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Probiotics, a Reality in Shrimp Culture Review Article

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ABSTRACT

Shrimp culture is one of the most lucrative and fast growing sectors in marine aquaculture; however, the intensification of cultures to meet the growing demands has incremented the incidence of diseases, causing substantial economic losses. Disease outbreak prevention and control is mostly based on antimicrobials, which has originated controversy, due to the accumulation of residues in the environment, increased resistance, and little consumer acceptance. Alternatively, new, more environmentally friendly methods are suggested, like the application of probiotics, a versatile procedure with broadly accepted benefits in shrimp production worldwide. Probiotics can control pathogens by means of multiple mechanisms; they can promote host growth, and improve the quality of the culture environment. Additionally, they can be administered through different routes, and in combination with other beneficial substances. This article offers an update on probiotic application in shrimp culture, with particular emphasis on productivity.

Key words: *shrimp culture, productivity, shrimp, probiotic, action mechanism*

INTRODUCTION

Aquaculture has been one of the fastest growing food producing sectors in the last decades. In crustacean culture, particularly, it is one of the most advantageous, profitable, and productive sub-sectors (Stentiford *et al.*, 2012). Shrimps are highly priced items mostly cultured in Asia and Latin America, which generate profits and jobs (FAO, 2004). The most widely cultivated species in those regions are *Litopenaeus vannamei* (Pacific white shrimp) and *Penaeus monodon* (giant tiger prawn) (Wang and Gu, 2010). Although this sector has a favorable growing trend, it faces difficulties, like availability of raw materials for feedstuff production and increased occurrence of diseases (Stentiford *et al.*, 2012).

In large scale production where animals withstand stress, the deterioration of optimum culture conditions leads to the emergence of diseases that cause significant economic losses (Mohapatra *et al.*, 2013). Disease control in aquaculture (prophylactic and therapeutic) was historically based on antimicrobial use (Cabello *et al.*, 2013); however, that practice is widely criticized today because of its impact on the accumulation of residues in the environment and resistance development, which also affects consumer product acceptance (Gothwal and Shashidhar, 2015; Kumar *et al.*, 2016; Liu *et al.*, 2017). Moreover, it demands good aquaculture practices to ensure efficient food use by the animals to increase productivity.

The administration of microorganisms to increase disease resistance and improve shrimp nutrition is an environmentally friendly and safer procedure (MartínezCórdova *et al.*, 2015). Probiotics are living organisms with beneficial effects on the host. They can transform the microbial community associated to the host or the environment, and they can enhance food intake or increase its nutritional value; and stimulate response to diseases or improve the quality of the surrounding environment (Verschuere *et al.*, 2000).

Several commercial probiotic products are available today (Miandare *et al.*, 2016, Xue *et al.*, 2016, Ferreira *et al.*, 2017, and Javadi and Khatibi, 2017). However, the isolation and characterization of new strains is an active field of research, particularly for strains isolated from the environment and/or the target host (Wang and Gu,

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2010, Franco *et al.*, 2016a, and Franco *et al.*, 2016b).

The aim of this paper was to review recent advances in probiotic application in shrimp culture, with particular emphasis on production yield increases.

DEVELOPMENT

Action mechanisms

Probiotics have beneficial effects through multiple mechanisms, not only on the target organisms, but also on the surrounding environment. The main action mechanisms described for probiotics in aquaculture are, the capacity to colonize and adhere to the intestinal tract, modulation of the immune system, production of beneficial compounds, production of pathogen antagonistic substances, and improvements in the aquatic environment.

Colonization and adhesion in the gastrointestinal tract

The ability of bacteria to adhere and survive in the enteric mucus is pivotal to establish the gastrointestinal microbiota. The adherence capacity is a feature of probiotic and pathogenic bacteria. It is one of the most important criteria for selection and application of probiotic bacteria in aquaculture (Lamari *et al.*, 2014, Vieira *et al.*, 2016), whereas for the pathogenic bacteria, it is associated with virulence and is considered the first sign of infection (Defoirdt, 2014). The information available in aquaculture indicates that bacteria isolated from continuously cultured animals or their surroundings, have a greater adhesion capacity to the gastrointestinal mucus and tissue, compared to foreign bacteria. Therefore, the action of many probiotics is often transient, and they should be administered continuously either as a supplement in the food or in the culture water so they can maintain their biological effect, unless they are grown from strains isolated from the very aquatic ecosystem (Nimrat *et al.*, 2011) (Table 1). However, microbial isolates from a microorganism were reported to colonize other cultured species, which indicates the lack of specificity for colonization in the digestive tract (Sánchez-Ortiz *et al.*, 2016).

