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Review

A review of the use of probiotics in freshwater prawn (*Macrobrachium* sp.) culture in biofloc systems

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ABSTRACT. This document is an updated review on the use of probiotics to the freshwater prawn *Macrobrachium* sp. culture in biofloc systems, pointing out the benefits in growth, survival, improvements in the immune system, pathogen control, and water quality. The review also emphasizes some aspects that need to be defined with greater accuracy, like the effect of doses and times of probiotic administration and refers to bacterial dynamics associated with biofloc. This review aims to enhance the knowledge of probiotics in commercially important species such as the freshwater prawn.

Keywords: *Macrobrachium* sp.; shrimp; probiotics, bacteria; biofloc system; water quality exchange; aquaculture

INTRODUCTION

Microbial communities in the aquatic habitat respond quickly to the changes in their environment. The changes can be subtle and manifest as activation or inactivation of the bacterial community's specific metabolic pathways or as changes in its composition, structure, and functionality, also called microbial loop (Bentzon *et al.*, 2016). The same happens in aquaculture production systems where the products generated in closed systems and under a continuous flow of water are diverse and can act positively in transforming the organic matter and the compounds generated in the production systems. At the same time, these products are used as a source of microbial biomass available for larger organisms. Still, they also exert detrimental effects and can develop virulence factors in response to environmental variations (De Schryver *et al.*, 2008). Recent studies suggest that proper management of microbial communities can help obtain better water quality, increase nutrient levels, and reduce pathogenic bacteria, thereby increasing the survival of the cultured species without using chemical substances or antimicrobials. Currently, in aquaculture, there are several concerns regarding the use of specific systems. Among these concerns are such as diseases in cultured animals, constant changes in water quality, and constant changes in the microbial community that can represent an input vector for pathogenic microorganisms. Also, these water changes lead to de indiscriminate use of this resource is made, which is limited throughout the world. Besides, discharge of this culture water leads to the contamination of water bodies, *i.e.*, lakes, rivers, seas, and oceans, because it contains feces, dead animals, hormones, antibiotics, pathogenic microorganisms, among others. Therefore, it is required to implement the use of technologies or systems that will allow avoiding these noxious effects on both animals and the environment (Prindle *et al.*, 2012; Bentzon *et al.*, 2016). One of the most used tech-

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nologies in aquaculture for manipulating microbiota in culture systems is the application of probiotics, like *Bacillus* and *Lactobacillus* (Pandiyan *et al*., 2013) showing benefits for animal health and water quality throughout different action mechanisms, immune response stimulation, segregation of substances that inhibit the growth of pathogenic microorganisms, enzyme production that induces absorption and improves the nutrition of fish and crustaceans, and reduction of compounds derived from the microbial metabolism (Yamashita *et al*., 2016; Das *et al*., 2017). Likewise, in the last years, the search for new sustainable aquaculture technologies has allowed the development of cultures in biofloc systems. Microbial oxidant reduction processes are carried out by the addition of a carbon source (Ferreira *et al*., 2015), allowing zooplankton promotion. It can serve as food for the cultured species, with a positive effect on water quality and control of pathogens without the need of chemicals and antibiotics and, as a consequence, less environmental impact (Ahmad *et al*., 2017).

Freshwater prawn importance

The *Macrobrachium* genus is composed of 238 species distributed from the tropical and subtropical fringe around the world to temperate regions (Bauer, 2011). In this genus, significant variations occur in terms of length, morphology, and habitat requirements (Pileggi & Mantelatto, 2010).

The larvae that belong to this genus are planktonic, and, once they pass to the juvenile stage, they begin to migrate from the coast to the deeper areas of freshwater, contributing to the energy flow that later becomes biomass along with the different habitats through which they transit. This characteristic is known as amphidromy (McDowall, 2007). It places the prawns in a prominent position in the list of adaptations and ecological roles present in aquatic organisms, even from an evolutionary perspective.

