The use of probiotics to control prawn diseases: administration methods, antagonistic effects and immune response

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Abstract

The giant freshwater prawn (*Macrobrachium rosenbergii*) is a high-yielding prawn variety well-received worldwide due to its ability to adapt to freshwater culture systems. *M. rosenbergii* is an alternative to shrimp typically obtained from marine and brackish aquaculture systems. However, the use of intensive culture systems can lead to disease outbreaks, particularly in larval and post-larval stages, caused by pathogenic agents such as viruses, bacteria, fungi, yeasts, and protozoans. White tail disease (viral), white spot syndrome (viral), and bacterial necrosis are examples of economically significant diseases. Given the increasing antibiotic resistance of disease-causing microorganisms, probiotics have emerged as promising alternatives for disease control. Probiotics are live active microbes that are introduced into a target host in an adequate number or dose to promote its health. In the present paper, we first discuss the diseases that occur in *M. rosenbergii* production, followed by an in-depth discussion on probiotics. We elaborate on the common methods of probiotics administration and explain the beneficial health effects of probiotics as immunity enhancers. Moreover, we discuss the antagonistic effects of probiotics on pathogenic microorganisms. Altogether, this paper provides a comprehensive overview of disease control in *M. rosenbergii* aquaculture through the use of probiotics, which could enhance the sustainability of prawn culture.

Keywords: *Macrobrachium rosenbergii*; innate immunity; disease management; prawn; microorganisms; probiotic administration; host-derived probiotics

1 Introduction

The global seafood market was valued at US$ 113 billion in 2020 and is projected to grow at an annual rate of 2.9%, reaching US$ 139 billion by 2027 (Research and Markets, 2022). Shrimp and prawn are commonly consumed, around 20% of the global market (Research and Markets, 2021). *Penaeus vannamei* (Pacific white shrimp or King prawn), *Penaeus monodon* (giant tiger shrimp), and *Macrobrachium rosenbergii* (giant freshwater prawn) are the most widely cultured species (Stankus, 2021).

Macrobrachium rosenbergii is a freshwater decapod crustacean belonging to the *Palaemonidae* family. It is cultured as a freshwater prawn offering an alternative to shrimp grown in brackish and marine aquaculture systems. Major countries producing prawn include India, China, Thailand, Bangladesh, and Malaysia (Kader et al., 2021). *M. rosenbergii's* aquaculture has attracted significant attention due to its high production yield, disease resistance, and ease of management in controlled freshwater systems such as rivers, lakes, canals, reservoirs, and ponds. Nevertheless, the intensification of culture practices driven by the increasing demand for prawn has also increased the susceptibility of *M. rosenbergii* to diseases, resulting in a substantial decline in the production (Chen-Fei, Chou-Min, & Jiun-Yan, 2020; Lee et al., 2022; Pillai & Bonami, 2012b). Diseases affecting the larval and post-larval stages of *M. rosenbergii* are primarily caused by viruses, bacteria, fungi, yeasts, and protists. Some of these diseases are exclusive to the giant freshwater prawn, such as *Macrobrachium* hepatopancreatic parvovirus (HPV) disease, rickettsia-like disease, white tail disease (WTD), as well as idiopathic diseases including idiopathic muscle necrosis, balloon disease, and appendage deformity syndrome (Pillai & Bonami, 2012b). White tail disease (WTD) caused by *M. rosenbergii* nodavirus (MrNV) is known to have a mortality rate of 100% (Sahul Hameed & Bonami, 2012).

Typical methods for disease control in aquaculture include implementing rigorous biosecurity protocols, adopting appropriate husbandry practices, administering antibiotics, vaccination, and using immunostimulants (Chen-Fei et al., 2020). Vaccination is not an effective method for invertebrates like prawns because they do not have adaptive immunity (Rowley & Pope, 2012). The rapid growth in demand for aquaculture products has decreased the efficiency of disease control measures and led to a substantial increase in antibiotics use (Henriksson et al., 2018). While antibiotics yield to quick recovery from diseases, their effects on the ecosystem and the emergence of antibiotic-resistant disease-causing agents have led to the investigation of alternative disease control remedies (Henriksson et al., 2018; Zorriehzahra et al., 2016). One of these approaches is the application of probiotics.

The aim of this review was to provide an in-depth analysis of disease etiology in giant freshwater prawn and explore the potential of probiotics as a sustainable substitute for disease control. We highlight various features that contribute to susceptibility of *M. rosenbergii* to diseases, including the rapid growth of aquaculture and the consequent increase in antibiotic use. Following this, we discuss the concept of probiotics and their significance in aquaculture, with a particular focus on their potential benefits for giant freshwater prawn.

2 Diseases affecting *M. rosenbergii* in aquaculture systems

Freshwater prawn diseases are influenced by environmental, nutritional, and physiological factors. These diseases can be caused by pathogenic or parasitic agents (Lane, Brosnahan, & Poulin, 2022). Pathogens, including viruses, bacteria, fungi, yeasts, and protists, are responsible for disease incidences with a significant impact on the economic viability of freshwater prawn production. Viral infections, in particular, are a major concern due to their high mortality rates. Viruses that affect freshwater prawns include Baculoviridae and Nimaviridae with dsDNA, Parvoviridae with ssDNA, Reoviridae with dsRNA, and Nodaviridae with +ssRNA (Pillai & Bonami, 2012b).

Several studies have investigated the diseases affecting *M. rosenbergii* in aquaculture systems, and recent reviews have focused on this topic (Lee et al., 2022; Pillai & Bonami, 2012b). **Table 1** summarizes common diseases affecting *M.rosenbergii* along with the agent, type, syndrome, and current control measures.