Production of antimicrobial and antiviral compounds

Microorganisms with probiotic activity can also have the capacity to generate extracellular products that inhibit or kill other potentially pathogenic bacteria, such as, antimicrobial substances (Pham *et al.*, 2014; Ming *et al.*, 2015), organic acids (Tejero-Sariñena *et al.*, 2012; Fakruddin *et al.*, 2017), and bacteriocines (Iyapparaj *et al.*, 2013; Muñoz-Atienza *et al.*, 2013; Ming *et al.*, 2015).

Probiotics not only have an antibacterial capacity, certain antiviral activity has also been described in some isolates, like *Pseudomonas* sp., *Vibrio* sp. and *Aeromonas* sp against the hematopoietic necrosis virus (IHNV) (Kamei *et al.*, 1988). Maeda *et al.* (1997) isolated a *Pseudoalteromonas undina* strain with antiviral effects, which increased survival of shrimps (*Penaeus* sp.), previously infected with the Simaaji Neuro Necrosis (SJNNV), Baculovirus, and Iridovirus. Sánchez-Ortiz *et al.* (2016) found that the administration of *Bacillus* spp in the diet of *L. vannamei* naturally infected with the white spot syndrome virus (WSSV) and IHNV could reduce the prevalence of the virus in the animals, and stimulated the growth and expression of genes of the immune system, such as pro-phenoloxidase (proPO), and superoxide dismutase (SOD), leading to a greater survival index in comparison to the untreated groups (Table 1).

Phagocytosis and apoptosis are the main mechanisms described during the antiviral immune response of shrimps (Wang and Zhang, 2008), though there are also reports on the effectiveness of therapeutic strategies based on interference RNAs and stimulation of the innate immune response of shrimp against viral envelope proteins (Thomas *et al.*, 2014; Taju *et al.*, 2015). However, the practical application of some of these findings is not feasible at the productive level. In that sense, the selection and use of probiotics, which are known enhancers of cell immunity, is a promising alternative.

So far, very few methods have been described for specific detection of the antiviral activity of probiotics, as well as their action mechanism. Some experimental suggestions are, cellular pretreatment with the probiotic, co-incubation of the virus and probiotic, virus absorption into the probiotic, and antiviral effect of culture supernatants Lakshmi *et al.*, 2013). Recently, a new eukaryotic line (Botić *et al.*, 2007) successfully used in human virology, but not in aquaculture, was reported as a model to achieve that purpose (Lakshmi *et al.*, 2013). The standardization of such techniques for probiotic-virus interaction studies in shrimp culture is an extremely urgent need to develop selection strategies for new strains, and more rational and effective therapies.

Production of beneficial compounds

Marine bacteria and yeasts may become important sources of protein to improve the nutritional contribution of certain cultured aquatic species (Achupallas *et al.*, 2015; Melo *et al.*, 2015; Gamboa-Delgado *et al.*, 2016; and Qiu and Davis, 2017).

Similarly, the lipids produced by marine microorganisms have been recommended to improve the nutrition of important aquatic species (Hoseinifar *et al.*, 2016). The production of lipases, chitinases, and proteases by selected microorganisms may contribute to the digestive process of cultured organisms, and have a positive impact on their productive behavior (Shen *et al.*, 2010; Zokaeifar *et al.*, 2012; Chai *et al.*, 2016; Seenivasan *et al.*, 2016; and Xue *et al.*, 2016) (Table 1).

Water quality improvements

Gram positive bacteria, especially genus *Bacillus* used as probiotics, can turn organic matter into $Co_{2.}$ On the contrary, the Gram-negative bacteria convert organic matter into bacterial biomass or slime (Dalmin *et al.*, 2001; Zokaeifar *et al.*, 2014). Mujeeb Rahiman *et al.* (2010) applied *Bacillus* sp. and *Vibrio* sp. in the diet and water of *Macrobrachium rosenbergii* at different doses and administration frequencies, which resulted in the reduction of ammonia and nitrate concentrations in the medium, and significantly increased survival, growth, and the immune system activity. After the application of commercially available EM (EM®, Japan), made of acid-lactic bacteria and yeasts, in the water of intensive culture of *L. vannamei*, Melgar Valdés *et al.* (2013) found that the treatment reduced organic matter and the concentration of nitrate, regulated pH, increased the availability of phosphorous in the water, and improved productivity indicators, like survival and the food conversion factor (Table 1).