Freshwater prawns are a group of aquatic animals that have an important ecological role for the environmental dynamics of the ecosystems of rivers and lagoons (Murphy & Austin, 2005). They are considered omnivores and scavengers; furthermore, they consume algae, remains of dead animals, and detritus (Albertoni *et al*., 2003). Some species of the genus *Macrobrachium* have a high economic value due to their high protein content, good taste, and visual appeal, making it a well-priced product as food for human consumption (García-Guerrero *et al*., 2013).

Difficulties in the culture

One of the central concerns in the culture of organisms such as tilapia, shrimp, and prawn (*Macrobrachium* sp.) is the intensive intervention during their production practices, which degrade the environment. First, the use of water produces enormous amounts of waste, such as not consumed food, excretion products, chemicals, even microorganisms, and parasites; second, because antibiotics and chemical substances are introduced into the ecosystem, which is necessary to carry out the activity. This impact has environmental, economic, and social costs (Buschmann, 2007).

For the production of aquatic organisms, including prawns, it is necessary to use water, even for small to medium systems that can reach up to several hundred cubic meters per day, so that water becomes a limiting factor for the activity (Borja, 2002). Also, diseases are a risk faced by prawns during their culture because they are vulnerable and under stress conditions. Among them are those caused by *Vibrio* species, which cause mortality of the organisms and, consequently, economic losses; for these reasons, technologies that solve these problems have been chosen (Ajadi *et al*., 2019).

Biofloc system

Biofloc technology (BFT) is a culture system consisting of microbial flocs made up of microorganisms, such as rotifers, nematodes, copepods, bacteria, and zooplankton. This technology is based on a minimum or zeroes replacement of water, which allows having fewer adverse effects on the environment, and, as an additional benefit, microbial protein is produced in the system that can be used as food by the organisms. According to Emerenciano *et al*. (2011), this technology was developed in the 70s by IFREMER-COP (French Research Institute for Exploitation of the Sea, Oceanic Center of Pacific) with different aquatic species such as *Penaeus monodon*, *P. vannamei*, *Fenneropenaeus merguiensis*, among others.

The flocs are formed by adding carbon sources to the water body. For the formation of flocs, an external carbon source is required at a 20:1 ratio of C:N to be added to the water, this can be achieved with molasses, rice flour, coffee, moringa, or tapioca, among others. The microorganisms that develop in this system have two main functions: 1) to maintain water quality by transforming toxic nitrogen into microbial protein, and 2) to serve as a natural food source for animals in culture (Emerenciano *et al*., 2013).

The microbial communities that make up the biofloc are developed by populations of cells of various species, which interact with each other by carrying out multiple functional activities within the community and with their host (Díaz & Wacher, 2003). In recent years, in the aquaculture of some crustaceans and fish, microbial consortia have been used as a food source and to improve the culture environment (Becerra *et al*., 2014).

One of the studies, Deng *et al*. (2018), describes the biofloc microbiota. They evaluated the effect of the addition of tapioca starch, cellulose, and their combination, on the microbial diversity of the biofloc, during the cultivation of the herbivorous carp through mass sequencing, reported that regardless of the source of carbon added to the culture system, the Proteobacteria, and Bacteroidetes phyla were the most abundant. It should be noted that the bacteria belonging to these phyla are reported in different studies as ubiquitous in aquatic environments and aquaculture production systems (Guo *et al*., 2011; Wei *et al*., 2016; Zhou *et al*., 2017).