Among the diseases caused by viruses, white tail disease (WTD) caused by *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus (XSV) are the most common and detrimental viral agents, leading to a significant reduction in prawn production. The virus responsible for WTD is a small (27 nm in diameter) non-enveloped icosahedral virus, MrNV, with a genome consisting of two linear positive-sense single-stranded RNA fragments, RNA-1 (3202 bp) and RNA-2 (1175 bp) (Sahul Hameed & Bonami, 2012). *M. rosenbergii* is more vulnerable to WTD compared to other prawn species, particularly in the larval, post-larval, and juvenile stages of development, with a mortality rate estimated at 100% in post-larval prawn within 2-3 days of infection (Pillai & Bonami, 2012b). WTD mostly affects the striated muscles of the cephalothorax, abdomen, and tail. Infected adults act as carriers of the disease without displaying any symptoms (Sahul Hameed & Bonami, 2012). Histological characteristics of

WTD in infected muscles of the abdomen and cephalothorax, and intratubular connective tissues of the hepatopancreas frequently appear as large oval or irregular basophilic cytoplasmic inclusions (Lee et al., 2022).

Besides WTD, other serious infections specific to *M. rosenbergii* include *Macrobrachium hepatopancreatic* parvovirus (MHPV) and *Macrobrachium nipponensis* reovirus (MnRV), which have a unique onset in the digestive tract. MHPV is caused by a parvo-like virus resulting in hepatopancreatic nuclear lesions in R and E-cells in hepatopancreas' and midgut's epithelial cells (Kumaresan, Palanisamy, Pasupuleti, & Arockiaraj, 2017). On the other hand, MnRV is caused by Reoviridae cardero-like virus exhibiting hepatopancreatic cytoplasmic lesions with large and round eosinophilic to pale basophilic inclusions in the connective tissues (K. F. Chen et al., 2021; Pillai & Bonami, 2012a). These viral infections are challenging due to ineffective treatment options and cause a mortality rate of 15 to 60% (Farook, H. M. Mohamed, N. Tariq, K. M. Shariq, & I. A. Ahmed, 2019a). These diseases can potentially be controlled by implementing biosecurity approaches, high-quality nutrition and high water quality standards, as well as adaptable stocking density (Pillai & Bonami, 2012b). Effective control measures for viral infections are lacking, making them difficult to manage.

In addition to viruses, certain bacteria such as *Vibrio* spp. and *Pseudomonas* spp. are causes of black spots, brown spot, shell diseases, bacterial necrosis, luminescent larval syndrome, white post-larval disease, and rickettsia (H. Ali et al., 2018; Muthukrishnan, Hoong, Chen, & Natrah, 2021; Pillai & Bonami, 2012b; Sasmita Julyantoro, 2015). Additionally, *Vibrio* spp. bacteria can invade body fluids, causing discoloration of body tissues, impaired wound repair, and blood clotting (Lu et al., 2022). The digestive tract in larval, post-larval, and adult prawns is highly vulnerable to bacterial invasion, especially rickettsias, which can disable the tubular structures of the digestive system leading to darkening and eventual death (M. Farook, H. M. Mohamed, N. M. Tariq, K. M. Shariq, & I. A. Ahmed, 2019b; Rowley, 2022). Other bacteria can invade the shell and use it for nutrition, resulting in eroded areas and black spots originating from the edges and tips of the exoskeleton (Farook et al., 2019b; Rowley, 2022).

Besides viruses and bacteria, oomycetes, such as *Lagenidium* sp., can enter the prawns through cracks or eroded areas of the cuticle, causing larval mycosis characterized by an extensive mycelial network visible throughout the exoskeleton of affected larvae (Farook et al., 2019b; Rowley, 2022). *Fusarium* spp., on the other hand, can result in fusariosis, burn spots, or black gill disease in *M.rosenbergii* (Johnson, 1995; Yao et al., 2022).

Protists, including *Zoothamnium, Epistylis, Vorticella, Opercularia, Vaginicola, Acineta, and Podophyra,* are considered external parasites that inhibit *M. rosenbergii's* swimming, feeding, and moulting in different life stages (Pillai and Bonami, 2012b; Ballester et al., 2017).

3 Probiotics

Probiotics are live active microbes that are introduced into a target host in an adequate number or dose to promote its health (Hill et al., 2014; Knipe, Temperton, Lange, Bass, & Tyler, 2021). They have increasingly been adopted as an eco-friendly substitute for enhancing aquaculture animals' well-being, given the growing concern with respect to antibiotic use and the desire to support disease resistance, growth performance, feed efficiency, and safety of aquatic products (Zorriehzahra et al., 2016). Probiotic *Bacillus licheniformis* was shown to significantly increase the survival of prawn challenged with pathogenic *Vibrio alginolyticus* (Nadella et al., 2018). Balasundaram et al. (2012) reported that inclusion of a commercial probiotic into the feed (3%) decreased the mortality of prawns injected with pathogenic *Vibrio parahaemolyticus* from 59% to 13%. Hindu et al. (2018a,b) reported that *Bacillus vireti*, isolated from the intestine of *M. rosenbergii* increased the survival of prawns challenged with pathogenic *Aeromonas hydrophila* and *Pseudomonas aeruginosa*. In addition to protecting from disease, probiotics offer several advantages, such as boosting digestive enzymes (amylase and protease activity), promoting growth performance, preventing the adhesion and colonization of harmful bacteria in the digestive tract, and regulating gut microbiota, in addition to elevating hematological parameters and the immune response (Sumon et al., 2018). Numerous mechanisms are involved in the health-promoting effect of probiotics, including the enhancement of innate immunity, provoking disease resistance, and competition with disease-causing microbes resulting in their elimination. Probiotics can also be used post-antibiotic treatment to restore the natural gut microflora.