However, some studies stressed that the application of probiotics did not improve the parameters evaluated in shrimp culture (Silva *et al.*, 2012; Bolívar Ramírez *et al.*, 2013). These results suggested that this effect is influenced by the frequency of application, the doses administered, and the production system (outdoor ponds, or more controlled conditions). This is a particularly interesting issue, considering that animal development and water quality parameters are less affected when the cultures are closer to the natural medium. The microbiological quality of water is a risk factor that may lead to outbreaks; hence, probiotics may also be used to reduce the prevalence of opportunistic pathogens in the environment (Chumpol *et al.*, 2017). The addition of *Bacillus* sp. as a supplement in the diet of *P. monodon* decreased the load of *Vivrio* sp. in the pond, which favored the prevalence of heterotrophic bacteria (Boonthai *et al.*, 2011). Silva *et al.* (2012) evaluated the addition of a commercial product made of *Bacillus* spp. at different stages of *L.vannamei*, and demonstrated that the treatment decreased the load of *Vivrio* sp., both in the intestine of animals and the water.

Additionally, new technologies based on microorganisms that contribute to optimum culture conditions have been suggested, including *Ibiofloc*, which aims to stimulate development and prevalence of heterotrophic microbial communities in the culture medium, which can remove organic matter through the addition of carbon sources (Crab *et al.*, 2012). This technology is also used in microbial biomass production as an alternative source of protein (Ahmad *et al.*, 2017). The literature shows several reports on the effectiveness of the procedure; also, several review articles discuss their particularities in detail (Crab *et al.*, 2012; Ekasari *et al.*, 2014; Kim *et al.*, 2015; Melo *et al.*, 2015; Suita *et al.*, 2015; Bossier *et al.*, 2016; Ahmad *et al.*, 2017; Ferreira *et al.*, 2017).

Immunomodulation

The defense against crustacean pathogens is mainly based on innate immunity mechanisms (Song and Li, 2014). The immune system of shrimps involves hemocytes (for encapsulation, nodule formation, and phagocytosis), several plasmatic components (antimicrobial peptides, histones, lysosomal enzymes, lipopolysaccharide binding proteins, and β-1.3 glucans, and recognition molecules), and multimeric systems (coagulation cascade proteins, and the prophenoloxidase system) (Aguirre-Guzmán *et al.*, 2009). The ever more frequent incidence of disease outbreaks and the ensuing economic losses, encourage the study of these defense mechanisms, since they offer novel alternatives to cope with diseases (Aguirre-Guzmán *et al.*, 2009).

Huang *et al.* (2013), in experimental disinfection trials in *L. vannamei*, using *V. harveyi* proved that the resistant animals showed a quicker, greater, and faster immune response in eliminating the pathogen, compared to normal animals. It suggests that the stimulation of the immune system by increasing the basal levels of some of its components may be relevant in the elimination of infectious agents and maintenance of homeostasis. The application of probiotics in shrimp culture with the purpose of stimulating the immune system is one of the most widely explored research areas. In that sense, various papers report the way in which the indicators of shrimp's immunological state are modulated by probiotics (Table 1) (Mujeeb Rahiman *et al.*, 2010; Shen *et al.*, 2010; Wang and Gu, 2010; Zokaeifar *et al*., 2014; Franco *et al.*, 2016b).

The pro-phenoloxidase system (proPO) is one of the main components of the immune system of peneid shrimp, whose final step in the enzymatic cascade ends with the activation of enzyme phenoloxidase through proteolysis, which induces toxic phenolic derivatives and melanin. That practically intact substance has microbicide activity, and it contributes to confinement of the pathogen at the entry site. Superoxide dismutase (SOD) is one of the main defense mechanisms against oxidative stress caused by pollution, infections, hypoxia, hyperoxia, temperatures, and immunostimulants (Neves *et al.*, 2000). Peroxidase and catalase are also important against oxygen reactive species (Castex *et al.*, 2010; and Sánchez Ortiz *et al.*, 2013). Moreover, the relevance of bacteriolytic enzymes against pathogenic bacteria, like lysozymes, is well documented (Burge *et al.*, 2007; Karthik *et al.*, 2014). Ezymatic activity and lysozyme expression in shrimp may be stimulated through the addition of probiotics, both in experimentally induced infections (Maeda *et al.*, 2013) and production-scale studies (NavinChandran *et al.*, 2014; Miandare *et al.*, 2016).