On the other hand, it has been shown that Proteobacteria, which are commonly found in biofloc systems, is a microbial group in charge of nutrient recycling and mineralization of organic components in aquatic systems (Cardona *et al*., 2016). Martins *et al.* (2013) indicate that the Proteobacteria group comprises several phototrophic and heterotrophic genera with a high degradative capacity of compounds such as methane and methanol in aquatic environments. Deng *et al.* (2018) mention that Betaproteobacteria, also commonly found in biofloc, is a group of aerobic or facultative bacteria responsible for transforming nitrogen compounds in aquatic ecosystems, the most important belong to the *Nitrosomonas* genus, which oxidizes ammonia. Moreover, bacteria such as Pseudomonadaceae (Ndi & Barton, 2012), Caulobacteraceae (Guo *et al*., 2011), Chitinophagaceae (Bartelme *et al*., 2017), and Sphingobacteriaceae (Bartie *et al*., 2005), are efficient in the transformation of various compounds generated during cultivation within the water column (cellulose, chitin, collagen, and nitrogen), thus promoting the use of products that otherwise could be considered pollutants.

Despite the relevance of bacteria in the functioning of the digestive tract in aquatic species, there are few investigations aimed at identifying microbial species that develop in the water and the intestine of the species in cultivation. In this regard, through an extensive sequencing study, Tzeng *et al*. (2015) determined that the main bacterial phylum in the intestine of *Macrobrachium nipponense* was that of Proteobacteria, followed by Firmicutes and Actinobacteria.

Chen *et al.* (2017) observed, through the technique of massive sequencing in water and the intestine of *Macrobrachium nipponense*, that changes in the environment can influence the intestinal microbiota, not only by providing microorganisms associated directly with the environment but also by indirectly interfering with the composition of established intestinal bacteria. Pérez-Fuentes *et al.* (2016) observed the development of different bacteria through the 16S gene of rRNA, in tilapia (*Oreochromis niloticus*) cultured in a biofloc system, in both the intestine and water, and found that the main pathogenic bacteria in water were: *Aeromonas hydrophila*, *A. salmonicida*, *Escherichia coli*, *Vibrio fluvialis*, and non-pathogens: *Bacillus subtilis*, *Enterobacter* sp., *Micrococcus* sp., they also observed that by decreasing the amount of food supplied to the fish, the number of recorded bacteria increased. In the intestine of the tilapia, they recorded 15 species of pathogenic bacteria (among them *Aeromonas hydrophila*, *A. sobria*, *E. coli*, *Pseudomonas cepacia*) and non-pathogenic (*Bacillus pumilus*, *B. subtilis*, *Micrococcus* sp.).

Although some studies have identified and classified the microbiota present in the biofloc during the culture of different prawn and shrimp species, research is still needed on the microbial communities that develop in both the water and intestine in the biofloc system during shrimp farming.

On the other hand, in recent years, research has been carried out to cultivate prawn (*M. nipponense*) in biofloc systems, due to the different benefits offered by this technology (Emerenciano *et al.*, 2017). Among these benefits are greater growth and survival in cultured animals, a decrease in commercial food investment, improvement in water quality, and fewer water refills. Ballester *et al.* (2017) cultivated prawns (*M. rosenbergii*) with two treatments, a biofilter recirculation system (RAS) and another with microbial flocs (F). Resulting in the high density of rotifers, ciliates, and flagellate, as well as bacteria, these were identified in treatment F, and attributed to the source of carbon used; because the carbon source promotes an increase in bacterial biomass, which, in turn, stimulates the development of other microorganisms, and these will be used as natural food *in situ* by cultured animals.

Another benefit of biofloc systems is pathogen exclusion, which is very important because, in aquaculture systems, infectious diseases caused by pathogen microorganisms are responsible for substantial economic losses, as they can cause mortalities higher than 90%. A strategy to reduce the diseases' impact is the use of antibiotics because they help to control some bacterial infections. However, there are many problems associated with their use. The inappropriate application of antibiotics has led to the development of resistance to them by bacteria; consequently, there is an urgent need for alternative sustainable control techniques (Kathleen *et al.*, 2016).