Potential probiotic candidates can be classified into host and non-host associated microorganisms (Lazado, Caipang, & Estante, 2015). Commercial shellfish production commonly uses non-host derived microbes as probiotics (Lakshmi, Viswanath, & Sai Gopal, 2013). However, host-associated probiotics are preferred as they lead to improved growth performance, higher feed efficiency, and enzymatic contribution to digestion (Ahmmed et al., 2020a; Khushi et al., 2020; Sumon et al., 2018). They also inhibit the adherence and colonization of pathogenic microorganisms in the gastrointestinal tract, increase haematological parameters, and boost the immune response (Adorian et al., 2019; Lazado et al., 2015). For prawn aquaculture, the probiotic candidates consist of the genera *Lactobacillus*, *Enterococcus*, *Bacillus*, *Aeromonas*, *Alteromonas*, *Arthrobacter*, *Bifidobacterium*, *Clostridium*, *Paenibacillus*, *Phaeobacter*, *Pseudoalteromonas*, *Pseudomonas*, *Rhodosporidium*, *Roseobacter*, *Streptomyces*, and *Vibrio* (Luis Balcázar, Decamp, Vendrell, De Blas, & Ruiz-Zarzuela, 2009).

4 Methods of probiotic administration

Probiotics can be administrated through several methods such as immersion, oral administration, direct administration into the body, administration in the environment, or a combination of these methods (Einar Ringø, 2020). Each method of administration has its advantages and disadvantages. For instance, the immersion method is a quick and effective approach to delivering probiotics, but it is not practical for large-scale aquaculture operations due to its high cost. Oral administration is more useful for large-scale operations but requires higher doses of probiotics to achieve similar results as the immersion method.

Oral administration is widely used for probiotic delivery in aquaculture through the diet or rearing water. In the early 1990s, single strains of probiotics were administered *via* feed. However, due to the diverse range of conditions and aquaculture species, multi-strain probiotics have gained interest for growth, immune enhancement, and environmental improvement of aquaculture species (Md Abul Kalam Azad et al., 2021; Md Abul Kalam Azad et al., 2019; Decamp, Moriarty, & Lavens, 2008; Fdhila et al., 2017; Ghosh et al., 2016; Hostins, Lara, Decamp, Cesar, & Wasielesky Jr, 2017; Sipra Mohapatra, Chakraborty, Prusty, PaniPrasad, & Mohanta, 2014; Vargas-Albores et al., 2017). Some of the commonly used probiotic strains in pelleted diets include *Bacillus* strain S11 (Rengpipat, Phianphak, Piyatiratitivorakul, & Menasveta, 1998), *Lactobacillus plantarum* (Gatesoupe, 1991), and *Carnobacterium divergens* (Gildberg, Johansen, & Bøgwald, 1995; Gildberg & Mikkelsen, 1998; Gildberg, Mikkelsen, Sandaker, & Ringø, 1997). In addition, non-pathogenic *Vibrio* spp., *Bacillus* spp., *Pseudomonas fluorescens*, *Aeromonas media* A 199, *Flavobacterium* sp., and *Lactobacillus lactis* are directly added to pond water to act as probiotics (Verschuere, Rombaut, Sorgeloos, & Verstraete, 2000).

Maintaining probiotic activity during oral administration can be challenging due to conditions in the gastrointestinal tract, such as acidity. To improve delivery efficiency, encapsulation methods have been developed, and live feed such as brine shrimp, rotifers, and copepods are used as encapsulation media (Gao et al., 2022). For instance, a shrimp larval feed was recently developed by enriching *Artemia franciscana* with *Bacillus* sp. B2, *Lactobacillus johnsonii* C4, *Bifidobacterium animalis subsp. lactis* strain BB-12, *and Streptomyces* sp. RL8 (Garcia-Bernal et al., 2020; Vázquez-Silva et al., 2017).

Encapsulation can protect probiotics from environmental conditions and improve their viability during storage, transportation, and delivery. Additionally, it can prevent the loss of probiotics by increasing their adhesion to the gut wall of the host organism. However, the cost and feasibility of encapsulation methods should be considered while selecting probiotics' administration method.

5 Antagonistic effect of probiotics against pathogenic microorganisms

5.1 Antibacterial activity

The use of probiotics is widespread in giant freshwater prawn aquaculture due to their potential to combat pathogenic bacteria (Miao et al., 2020; Xue, Liu, Liu, Wang, & Xu, 2021). Probiotics play a crucial role in enhancing the essential gut microflora of prawns by producing bacteriocins and organic acids that counteract harmful microbes (Chauhan & Singh, 2019; E Ringø, Olsen, Vecino, Wadsworth, & Song, 2012). **Table 2** summarizes the antagonistic effects of probiotics on pathogenic microbes in freshwater giant prawn culture.

Lactic acid bacteria are amongst the most commonly used probiotics in prawn culture, primarily due to their exceptional ability to inhibit the proliferation of pathogenic microbes by producing antibacterial components such as hydrogen peroxide and organic acids (Zorriehzahra et al., 2016; Zoumpopoulou et al., 2013). Additionally, some lactic acid bacteria, including *Streptococcus* spp. and *Lactobacillus* spp., produced antibiotics and decreased pH to suboptimal levels for pathogenic bacteria. For instance, *Lactobacillus* spp. isolated from the gut of prawns demonstrated inhibitory activity against *V. harveyi* (Ahmmed et al., 2020b). Moreover, *B. cereus*, isolated from the intestine of adult giant freshwater prawn, showed antibacterial activity towards *A. hydrophila* and could be used as a probiotic in *M. rosenbergii* aquaculture (Wee, Mok, Romano, Ebrahimi, & Natrah, 2018). In a modern biofloc culture system, *B. licheniformis* and *B. subtilis* showed an antagonistic effect against *Vibrio sp.* when used as probiotics in the rearing of *M. rosenbergii* (Frozza et al., 2021). Furthermore, *B. vireti 01*, isolated from the gut of healthy prawns, can be considered as an alternative to antibiotics in freshwater prawn cultures since it inhibits the growth of *P. aeruginosa* growth (Vidhya Hindu, Chandrasekaran, Mukherjee, & Thomas, 2018). Finally, *B. licheniformis* exhibited antibacterial activity against *V. alginolyticus* (Nadella et al., 2018), and *P. acidilactici GY2* and *S. cerevisiae* promoted the growth and survival of giant freshwater prawns (Miao et al., 2020).