Quantification of enzymatic activity and gene expression of proteins involved in the immune response, are often analyzed as indicators of the immunological state of shrimp (Miandare *et al.*, 2016; Sánchez-Ortiz *et al.*, 2016). Zokaeifar *et al.* (2014) evaluated the behavior of gene expression of the immune system in experimental conditions, in *L. vannamei* treated with *Bacillus subtilis* L10 and G1 for eight weeks, and then infected with *Vivrio harveyi*. Thus, the expression of proPO, and other genes of pathogen-associated molecular pattern-binding proteins was observed to increase in comparison to the established controls. Additionally, accumulated mortality was found to decrease among the animals treated in comparison to the control (36.7-50% vs 80% for the control). These results indicated that the increase in the expression of the genes evaluated is linked to the application of probiotics, which led to higher resistance in animals. Wang and Gu (2010) made a study in which they evaluated the effects of *Lactobacillus acidophilus* RS058, *Rhodopseudomonas palustris* GH642, and *Bacillus coagulans* NJ105 on the growth and immune response of *L. vannamei* juveniles in 12 500 L tanks, for 35 days. The enzymatic activity of PO and SOD, and the growth parameters evaluated were higher in the shrimp treated with probiotic strains in relation to the controls. However, peroxidase activity showed no differences among the groups mentioned.

Cell immunity was another parameter of interest associated to the immunological state of peneid shrimps; the increase in the number of circulating hemocytes is linked to a greater phagocytic capacity and elimination of foreign agents (Sánchez-Ortiz *et al.*, 2015). Xia *et al.* (2014) upon administration of a strain of *Arthrobacter* sp. CW9 in the culture water of *L. vannamei* for 24 days, detected an increase in phagocytic activity, increased effectiveness in the elimination of pathogens, and better growth and survival, in comparison to the control group. Similar results were described by NavinChandran *et al.* (2014) for *P. monodon* treated with *Bacillus cereus*.

Many recent studies suggest the use of immunostimulants, based on previous results of probiotic or probiotic derivatives administration; however, some authors warn about the adverse effects of prolonged immunostimulation in shrimp (Smith *et al.*, 2003).

Anti-Quorum sensing activity

Pathogen antagonism is one of the most broadly used selection criteria to achieve new strains with probiotic potential (Bright Singh *et al.*, 2014; Shazwani *et al.*, 2015). Nevertheless, some researchers suggest an alternative approach based on a reduction of pathogenic virulence, without compromising growth directly (Czajkowski and Jafra, 2009; Brackman *et al.*, 2011; and Defoirdt *et al.*, 2012). This perspective, known as antivirulence therapy relies on the expression of many genes involved in bacterial pathogenicity, which is regulated by quorum sensing (QS): a process of cell-to-cell communication in bacteria, mediated by signaling molecules with low molecular weight, which cause population-density dependent responses (Defoirdt *et al.*, 2010). QS interference, or quorum quenching might allow bacterial disease control with a minor tendency to develop resistance, and alter the normal microbiota in the host, in comparison to antimicrobials (Table 1) (Defoirdt, 2016). The strategies described to achieve that purpose include the utilization of inhibitors and degrading enzymes of AHL (acyl-homoserine-lactones; signaling molecules) (Brackman *et al.*, 2008; Pande *et al.*, 2013; Torres *et al.*, 2013; Pande *et al.*, 2015; and Torres *et al.*, 2016). The AHL degrading enzymes are widespread among bacteria, particularly genus *Bacillus* (Defoirdt *et al.*, 2011). Ramesh *et al.* (2014) isolated *Bacillus* strains from the intestine of *Penaeus monodon* and their activity was evaluated against *Vivrio* spp. and anti-QS. Of the 12 isolates, only APV03 and APV07 showed both mechanisms. The administration of the strains alone or combined protected the postlarvae and juveniles of *P. monodon* against *Vivrio harveyi* infection, whereas the quorum quenching activity of the two strains was evaluated *in vitro* against the marker strain *Chromobacterium violaceum.* Yuniarti *et al.* (2015) demonstrated that a strain isolated from the intestine of *Penaeus monodon,* identified as *B. subtilis,* and producer of AHL degrading enzymes, protected *P. monodon* juveniles from *V. harveyi* infection. The strain was grown in coculture with *V. harveyi*, and it was able to inhibit pathogen growth and reduce AHL *in vitro* concentration in the medium. However, the *in vitro* results did not coincide with the ones observed in the *in vivo* protection assay. No differences were observed between the AHL concentrations between the *B.subtilis* treated groups and the control, though the concentration of AHL tended to decrease when the *B.subtilis* concentration increased in the medium.