Biofloc technology applied to aquaculture is a tool for pathogen control in the aquatic environment. In contrast to conventional approaches, it does not generate resistance to pathogens. Its action derives from a competitive exclusion effect by probiotic bacteria against other microbial groups, by secreting a wide variety of exoenzymes and polymers that generate an environment hostile to bacteria, especially to pathogens (Monroy *et al*., 2015). The probiotic potential of the biofloc maybe since part of the intestinal microbiota of aquatic organisms is released through the feces to the environment, which, due to its nature, has high nutrient loads, allowing for their proliferation. The cultivated species take advantage of the benefits provided by these microorganisms (Crab *et al*., 2010). In this regard, Maya *et al.* (2016) identified bacteria of the *Bacillus, Lactobacillus, Lactococcus, Saccharomyces* genera known for their probiotic potential in a biofloc system, which indicates that, in the biofloc, various genera of probiotics are developed that provide benefits to both animals and culture water.

The presence of natural probiotics in biofloc culture systems reduces water treatment costs by up to 30% since this system can operate with a low water exchange, with rates of 0.5 to 1% per day. At the same time, aeration is maintained at 4.0 mg L^{-1} to keep flocs suspended (Crab *et al*., 2009).

The water quality in a biofloc system is regulated by the bacterial community and its development from the carbon-nitrogen relationship, which guarantees the proliferation of heterotrophic bacteria that transform nitrogen compounds, such as ammonia, into simpler compounds that are not harmful to animals in culture (Samocha *et al*., 2007; Asaduzzaman *et al*., 2010). Biofloc systems are complex ecosystems subjected to various biochemical processes that vary relative to the conditions in which the culture develops and is dependent on water quality values (Table 1).

In recent decades, the massive expansion of aquaculture has begun to face some important limitations, such as the increase in demand and price of fishmeal, which is the primary raw material for the preparation of aquaculture diets. However, due to overfishing, natural populations are unable to meet the demand. Aquaculture needs to obtain alternative protein sources to replace the use of fishmeal (Avnimelech, 2012; Crab *et al*., 2012).

Biofloc cultures are a technological alternative to optimize the use of aquaculture diets, leading to the reduction of fishmeal inclusion in the formulations. In this type of systems, a wide range of microorganisms (bacteria, phytoplankton) is developed, as well as organic matter aggregates, in addition to rotifers, ciliates, protozoa, and copepods that form macro-aggregates (biofloc) a rich natural source of protein: lipid "*in situ*" available 24 h a day for organisms in culture. It is known that the potential feed gain with this technology is 10 to 20%, with the consequent 40 to 50% reduction in feed costs (Azim & Little, 2008; Poleo *et al*., 2011; Hargreaves, 2013); furthermore, the biofloc has a protein content of between 25 and 50% and the fat content ranges from 0.5 to 15% (Emerenciano *et al*., 2013).

Probiotics: definition and benefits

This term was used for the first time in 1965 by Lilly & Stillwell as a modification of the original word "probiotika." In 1989, Fuller expanded the definition to "live microbial food supplement that benefits the host (human or animal) by improving the microbial balance of the body." There are several definitions for the word "probiotics" that have been modified over time. However, a more general and shared concept of probiotic is proposed by Irianto & Austin (2002) "one or more microorganisms with beneficial effects for the host, able to persist in the digestive tract because of its tolerance to acid and bile salts." Furthermore, a probiotic can be just one microorganism or a mix of microorganisms that present a synergistic effect potentiating the benefits (Martínez-Cruz *et al*., 2012).

To consider microorganisms as probiotics, they must comply with specific requirements such as: being safe for the animal, *i.e.*, not to cause disease, they must arrive alive to the gastrointestinal tract and be able to colonize it to achieve an effective competitive exclusion. Also, it must inhibit the growth of pathogenic microorganisms, both Gram-positive and Gram-negative, by producing acids or other substances that inhibit their growth. Likewise, they must have a short reproduction time, tolerate gastric pH and bile salts; they must be stable when in contact with bile, acids, and enzymes, and, finally, they must be stable and viable during storage (Gutiérrez, 2013).

