics, prebiotics, and medicinal plants (c) Safe to environment	(a) Cannot provide curative effect (b) Success rate is subject to environmental conditions (c) Strain variations are there which question the uniform efficacy
5	Chemical control	(a) It uses chemical with biocide potential (b) Immediate curative effect (c) Much effective and less cost intensive	(a) Spoil environment (b) Usage is banned/highly restricted (c) Cannot be preventive (d) Residue effects in the animals leads to long-term health effects in consumers
6	Biosecurity methods	(a) Biosecurity measures include stringent animal quarantine protocols, disinfection of eggs, water sanitation, appropriate feed, and destroying dead animals (b) Much effective	(a) Needs much technical expertise (b) High cost involved (c) Curative effect is not possible (d) Labour intensive

18.4 QS and QQ as Inevitable Attributes for an Alternative Disease Control Strategy

Antibiotic agents are the common choice for the management of bacterial infections in aquaculture hatcheries and farms. These agents were applied as feed fortified with antibiotics and also by adopting the immersion therapy (adding directly to pond water) (Rodgers and Furones 2009). However, frequent and intense use of antibiotics in aquaculture hatcheries and farms develops a selective pressure on the aquaculture animals. It also adversely affects the environment, creating reservoirs of drug-resistant bacteria and transferable resistance genes in pathogens and other bacteria in aquatic environment. From these reservoirs, resistant genes may disseminate by horizontal gene transfer and reach human pathogens or drug-resistant pathogens from aquatic environment which ultimately would be biomagnified and affect public health (Heuer et al. 2009). So, alternative strategies, which will not induce resistance in pathogens, are desirable (Defoirdt et al. 2007) in the current scenario on controlling shrimp diseases. Inhibition of quorum sensing (QS) has been proposed as an alternative strategy for the management of bacterial infections. Several approaches were being proposed to negatively affect the bacterial communication to stop their quorum sensing system (Jayaprakashvel and Shanmugaiah 2015). Researchers in other parts of the world have speculated and demonstrated the effectives of interfering QS in aquaculture (Defoirdt et al. 2007). However, comprehensive and conclusive studies on the inhibition or modification of bacterial QS system still hold much promise.

Most of the pathogens regulate their virulence factors such as biofilm formation, peptide synthesis, and production of certain enzymes through a population density-dependent intercellular bacterial communication system called QS (de Kievit and Iglewski 2000). When these QS systems are disrupted, the bacterial pathogens lose their disease-causing potential (Defoirdt et al. 2006, 2010). Autoinducer (AI) molecules are produced as signaling molecules to sense the bacterial populations in the vicinity by the QS organisms. However, the organisms that combat the QS are reported to produce quite a few QSI enzymes and higher organisms are proved to disrupt this QS phenomenon in many systems (Teplitski et al. 2000; Bauer and Robinson 2002; Bauer and Mathesius 2004). Many researchers elsewhere in the world (Manefield et al. 2000; Defoirdt et al. 2006; You et al. 2007) have studied this novel and impressive way of disease control in aquaculture. Nonetheless, this promising strategy has not been comprehensively attempted in India. So, it is much anticipated that one could get some potent novel QS inhibitors from our marine resources like marine microbes and other organisms from the vast coastal and marine resources of India. The information about the different bacterial pathogens associated with the shrimp diseases is not yet conclusive. So, it is very essential to isolate pathogenic bacteria associated with shrimp larvae in the hatchery. This would provide information about the dominance of bacterial pathogens in shrimp hatcheries and the same would help to form unique strategies for their management.