5.2 Antiviral activity

In aquaculture including the culture of giant freshwater prawns, probiotics have been applied to combat viral disease. However, the actual antiviral mechanism in prawn farming is not yet fully understood (Lakshmi et al., 2013; S Mohapatra et al., 2012). As discussed in section 2, one of the most common viral diseases affecting freshwater prawns is white tail disease, caused by *M. rosenbergii nodavirus (MrNV)* with significant production losses in prawn farms (Lakshmi et al., 2013). Despite the lack of a complete understanding of the mechanism of action, certain strains of bacteria, such as *Vibrio* spp., *Pseudomonas* spp., *Coryneforms* and *Aeromonas* spp. groups, have been identified as potential probiotics for the treatment of viral diseases in shellfish (Chauhan & Singh, 2019; Zorriehzahra et al., 2016). For instance, *B. megaterium* and *Vibrio* species have been shown to exhibit antiviral activity against white-spot syndrome virus in various shellfish species (Li, Tan, & Mai, 2009). In addition, studies have shown that certain strains of *Lactobacillus* spp. can be used as probiotics in a single strain or combined with commercial probiotic products like Sporolac® to provide resistance against lymphocystis viral disease (Harikrishnan, Balasundaram, & Heo, 2010). Furthermore, lactic acid bacteria, including *L. paracasei A14*, *L. plantarum YU*, *L. pantarum L-137* and *L. casei Shirota*, have also shown promise in the remediation of viral diseases (Al Kassaa, Hober, Hamze, Chihib, & Drider, 2014).

5.3 Antifungal activity

In the aquaculture of shellfish and finfish species, even though the antifungal activity of probiotics was reported, there is currently no research on the potential of using probiotics for their antifungal properties. However, there have been studies on the antifungal effects of certain probiotic strains that could be applicable in freshwater prawn culture. *Aeromonas* A199, isolated from eel rearing water, as well as *Lactobacillus plantarum FNCC 226*, *Janthinobacterium M169*, and *Pseudomonas M174*, have been documented to decrease the growth of *Saprolegnia* species (Lategan, Torpy, & Gibson, 2004; Nurhajati, Aryantha, & Kadek Indah, 2012; Zorriehzahra et al., 2016). Additionally, certain probiotic strains isolated from commercial fermented cheese products, such as *RC4b2, RC2b4, RC4a3, RC1b8, FCb1, RC2b3, SCa4, SCb2, LZb8, LZa7, S2a3, S4b1, Kb2,* and *Y2a5*, have demonstrated antifungal activity against *Fusarium oxysporum* and *Rhizoctonia solani* (F. S. Ali, O., & Hussein, 2013).

Lactic acid bacteria strains, including *Lactobacillus fermentum L23* and *Lactobacillus rhamnosus L60*, have been found to decrease the production of aflatoxin B1 and the growth of Aspergillus section Flavi, while strains such as *KCC-28, KCC-27, KCC-26,* and *KCC-25* have shown strong antifungal activity against *Fusarium oxysporum*, *Botrytis elliptica, Penicillum roqueforti, Penicillum chrysogenum*, and *Aspergillus fumigatus* (Gerbaldo, Barberis, Pascual, Dalcero, & Barberis, 2012) (Ilavenil et al., 2015). Further research is needed to determine the potential use of these probiotic strains in the aquaculture of giant freshwater prawns for their antifungal activity.

6 Probiotics as immunity enhancers in prawn

6.1 Impact of probiotics on immunological parameters of *M. rosenbergii*

The cultivation of fish and shellfish is highly dependent on maintaining a fully functional and well-balanced immune system in order to protect and sustain their health. Accordingly, there has been much interest in identifying compounds or agents capable of enhancing the performance of the host's immune system (Dawood, Koshio, Abdel-Daim, & Van Doan, 2019; Lazado et al., 2015). To this end, numerous studies have investigated the impact of probiotics on the immune response of aquatic animals, particularly finfish, with extensive research being conducted in this area (Dawood & Koshio, 2016; Hasan et al., 2019; Jamal et al., 2020; Merrifield et al., 2010; Van Doan et al., 2020). **Table 3** summarizes the effects of probiotics on immunological parameters of giant freshwater prawn. The studies summarized in the table highlight the various probiotics used, their sources, mode of use, doses, trial durations, and the resulting effects on immunological parameters.

Most immunomodulatory investigations in prawns have employed probiotic mixes and culture collections from commercial sources (Md Abul Kalam Azad et al., 2019; Dash et al., 2016; Gupta, Verma, & Gupta, 2016; Zhao et al., 2019). Invertebrates like giant freshwater prawns rely solely on innate or non-specific immunity composed of cellular and humoral elements to detect and suppress the proliferation of pathogenic microbes. Indeed, the immunity of *M. rosenbergii* relies on the clearance efficiency of haemocytes and the activities of prophenoloxidase, superoxide dismutase as well as phagocytic activity (Amparyup, Charoensapsri, & Tassanakajon, 2013; Md Abul Kalam Azad et al., 2019; Kader et al., 2021; Wei, Tian, Wang, Yu, & Zhu, 2021).