Some studies assessed the effect of probiotics on the growth of fish and crustaceans, among them are: Seenivasan *et al*. (2016), who observed the effect on survival and growth, for 60 days of three probiotics (*Lactobacillus sporogenes, Bacillus subtilis*, and *Saccharomyces cerevisiae)* given in the diet for postlarvae of *M. rosenbergii*. The results indicated that all the probiotics promoted significant growth and enzyme production (protease, amylase, and lipase) in *M. rosenbergii,* but the best was *S. cerevisiae*.

During a 240 day culture with juvenile *M. rosenbergii* prawns, Ghosh *et al.* (2016) observed that by providing a mixture of two commercial probiotics (Zymetin: *Bacillus mesentericus,* and Super PS: *Rhodobacter* sp. and *Rhodococcus* sp.) the growth and productive yield of the prawns were significantly higher (30%) compared to the other diets (without probiotics, or with probiotics added separately), indicating that their mixture yields better results than their separate addition. (Table 2).

Table 1. Effect of a biofloc system on the water quality in crustaceans' culture.

Days of the experiment	Water quality results	Author
90 days	Treatment with molasses induced significant differences in ammonia, nitrites, nitrates, and total nitrogen in the water used for the control prawn (*M. rosenbergii*) culture.	Miao *et al*. (2017)
30 days	Water quality parameters (total ammonium, nitrite, and nitrate) were within the optimal range when adding a carbon source to shrimps (*P. vannamei*) culture.	Suita *et al.* (2015)
21 days	Concentrations of TAN and N-NO_2 were significantly lower ($P < 0.5$) in the biofloc system in shrimps (*P. vannamei*) culture than in the control. A significant difference ($P < 0.5$) was obtained in the individual final weight, an increase in biomass, and a better protein conversion rate for biofloc treatments compared to the control.	Luis-Villaseñor *et al.* (2015)
42 days	Parameters, like nitrite, nitrate, and total ammonium, were optimal for the culture of shrimps (*P. vannamei*) in the treatments with three different probiotics (9.48×10^4, 1.90×10^5, and 3.79×10^5 CFU mL^{-1}). The benefits of zero water exchange were observed, and the parameters were kept stable.	Llario *et al*. (2020)

Table 2. Results on the significant growth of prawns by the administration of different probiotics.

Probiotics	Species	Results in growth	Author
Bacillus subtilis and *Pseudomonas fluorescens*	*Macrobrachium malcolmsonii*	With *B. subtilis*, the initial weight was 5.86 g and final 20.25 g, and with *P. fluorescens*, the initial weight was 6.8 g and final 18.5 g after 60 days.	John *et al*. (2018)
Clostridium butyricum	*Macrobrachium rosenbergii*	The probiotic induced significantly higher ($P < 0.05$) weight and growth rate than in the control group after 60 days.	Sumon *et al*. (2018)
Bacillus Cereus	*Macrobrachium rosenbergii*	The probiotic concentration (1×10^4 CFU g^{-1}) induced a significantly higher growth than the control group after 28 days.	Wee *et al.* (2018)
Commercial probiotic: Prosol	*Macrobrachium rosenbergii*	The final body weight increased, the net body weight gain and the specific growth rate with the probiotic was significantly higher ($P < 0.05$) after 105 days.	Gupta & Dhawan (2011)

One of the most important aspects of promoting the use of probiotics in aquaculture is the water quality of the systems to which they were applied. To this regard, Akter *et al*. (2017) observed that when supplying commercial probiotics to the prawns (*M. rosenbergii*), water quality parameters (ammonium and nitrogen compounds) were in their optimal range for the species. Thus, not needing continuous water replacement for the microbial loop action since capturing the nitrogenous and transformed in less toxic compounds and generated microbial protein that will provide a supplemental source of nutrition for the animals in culture.