18.5 Shrimp Diseases and QS of Aquaculture Bacterial Pathogens

Until very recently, it was strongly believed that bacteria were existing as monocellular forms only to find their nutrients from the environment and also to reproduce by binary fission. During the 1970s, scientists have discovered a cell-to-cell communication mediated by signaling molecules among bacteria. This has prompted scientists across the globe that bacteria could exist as microbial communities to evade the harsh environmental conditions or to exhibit their virulence factors in a coordinated manner. The discovery of intercellular communication among bacteria has led to a realization that bacteria are capable of exhibiting coordinated activities such as biofilm formation, luminescence, virulence factor production, nodule formation, etc. The phenomenon of quorum sensing or autoinducer-mediated intercellular bacterial communication has been found to exist among many environmental bacteria, especially in the aquatic environments. The concept of quorum sensing strongly depends on the principle that at low cell densities, the QS operating bacterium produces lesser quantity of the AIs which can be hardly detected by bacteria of the same nature. Similarly, when the bacterial community reaches a threshold population, their production of AIs increases considerably so that all the similar bacteria in the vicinity effectively sense the levels of AIs in turn the direct proportionate population of the producing bacteria. This allows the bacteria to sense a threshold cell mass, and in response, to activate or repress set of genes involved in the exhibition of certain phenotypes, for example, biofilm formation (Taga and Bassler 2003). Interestingly, acylated homoserine lactones (AHLs) and AI peptides are the most studied signaling molecules of gram-negative and gram-positive bacteria, respectively (Taga and Bassler 2003). Most of the bacteria so far identified as QS system operators are found to have some synergistic or pathogenic association with higher-order organisms such as plants and animals (de Kievit and Iglewski 2000; You et al. 2006). The genes identified in relation to QS system are found very critical for their virulence, biofilm formation, and colonization of eukaryotic hosts (Bauer and Robinson 2002).

It has been evidently understood that the QS system in pathogenic bacteria regulates the synthesis of virulence factors. Hence, it is now proposed and successfully demonstrated in many host systems that if we could alter or inactivate AIs and suppress QS signal generation, we could modify the gene response in pathogens to operate virulence factors and thus could avoid the pathogen to express its virulence mechanism. Thus, it could be useful in controlling infection development and persistence of human, animal, and plant bacterial pathogens (Mäe et al. 2001; Fray 2002; Hentzer et al. 2002; Zhang 2003; Ozer et al. 2005).

Hence, researchers targeted QS systems of pathogens as a novel strategy to combat the infectious diseases in aquaculture animals (Defoirdt et al. 2004) such as *Macrobrachium rosenbergii* (Baruah et al. 2009); Artemia (van Cam et al. 2009), rotifer *Brachionus plicatilis* (Tinh et al. 2007), and on first-feeding turbot larvae

Scophthalmus maximus L. (Tinh et al. 2008). In this scenario, it can be concluded that the disruption of QS has been considered as a novel disease control strategy in aquaculture in which the international researchers are progressing steadily. However, this novel disease control strategy is yet to be studied more comprehensively with special reference to Indian aquaculture.

In India, the profiling of shrimp pathogens at hatchery and farms has been carried out to some extent. But our literature survey has suggested that there has not been much work on QS of aquaculture pathogens. Moreover, the use of QS inhibitors as disease control molecules in shrimp aquaculture has been a relatively unexplored strategy in India. Nayak et al. (2010) identified unregulated immune-related genes in *P. monodon* postlarvae which are produced against artificial infection of *V. harveyi* (Bramhachari and Dubey 2006). Remarkably, Sharma et al. (2010) evaluated the immune response and resistance to diseases in tiger shrimp, *P. monodon.* They have initially fed the animals with biofilm forming *V. alginolyticus* and concluded that the immunization of animals resulted in the quick reduction of *V. alginolyticus* and WSSV from the internal systems of the shrimps and thus had provided effective resistance by shrimp juveniles against viral and bacterial pathogens. Probiotics for aquaculture are studied extensively in India. Very recently, Soundarapandian and Babu (2010) studied the effect of probiotics on the hatchery seed production of black tiger shrimp, *Penaeus monodon* (Fabricius). Selvin and Lipton (2004) have worked on *Dendrilla nigra*, a marine sponge, and demonstrated its effective usage as a resource of antibacterial substances for managing shrimp diseases.