Supplementing diets with host-associated microbiota, such as *Enterococcus faecalis*, *L. lactis I*, and *L. lactis II*, isolated from the intestine of giant freshwater prawns, enhanced the innate immunity of prawns with a significant increase in total haemocyte counts and phenoloxidase activity when compared to the control group (Kader et al., 2021). Similarly, supplementing diets with potential probiotic bacteria, *Lactobacillus sp*. and *Enterococcus faecalis,* isolated from *M. rosenbergii's* digestive tract, improved cellular immunity with significantly higher levels of small granular haemocytes and non-granular haemocyte counts than prawns fed with non-supplemented diets (Ahmmed et al., 2020b; Kader et al., 2021; Sumon et al., 2018; Vidhya Hindu et al., 2018). *Bacillus NL110* and *Vibrio NE17* applied as probiotics in the feed and rearing water of freshwater prawns resulted in significant improvements in immune indices, including total haemocyte counts, phenoloxidase activity and respiratory burst (2021). Additionally, *B. vireti 01*, a putative probiotic isolated from the gastrointestinal tract of freshwater prawns, has increased several immunological parameters, including superoxide dismutase, catalase and serum glutathione of freshwater prawns (Vidhya Hindu et al., 2018). Similarly, *B. cereus* isolated from the gut has boosted superoxide dismutase activity in the haemolymph of freshwater prawns (Wee et al., 2018). In addition to this, *B. cereus* increased the level of intestinal short-chain fatty acids, which ameliorate the gut epithelium of shrimp by maintaining structural stability and reducing the intestinal pH, thereby inhibiting the growth of harmful bacteria (Duan et al., 2017).

Non-host-derived probiotics, such as *L. plantarum* from a culture collection, have also increased phenoloxidase activity, respiratory burst, total haemocyte counts, and clearance efficiency in a dose-dependent manner (Dash et al., 2014; Dash et al., 2016). Similarly, *B. pumilus* improved immune enzymes such as catalase, acid phosphatase , nitric oxide synthase and phenoloxidase as well as elevated respiratory burst and phagocytosis of *M. rosenbergii* (Zhao et al., 2019). Additionally, the commercial probiotic Zymetin® also exhibited immunomodulating effects on freshwater prawns with a significant increase of total haemocyte counts, phagocytic activity, and clearance efficiency (Md Abul Kalam Azad et al., 2019).

While investigating the immunomodulatory effects of probiotics on prawn species, probiotics were applied as feed additives (Alavandi et al., 2004; Liu, Chiu, Shiu, Cheng, & Liu, 2010; Zokaeifar et al., 2014). On the other side, probiotics applied to the rearing water have also revealed efficiency in enhancing the immune response in shrimp species. Therefore, future research is needed to evaluate the effectiveness of water-supplemented probiotics in modulating the immune system of prawns.

6.2 Effects of host-derived probiotics on the expression of immune genes

Recently, there has been an increased interest in the immunomodulation of aquatic animals through regulating immune-related genes. Indeed, gene alteration for immune and antioxidant activities is considered as a reliable indicator for improved immunity in aquaculture species following probiotic treatment (Van Doan et al., 2020). In *M. rosenbergii*, various immune and antioxidant genes have been identified in protection against numerous infectious pathogens and foreign compounds. The functionalities of these genes have been comprehensively reviewed by (Kumaresan et al., 2017). Hepatopancreas, haemocytes, and gills have been considered as main tissues expressing immune-related proteins (X. Zhang et al., 2014). Lipopolysaccharide and β-1,3-glucan binding protein, anti-lipopolysaccharide factors, prophenoloxidase, peroxinectin, penaeidin, heat shock protein, superoxide dismutase, and catalase are some of the immune genes of crustacean shellfish reported to be upregulated upon probiotic supplementation. This field of research regarding the modulation of gene transcription *via* probiotics in freshwater prawn aquaculture is still in its early stages. Kader et al. (2021) reported that freshwater prawn treated with three probiotics collected from the host's intestine, *E. faecalis*, *Lac. lactis* I, and *Lac. lactis* II. The study showed a significant upregulation of expression of both immune and antioxidant genes, including β-1,3-glucan binding protein, superoxide dismutase, prophenoloxidase, peroxinectin, acid phosphatase and alkaline phosphatase.

Various probiotics exhibit distinct impacts on the transcription of similar or varying immune-related genes in shellfish (Yarahmadi, Miandare, Fayaz, & Caipang, 2016). These discrepancies could be attributed to variations in experimental circumstances and shellfish species employed. However, previous research suggested that evidence involving gene expression in other aquatic animals caused by probiotics could allow to understand their mode of action in disease prevention and control (Hao et al., 2014; Wu et al., 2014). For instance, the diet of whiteleg shrimp was supplemented with three putative host microbiota, *Shewanella haliotis*, *B. cereus,* and *A. bivalvium* for 28 days. The shrimps fed a probiotic-supplemented diet exhibited significantly elevated expression of prophenoloxidase, β-1,3-glucan binding protein, and penaeidin 3 genes compared to the shrimp fed the non-probiotic diet (Hao et al., 2014). Similarly, three *Bacillus* strains, including *B. subtilis*, *B. pumilus*, and *B. cereus* collected from the intestinal tract of mud crab *Scylla paramamosain* were evaluated as probiotics for the host animals. In addition to protecting against *V. parahaemolyticus*, probiotic strains significantly upregulated the transcription of several antioxidant genes of mud crab, including prophenoloxidase, superoxide dismutase and catalase (Wu et al., 2014) .

In summary, probiotics supplementation, using bacteria such as *Bacillus* spp., *Lactobacillus* spp., *Limosilactobacillus fermentum*, *Clostridium* spp., *Lactococcus* spp., and commercial probiotics such as Zymetin®, have been shown to increase immune parameters such as total haemocyte counts and differential haemocyte counts, enhance phagocytic activity and clearance efficiency in addition to increasing prophenoloxidase and superoxide dismutase activities, and the expression of immune-related genes (Amparyup et al., 2013; Md Abul Kalam Azad et al., 2019; Kader et al., 2021; Wei et al., 2021). It should be mentioned, however, that it is not clear whether increasing these parameters in the absence of pathogens really is beneficial for the prawns. Indeed, the immune system should only be enhanced in case of an infection, and an increase of immune parameters in the absence of a pathogen might not be advantageous after all. Therefore, further research is needed in order to determine the optimal levels of these immune parameters in healthy and diseased prawns.