Modes of administration

Probiotics produce beneficial effects in the gastrointestinal tract, especially. Therefore, many of the administration modes developed are oriented to increasing stability and facilitate assimilation (Table 1). The addition of probiotics in the diet is one of the most commonly used ways (Shen *et al.*, 2010; Boonthai *et al.*, 2011; Liu *et al.*, 2014), since probiotics are simultaneously incorporated in the food. It is associated with an enzymatic contribution to digestion and better use of the nutrients ingested (Zokaeifar *et al.*, 2012; Nimrat *et al.*, 2013). Additionally, periodicity in probiotic administration produces a favorable balance in the intestine of shrimp, with beneficial microorganisms that compete with other intestinal colonizers, such as *Vivrio* spp. and other pathogens (Luis-Villasenor *et al.*, 2013). However, in this type of application, the probiotic microorganisms are exposed to extreme physical and chemical conditions which might affect viability and reduce their effects on the host (Nimrat *et al.*, 2011).

Microencapsulation is an alternative method consisting in covering the cells with a polymer matrix, mainly alginates, thus enabling extension of culture storage periods, improving its viability in the feed and the intestinal tract of hosts, and protecting from bacteriophages (Nimrat *et al.*, 2012). Some live foods with high nutritional value and broad use in shrimp culture, like rotifers and *Artemia* spp. have also been evaluated as vehicles for probiotic administration (Hadiroseyani and Sutanti, 2014; Jamali *et al.*, 2015). This process is known as encapsulation, and it makes use of the filtering capacity of the organisms introduced by probiotics when they are added to the culture medium. Even when various authors refer to the functionality of encapsulations (Ziaei-Nejad *et al.*, 2006; Nimrat *et al.*, 2011; Jamali *et al.*, 2015), their moderate influence on the productive parameters of shrimp culture, their complex scale-up, and considerable cost, suggest the need to perform cost-effectiveness studies for application in the productive stage (Kumar *et al.*, 2016). Moreover, probiotics can be added directly in the water where the animals are cultured, especially those that can remove organic matter (Dalmin *et al.*, 2001) and toxic substances, thus improving the quality of the aquatic environment (Wang y Gu, 2010; Silva *et al.*, 2012; Laranja *et al.*, 2014; Franco *et al.*, 2016b).

Influence of probiotics on the productive parameters of shrimp culture

Disease control and prevention, mainly in the larval stages, is another critical issue of production in shrimp culture. In that sense, the antagonistic activity of probiotics to achieve increased animal survival has gained acceptance (Vaseeharan and Ramasamy, 2003; Luis-Villaseñor *et al.*, 2011). Balcázar and Rojas-Luna (2007) demonstrated the potential of *Bacillus subtilis* UTM 126, isolated from the intestine of *L. vannamei*, to control *V. harveyi* in shrimp culture. The treatment of *L. vannamei* juveniles using diet supplementation with 105 CFU.g-1 of the above mentioned strain produced a decrease in accumulated mortality of up to 18.25%, compared to 51.75% in the control group.