Vaseeharan et al. (2007) isolated a novel *Photobacterium damselae* ssp. *damselae* strain from the infected *P. monodon*, a tiger shrimp, in India. Immanuel et al. (2004) have isolated solvent extracts (butanol extracts) from some of the land-based herbal plants and seaweeds. They have demonstrated the effect of those extracts on the survival, growth, and pathogen (*V. parahaemolyticus*) load on shrimp *Penaeus indicus* at larval stages. Chrisolite et al. (2008) have found the presence of bioluminescent *V. harveyi* from the operational shrimp hatcheries in the southern part of India. They also have isolated and established the inhibitory effect of bacteriophages against shrimp pathogenic bacteria. Gopala et al. (2005) have studied the presence of pathogenic vibrios from the shrimp industry and demonstrated their ill effects in the aquaculture environments. It is noteworthy that Karunasagar et al. (2007) worked extensively on biocontrol of pathogens in shrimp hatcheries using bacteriophage which is one of the recent disease control strategies. In almost all types of shrimp hatcheries, *Vibrio* infections are found in India including the semi-intensive penaeid shrimp hatcheries of Tamil Nadu (Abraham and Palaniappan 2004). Tyagi et al. (2007) envisaged the transgene technology to control the vibriosis of shrimps. They have developed a lysozyme to be expressed from *P. monodon* through recombinant DNA technology. These recombinant lysozymes have been reported to have antibiotic potential against vibrios in shrimp.

18.6 Quorum Quenching in Aquaculture Pathogens as a New Disease Management Strategy

Aquaculture and fisheries are considered to be the best approaches for the sustenance of global food security in the population explosion era. Aquaculture, especially shrimp aquaculture, has been followed intensively owing to technical advancements. However, the bacterial and viral diseases of shrimps are found to be the chief restraining factors of shrimp aquaculture. Among the various diseases caused by pathogens, bacterial diseases of shrimps at their larval stages in hatcheries are reported to be very tough to manage. Several species of luminescent *Vibrio* spp. and quite a few *Bacillus* spp. are found associated with devastating bacterial infections in shrimp larvae (Vaseeharan et al. 2007; Sung et al. 2001). Nonetheless, antibiotics could offer better control against bacterial infections. However, due to increasing awareness against chemical usage, regulations against antibiotics, emergence of antimicrobial-resistant bacteria in fish and other aquatic animals, and fear over antibiotic residues in animals necessitate developing novel disease management technologies for the effective management of shrimp production through aquaculture.

It has been demonstrated that the major bacterial pathogens of shrimps were found to operate the quorum sensing system to exhibit their virulence factors and pathogenicity. They were found to operate quorum sensing for the establishment of biofilms. Hence, it could be understood that quorum sensing could be the potential target to inhibit the disease-causing ability of bacterial pathogens in shrimp (Zhao et al. 2015). Moreover, QS is a classical gene regulation system of many pathogens in humans, plants, and animals. Disruption of QS molecules in the pathogens by antiquorum sensing metabolites such as lactonoses, halogenated furanones, etc. are considered as a novel disease control strategy in many ecosystems. Moreover, in a more controlled environments such as shrimp hatcheries, the disruption of QS could be a reliable and ecofriendly disease management strategy. The disruption of QS by AI inhibitors completely blocks the QS system in bacterial pathogens. They not only prevent the expression of virulence-associated genes but also could attenuate the disease-causing ability of shrimp pathogenic bacteria. Hence, inhibition of QS has been proposed as a new anti-infective strategy. Several techniques that could be used to disrupt QS were investigated with reference to shrimp aquaculture in other parts of the world (Defoirdt et al. 2007). However, not much work has been done on this novel disease control strategy in India. Scores of currently studied QS inhibitors are from marine resources. Actinobacteria, especially marine actinobacteria, are prolific producers of effective antibiotics. Nearly 70% of recently discovered antibiotics are from actinobacteria. The marine actinobacteria are considered as a sustainable and effective bioresources for the search source of novel bioactive metabolites (Subramani and Aalbersberg 2012). Since the marine actinobacteria are relatively less explored microbial community, chances are plenty to obtain potent antimicrobial metabolites that could disrupt the QS system and attenuate the virulence factors of shrimp pathogens.

18.7 Future Perspectives

The aquaculture sector is the fastest growing agriculture sector which is directly related to future food security. The heavy use of antibiotics in this sector for control of bacterial infections has been proven to have environmental and human health hazards. Recent advancement in scientific research has paved the way to use alternative strategies for disease management in aquaculture. The bacterial infections in aquaculture can be controlled with QS inhibitors, a relatively effective yet a novel disease control strategy. This alternative, new strategy would pave way for the use of environment-friendly disease control strategy in aquaculture sector with no direct or indirect negative impacts on human health and environment.

18.8 Conclusion

Based on the fact that nearly the virulence factors of bacterial pathogens in aquaculture are being controlled by QS, the microbial metabolites with the ability to block the QS can be used to prevent bacterial infection in aquaculture.

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