7 Conclusions and future directions

In the production of aquaculture shellfish species, probiotics' application has emerged instead of harmful chemicals and antibiotics (Jahangiri & Esteban, 2018). However, the use of probiotics in giant freshwater prawn culture is still in its early stages and only a limited number of commercial probiotic products are available in local and international markets (Adel & Dawood, 2021). Consequently, more studies are needed for profiling a wide range of probiotic strains for application in the culture of various aquaculture shellfish species. Moreover, most of the probiotics currently used in shellfish culture are based on lactic acid bacteria and *Bacillus* spp. Hence, further studies are required to identify other potential probiotics that can offer benefits such as physiological responses, improved growth performance, and infection resistance (Einar Ringø et al., 2020).

Choosing the right probiotics and determining the effective dosage can be challenging due to the species-specific nature of probiotics (Hoseinifar, Sun, Wang, & Zhou, 2018). Therefore, further researches are compulsory to increase the effectiveness of feed- and water-administered probiotics. In shellfish aquaculture, the antagonistic effects of probiotics on microbes, especially bacteria, have been reported. However, research on the antiviral and antifungal activity of probiotics in shellfish aquaculture is still limited. Hence, additional investigations are vital to understand the mechanism of antifungal and antiviral activity and to identify suitable probiotics. Recent advances in high-throughput sequencing techniques enable studying the impact of probiotics on prawn-associated microbiomes. Some recent studies reported shifts in the prawn-associated microbiota after probiotic treatment (Cienfuegos-Martinez et al., 2022; Zheng et al., 2022; Qiu et al., 2023). However, in order to determine whether probiotics have a beneficial impact on the prawn microbiome, we first need to obtain a better understanding of what can be considered a healthy prawn microbiome (by analysing microbiomes of healthy and diseased prawns grown in different culture systems).

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Conflict of interest statement

The authors declare that they have no competing interests.

Data availability statement

No data were generated for this paper.

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Tables

Table 1. Common diseases affecting *M. rosenbergii* along with the agent, type of agent, symptoms and current control measures.

Disease	Agent	Type of agent	Symptoms	Current control measures	References
White tail disease (WTD)	*Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus (XSV) Nodavirus and satellite	Virus	Lethargy and opaqueness of the abdominal muscle. Whitish tail and muscle. Affects hatchery and nursery stages. Approximately 100 % mortality rate in post-larvae within 2-3 days of infection	Screening of brood stock and postlarvae. Use of specific pathogen free brood stock	(Gangnonngiw, Bunnontae, Phiwsaiya, Senapin, & Dhar, 2020; Hameed & Bonami, 2012; Sahul Hameed & Bonami, 2012)
Macrobrachium Muscle Virus (MMV)	Parvo-like virus	Virus	Infected tissue becomes opaque, with progressive necrosis; accompanied by progressive weakening of feeding and swimming ability. Affects juveniles.	Improve prevention methods including nutrition and water quality management.	(Pillai & Bonami, 2012b; Tung, Wang, & Chen, 1999)
White spot Syndrome Baculo Virus (WSBV)	Baculovirus	Virus	White spots on the cuticle; affects larvae, juveniles and adults.		(Arockiaraj et al., 2013; Hameed, Charles, &

					Anilkumar, 2000; Li et al., 2009)
Infectious hypodermal and hematopoietic necrosis (IHHN) disease	IHHN virus	Virus	Characterized by high mortality rate (approximately 100%). Affects post-larval stage.	Screening the viral infection in shrimp larvae before culture, good water quality management.	(Arockiaraj et al., 2015; Hameed & Bonami, 2012; Hsieh et al., 2006)
Monodon baculo virus (MBV)	MBV Baculovirus	Virus	Eosinophilic intranuclear inclusions that contain enveloped, bacilliform virions in the hepatopancreas of the larvae.	Improve management in hatchery.	(Gangnonngiw et al., 2010)
White spot syndrome virus (WSSV)	WSSV Nimaviridae, Whispovirus	Virus	White spots on the exoskeleton and appendages; accumulation of cuticular substances on the inner surface of the cuticle; pink-red colouration on the cephalothorax cuticle; reduction in feeding and increased lethargy; yellow hypertrophied hepatopancreas.	Improve management in hatchery, particularly water quality.	(Chiew, Salter, & Lim, 2019)
Macrobrachium nipponensis Reovirus (MnRV)	MnRV Reoviridae Cardero-like virus	Virus	Develop in the connective tissue of the host.	Improve management in hatchery, optimum water quality management.	(S. Zhang, Shu, Zhou, & Fu, 2016)
Macrobrachium hepatopancreatic parvovirus (MHPV)	Parvo-like virus	Virus	Hepatopancreatic nuclear lesions and epithelial cells. Opacity of abdominal muscles Reduced growth rates, anorexia, reduced preening activity.	No appropriate treatment available, needs prevention methods, screening the viral infection in shrimp larvae	(Pillai & Bonami, 2012b)

				before culture ensuring high standard in nutrition and water quality, low farming density.	
Decapod iridescent virus 1 (DIV1)	*Cherax quadricarinatus* iridovirus (CQIV). Shrimp hemocyte iridescent virus (SHIV))	Virus	“Peppered” appearance	Screening the viral infection in shrimp larvae before culture, water quality management.	(Srisala et al., 2020)
Penaeus vannamei nodavirus (PvNV) (white tail disease-like muscle necrosis)	*Penaeus vannamei* nodavirus	Virus	Whitish, opaque lesions in the tail; affects larvae, 50 % mortality rate.	Improved management in hatchery.	(Tang, Pantoja, Redman, Navarro, & Lightner, 2011)
Acute hepatopancreatic necrosis disease (AHPND)	*Vibrio* spp. *(V. parahaemolyticus, V. punensis, V. harveyi, V. owensii, V. campbelli*) and *Shewa nella sp.* that contain pVA1 plasmid	Bacterium	Appearance of empty stomach and gut in tandem with a light-coloured; severe atrophy of hepatopancreas; lethargy; up to 100% mortality with 20-30 days; early life stages are more susceptible.	Screening the viral infection in shrimp larvae before culture, water quality management.	(Chiew et al., 2019; Kumar, Roy, Behera, Bossier, & Das, 2021)