Rubia *et al.* (2017) reported that during prawn (*M. rosenbergii*) culture with the individual addition of three commercial probiotics, the water quality parameters remained in their optimal range without significant variations, helping the farmer to keep the water quality. Furthermore, to stabilize water quality during the culture, probiotics compete, and displace pathogenic microorganisms that cause high mortality. Mujeeb *et al*. (2017) detected that *Brevibacillus laterosporus* bacteria, isolated from a sample of *M. rosenbergii* larvae, presented antibacterial activity against pathogens, *Aeromonas hydrophila* and *Vibrio parahaemolyticus*, hence it is possible to isolate specific probiotics to be used on the same species. Besides, the bacterium *B. laterosporus* was also able to inhibit other bacterial genera, such as *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Vibrio*, *Bacillus*, *Streptococcus*, and *Enterobacteriaceae*.

Azad *et al.* (2019) administered the commercial probiotic Zymetin during the culture of juvenile prawns that were challenged against a pathogenic strain of *Vibrio* spp. and *Aeromonas* spp. They observed, at the end of the experiment, that there was a significant increase of total and beneficial bacterial density (*Bacillus* spp., *Enterococcus* spp., *Lactobacillus* spp.), and significant curtailment of some harmful bacteria (*Aeromonas* spp. and *Vibrio* spp.) in the water and prawn intestine of all tested groups ($P < 0.05$). In other words, an exclusion of pathogenic microorganisms by probiotics occurred.

Another of the main benefits of probiotics is that they stimulate the host's immune system. They act on the cells involved in natural and specific immunity (Balcázar *et al*., 2006); thus, probiotics have been tested in different aquatic species and varied results, although, in general, an improvement in the health or growth of organisms has been reported (Table 3).

Probiotic concentration and dose

Some relevant aspects of the use of probiotics are the concentration and the dose set up at which probiotics can exert the most significant possible benefit on the host. Some authors have stated the effective concentration to obtain the best results in growth, survival, as well as in the prawn's immune response. In this sense, Dash *et al*. (2016) evaluated the growth, food efficiency, biochemical composition, and response to three different concentrations (1×10^7, 1×10^8, 1×10^9 CFU L^{-1}) of a probiotic (*Lactobacillus plantarum)*. They found that they were significantly higher than in control (without probiotic).

Furthermore, Kumar *et al.* (2013) observed that the inclusion of a probiotic (*Bacillus licheniformis*) in diets led to increased growth and immune response with the highest concentration of probiotics (1×10^9 CFU g^{-1}), and a significant increase of bacteria in the intestinal tract ($P < 0.05$) and the simultaneous decrease of pathogenic species, such as *Aeromonas* spp. and *Pseudomonas* spp., in all experimental groups of *M. rosenbergii*. Some investigations are described in which beneficial effects were obtained concerning growth, exclusion of pathogens, and enzymatic activity with different probiotic concentrations (Table 4).

Perspectives

In the last two decades, research on the use of probiotics in aquaculture has increased. However, there is little information about the concentration and the appropriate dose for the growth of cultured organisms and the multiple benefits they provide (Seenivasan *et al*., 2011; Kumar *et al*., 2013).

Due to the scarce research to this regard, it is necessary to face this topic specifically with a clear view of the dose to be used and the risks involved in specific objectives of the studies. On the other side, the increase in the quality and quantity of the national aquaculture production implies an improvement in the production processes involving environmental, social, and economic aspects, that is, the sustainable development of the activity (Grealis *et al*., 2017). In this sense, the development and implementation of probiotics in the world and Mexico, is a reality and represent one of the best options to improve aquaculture production (Cienfuegos *et al*., 2017). However, the functionality and, thus, the safety of this biotechnology involve research and responsible application. In Mexico, there is no regulatory framework for this biotechnology; however, there is the Official Mexican Standard NOM-061-ZOO-1999, which states that: "in the case of probiotic additives, prepared with microorganisms producing lactic acid or similar, prior previous to their regulation, you must ensure its verification to determine the genus and species used, as well as specify the concentration of viable microorganisms expressed in colony-forming units per milliliter (CFU mL^{-1}) or gram of finished product". As this is the only item specified for the management of probiotics in Mexico, there is much work to do for the probiotic additives in the field of aquaculture regulations to ensure their proper use. It must be emphasized that, in the first instance, it refers to the processes involved in the probiotics' development and application, that is, guidelines for their importation and evaluation of their functionality. The fact of not having adequate regulations generates misinformation and an erroneous conception of this biotechnology scope. For example, in some products available in the market, no information is given about storage, dosing, and shelf life, which can, consequently, cause a decrease in their functionality.