Black spot; brown spot; shell disease	*Vibrio*; *Pseudomonas* ; *Aeromonas*	Bacterium	Melanized lesions; affects all life stages, but more frequently observed in juveniles & adults.	Improved hatchery management; oxolinic acid; nifurpurinol	(Pillai & Bonami, 2012b)
Bacterial necrosis	*Pseudomonas*; *Leucothrix*	Bacterium	Similar to black spot but only affects larvae, especially Nauplius, Protozoea, Zoea/Mysis	Improved hatchery management; nifurpurinol; erythromycin; penicillin-streptomycin; chloramphenicol	(Pillai & Bonami, 2012b)
Luminescent larval syndrome	*Vibrio harveyi*	Bacterium	Moribund & dead larvae, luminescence	Improved hatchery management; chloramphenicol; furazolidone	(Gupta et al., 2016)
White postlarval disease; rickettsia like disease	*Rickettsia*	Bacterium	White larvae, especially stages IV and V	Improved hatchery management; oxytetracycline; furazolidone; lime prior to stocking	(Pillai & Bonami, 2012b)
Mid-Cycle Disease (MCD)	*Alcaligenes* sp. and *Enterobacter* sp.	Bacterium	Lethargy; spiralling swimming; reduced feeding and growth; bluish-grey body colour; affects larvae, especially stages VI and VII	Improved hatchery management; hatchery disinfection Improve management in hatchery, particularly water quality	(Phatarpekar, Kenkre, Sreepada, Desai, & Achuthankutty, 2002) (Pillai & Bonami, 2012b)

Lactococcosis	*Lactococcus garvieae*	Bacterium	Hyperacute haemorrhagic septicaemia	Vaccine, medical herbs, antibiotics (such as lincomycin, oxytetracycline and macrolides)	(S.-C. Chen, Lin, Liaw, & Wang, 2001), (Kawanishi et al., 2005)
Larval mycosis	*Lagenidium* spp.	Oomycete	Extensive mycelial network visible throughout exoskeleton of larvae	Improved hatchery management; trifluralin; merthiolate	(Pillai & Bonami, 2012b), (Owens & Hall, 1989)
Burn spot disease, black gill disease, fusariosis. Fungal infection	*Fusarium solani*	Fungus	Secondary infection; affects adults	Improved management	(Yao et al., 2022), (Pillai & Bonami, 2012b), (Cantrell & Betancourt, 1995),
Yeast infections	*Debaryomyces hansenii*; *Metschnikowia bicuspidate; Candida albicans Candida sake; Metschnikowia artemia*	Fungus	Yellowish, greyish, or bluish muscle tissues in juveniles (Does not cause significant disease)	Improved hatchery management	(S.-C. Chen et al., 2007), (S.-C. Chen et al., 2003)
Black spot disease	*Fusarium* spp.	Fungus	black spot cuticular lesions		(Yao et al., 2022),

					(Cantrell & Betancourt, 1995)
Protozoan infestations	*Zoothamnium*; *Epistylis*; *Vorticella*; *Opercularia*; *Vaginicola*; *Acineta*; *Podophyra*; etc.	Protozoan	External parasites that inhibit swimming, feeding, and moulting; affect all life stages	Improved management; formalin; merthiolate; copper-based algicides	(Pillai & Bonami, 2012b), (Ballester et al., 2017)
Idiopathic Muscle Necrosis (IMN)	Environmental disease	Unknown	Whitish colour in striated tissue of tail and appendages; when advanced, necrotic areas may become reddish; affects all life stages	Improved management; Improve Pond management	(Nash, Chinabut, & Limsuwan, 1987)
Exuvia Entrapment Disease (EED), sometimes known as Moult Death Syndrome (MDS)	undetermined aetiology	Unknown but probably multiple causes, including nutritional deficiency	Localised deformities (rostrum, antennae, legs); failure to complete moulting; affects late larval stages; also seen in post-larvae, juveniles & adults	Dietary enrichment, carotenoid supplementation. Improve management in hatchery, particularly water quality	(Pillai & Bonami, 2012b)
Balloon disease			Swelling of the branchiostegal region; hypertrophy of some gill filaments	Improve quality of water and pond bottom	(Pillai & Bonami, 2012b)
Appendage deformity syndrome			Deformities (rostrum, antennae, legs, etc.) and mortalities	Carotenoid supplementation	(Pillai & Bonami, 2012b)

Table 2. Antagonistic effects of probiotics on pathogenic microbes in the sustainable aquaculture of *M. rosenbergii*.