On the other hand, in recent decades, there has been a growing interest in the development of sustainable biotechnologies and, therefore, in the use of probiotics, which for aquaculture represents a tool that has gained relevance internationally (Pandiyan *et al*., 2013). However, the benefits obtained from these microorganisms, the specific aspects of the mechanisms of action by which these bacteria (mainly) exert their positive effects are still unknown. In this regard, the main lines of research have been associated with the control and prevention of pathogens, growth, digestive

Table 3. Benefits obtained in the immune system by adding probiotics to prawn and shrimp culture.

Microorganism used	Results obtained	Author
Bacillus amyloliquefaciens	With the probiotic, a higher significant stimulation of the immune response of shrimps *Penaeus vannamei* (total protein concentration, 128 mg mL^{-1}; cell number with apoptosis, 1; and percentage of granular cells, 81%) was obtained as compared with the control (104 mg mL^{-1}, 3, 51% respectively).	Llario *et al.* (2020)
Bacillus pumilus	The probiotic was evaluated in a prawn culture (*Marsupenaeus japonicus*) at doses of 1×10^7, $1\times10^{8,}$ and 1×10^9 CFU g^{-1}. The immune response was stimulated, as the activity of catalase, nitric oxide synthase, and acid phosphatase increased significantly in probiotic treatments as compared with the control.	Zhao *et al.* (2019)
Commercial probiotics	Supplementation with a commercial probiotic enhanced the prawns' immune activity (*Macrobrachium rosenbergii*); the hematological profile reflected the effect of the probiotic.	Jakhar *et al.* (2016)
Bacillus subtilis and *Lactobacillus* sp.	*M. rosembergii* grown in biofloc significantly increased (P < 0.05) the number of hemocytes, phagocytes, serum superoxide dismutase, and lysozyme activity, when adding a mixture of the two probiotics due to the increase (P < 0.05) of the two probiotics in both the culture water and the intestine.	Miao *et al*. (2017)

Table 4. Results of the different probiotic concentrations used in prawn (*Macrobrachium rosenbergii*).

Probiotic	Concentrations	Results	Author
Lactobacillus plantarum	1×10^7, 1×10^8 and 1×10^9 CFU L^{-1}	Prawns from the three probiotic doses had significantly better growth, feeding efficiency, biochemical composition, and immune response compared to the control.	Dash *et al.* (2016)
Bacillus subtilis	1×10^8 cells mL^{-1}	The probiotic had a significant effect on larval growth. Survival was significantly higher in the probiotic group (55.3 ± 1.02) than in the control group (36.2 ± 5.02).	Keysami *et al*. (2007)
Bacillus licheniformis	1×10^9 CFU g^{-1}	The highest growth and best immune response were registered with the highest bacterial density. In the intestine, microbial counts in the presence of *B. licheniformis* were significantly (P < 0.05) higher in the groups with probiotics that were in control.	Kumar *et al.* (2013)
Commercial Probiotics BinifitTM	0.5, 1.0, 1.5 and 2%	The best results in survival, growth, biochemical components, and energy budget, were observed at the 2% concentration.	Seenivasan *et al.* (2011)

physiology, and water quality, specifically of the species in question: fish, mollusks, or crustaceans (Villamil & Martínez, 2009).

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