Probiotics type	Sources	Doses/duration	Key research findings	References
Pediococcus acidilactici PA-GY2 and or Saccharomyces cerevisiae	Gut of prawn	60 days	– Inhibits the growth of *Aeromonas hydrophila* – Decreased the mortality rate of prawn (~50%)	(Miao et al., 2020)
Lactobacillus spp.	Gut of prawn	9 log CFU/g for 8 weeks	– Inhibitory activity against *Vibrio harveyi* – Improved weight gain (550%) in a short period of culture	(Ahmmed et al., 2020b)
Lactobacillus acidophilus 04	Homemade curd	10^6 Cells/g for 30days	– Antibacterial activity against *Vibrio anguillarum*, *V. vulnificus* and *V. harveyi* – Improved growth and survival rate (86%) of freshwater prawn	(Khan & Mahmud, 2021)
Lactobacillus plantarum DM5	Culture collection	10^7, 10^8 and 10^9 CFU/g	– Inhibitory activity towards *Aeromonas hydrophila*	(D. Das, Baruah, & Goyal, 2014)
Bacillus subtilis	Juvenile of freshwater prawn	10^8 CFU/g feed for 60 days	– Potential inhibitory activity against *Aeromonas hydrophila* – Enhanced growth and survival rate	(Keysami & Mohammadpour, 2013)
Zymetin *(Bacillus mesentericus, Clostridium butyricum* and *Enterococcus faecalis)*	Commercial probiotic	5 g/kg for 60 days	– Hinders the growth of *Vibrio* spp. and *Aeromonas* spp.	(Md Abul Kalam Azad et al., 2019)
Lactobacillus plantatum MTCC 1407	Culture collection	-	– Inhibits the proliferation of *Pseudomonas fluorescens* and *Aeromonas hydrophila*	(P. Das, Khowala, & Biswas, 2016)
Bacillus cereus	Gut of healthy prawn	10^4/g for 28 days	– Inhibits the growth of *Aeromonas hydrophila*	(Wee et al., 2018)

			– Probiotic-fed prawns exhibited an overall better hepatopancreatic condition (no hemocyte infiltrations and necrosis)	
Bacillus coagulans MTCC 2302	Culture collection	-	– Inhibits the growth of *Vibrio parahaemolyticus*	(M Karthik, Bhavan, & Manjula, 2018)
Bacillus licheniformis	Culture collection	1 x 10^9/g for 60 days	– Inhibits the growth of *Vibrio alginolyticus*, *Aeromonas* spp. *and Pseudomonas* spp. – The growth of experimental group of prawn was 25% – 75% higher than control	(Ranjit Kumar et al., 2013)
Clostridium butyricum	Intestine of prawn	2 x 10^9/g for 60 days	– Inhibits the growth of *Vibrio harveyi* – 28% higher weight gain compared to control group	(Sumon et al., 2018)
Bacillus licheniformis	Culture collection	1 x 10^9 CFU/g for 45 days	– *B. licheniformis* in feed help in reducing the growth of *Vibrio alginolyticus*	(Nadella et al., 2018)

Table 3. Effects of host-associated and non-host-derived probiotics on immunological parameters of giant freshwater prawn (*M. rosenbergii*).

Probiotics	**Source**	**Mode of use**	**Dose and trial duration**	**Effects on immunological parameters**	**Reference**
Enterococcus faecalis, *Lactococcus lactis* I, & *Lac. lactis* II	Intestine of *M. rosenbergii*	Diet	10^8 CFU/g 50 days	THC and PO ↑ α2-M, LGBP, proPO, Cu, Zn-SOD, TG, PE, AKP and ACP ↑	(Kader et al., 2021)
B. cereus	Intestinal tract of prawn	Diet	10^4 CFU/g 28 days	SOD ↑ MDA →	(Wee et al., 2018)
B. vireti 01	Intestinal tract of prawn	Diet	10^8 cells/mL 14 days	SOD, CAT and GSH ↑	(Vidhya Hindu et al., 2018)
Bacillus NL110 & *Vibrio* NE17	Egg, larvae, and intestine of *M. rosenbergii*	Diet and water	~ 10^9 CFU/g (Feed) ~ 10^9 CFU/mL (Water) 60 days	THC, RB and PO ↑	(Mujeeb Rahiman et al., 2010)
Lactobacillus plantarum	Culture collection	Diet	10^7, 10^8 & 10^9 CFU/g 90 days	THC, PO, RB, CE ↑	(Dash et al., 2014)
L. plantarum (Heat killed)	Culture collection	Diet	10^7, 10^8 & 10^9 CFU/g 90 days	THC, PO, RB, CE ↑	(Dash et al., 2015)
L. plantarum	Culture collection	Water	10^7, 10^8 & 10^9 CFU/L 90 days	THC, PO, RB, CE ↑	(Dash et al., 2016)
B. pumilus	Culture collection	Diet	10^7, 10^8, & 10^9 CFU/g 60 days	RB, CAT, PcA, ACP, NOS and PO ↑ SOD →	(Zhao et al., 2019)
B. coagulans	Culture collection	Diet	10^5, 10^7 & 10^9 CFU/g 60 days	RB and LZ ↑	(Gupta et al., 2016)

B. licheniformis	Culture collection	Diet	10^6, 10^7, 10^8 & 10^9 CFU/g 60 days	THC, SOD, PO ↑	(Ranjit Kumar et al., 2013)
Zymetin® *(Bacillus mesentericus, Clostridium butyricum, Enterococcus faecalis)*	Commercial	Diet	5 g/kg 60 days	THC, DHC, PcA and CE ↑	(Md Abul Kalam Azad et al., 2019)
Saccharomyces cerevisiae	–	Diet	5, 10 & 20 g/Kg 75 days	THC, RB and PO ↑	(Parmar, Murthy, Tejpal, & Naveen Kumar, 2012)

Increased (↑); No change (→); Total hemocyte count (THC); Phenoloxidase (PO); α2-Macroglobulin (α2M); Lipopolysaccharide and β-1,3-glucan-binding protein (LGBP); Prophenoloxidase (proPO); Superoxide dismutase (SOD); Transglutaminase (TG); Peroxinectin (PE); Alkaline phosphatase (AKP); Acid phosphatase (ACP); Large granular haemocytes (LGH), Small granular haemocytes (SHG); Non-granular haemocyte (NGH); Malondialdehyde (MDA); Catalase (CAT); Glutathione (GSH); Respiratory burst (RB); Clearance efficiency (CE); Phagocytic activity (PcA); Nitric oxide synthase (NOS); Lysozyme (LZ); Differential haemocyte counts (DHC).