However, even when various studies prove the protection resulting from probiotic administration during a specific growth stage (either larval or post-larval), there are few references of their effect during shrimp ontogeny, and their importance to production. Ziaei-Nejad *et al.* (2006) evaluated the effect of a commercial probiotic application made of *Bacillus* spp. during growth and survival of *Fenneropenaeus indicus* in several stages of development (Table 1). A comparison of the effects of different ways of administration of a product (added to water or bio encapsulated in Artemia), between stages M-I and PL14, concluded that *Bacillus* spp. colonized the digestive tract of animals, which contributed to higher digestive enzymatic activity (protease, lipase, and amylase), humid weight, and survival, in relation to their respective controls. However, the different ways of administration evaluated for the probiotic did not show significant differences in survival or weight, even when *Bacillus* spp counts were slightly higher in the larvae treated with enriched Artemia. Besides, the application of probiotics in larval stages proved to be critical in improving growth parameters in ponds during the productive phase; the administration of probiotics by addition in the water resulted in low colonization of the intestine of adult shrimp. Another study involving larval and post-larval stages of *L. vannamei* made by Franco *et al.* (2016b), compared the effects on immunity and larval quality of CIGBC-232, isolated from the intestine of healthy shrimp, and commercial probiotic EPICIN-3W in production conditions. The results revealed that the application of CIBGC-232 significantly reduced the load of *Vivrio* spp. in the ponds. Besides, it increased animal weight and size, in relation to the commercial product.

Furthermore, Rengpipat *et al.* (2003) evaluated the effect of probiotic inclusion in the diet of *P. monodon* during production, in mud-bottomed ponds, for 100 days, in the warm and cool seasons. The administration of supplemented food resulted in greater survival and size of the animals treated in the two seasons, compared to the control. The application of the probiotic as feed additive during the trial resulted in more than 30% daily weight gain (DWG), and 28% more of survival, in relation to the control. The improvement of these parameters led to 49% higher annual estimated yields (two 100-day culture cycles) in shrimps on supplemented diet, whereas Melgar Valdés *et al.* (2013) concluded that the application of a commercial probiotic improved the production parameters of *L. vannamei*, as well as the quality of pond water under intense culture conditions.

Although most papers report the positive effects of probiotics in shrimp culture, some authors refer to the inefficiency or poor activity of commercial products at a larger scale (Xue *et al.*, 2016). Hence, quality assessment analysis of commercial products is required, along with determination of the optimum dose and mode of use before production.

CONCLUSIONS

Probiotics are involved in the productive parameters of shrimp, such as greater nutrient intake, enhanced immune system, and greater animal survival. Easy handling and safety make probiotic use an increasingly accepted practice; it improves the culture medium conditions and offers advantages for expansion and optimization of sustainable shrimp culture.

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Table1. Probiotics used in shrimp culture and their main effects

Stage	Probiotic species	Crustacean species	Administration	Beneficial effects	Reference
Post-larval	*Bacillus* sp.	*Penaeus monodon*	Addition to water	Improves survival against *Vivrio* spp. anti-QS activity	(Ramesh *et al.*, 2014)
Post-larval	*Bacillus subtilis*	*Penaeus monodon*	Addition to water	Improves survival against *Vivrio* spp. anti-QS activity	(Yuniarti *et al.*, 2015)
Post-larval	*Bacillus* spp.	*Penaeus monodon*	Added to food	Improves survival against *Vivrio* spp. and stress resistance, and stimulates growth	(Laranja *et al.*, 2014)
Post-larval	*Bacillus* sp.	*Penaeus monodon*	Added to food	Improves survival against *Vivrio*. sp and stimulates growth (WG)	(Rengpipat *et al.*, 2003)
Post-larval	*Bacillus subtilis*	*Penaeus monodon*	Addition to water	Improves survival against *Vivrio* spp.	(Vaseeharan and Ramasamy, 2003)
Larval and post-larval	*Bacillus* spp. (INVE Sanolife® MIC)	*Litopenaeus vannamei*	Addition to water and micro algae cultures, and bio-encapsulation	Improves survival and growth, decreased the load of *Vivrio* spp.	(Silva *et al.*, 2012)
Larval	*Bacillus* spp. (9 commercial products from China)	*Litopenaeus vannamei*	Addition to water	Improves survival and stimulates growth (DI)	(Xue *et al.*, 2016)
Post-larval	*Bacillus subtilis*	*Litopenaeus vannamei*	Added to food	Improves survival and stimulates growth (PP, WG, and AED)	(Zokaeifar *et al.*, 2012)
Post-larval	*Bacillus subtilis*	*Litopenaeus vannamei*	Addition to water	Improves water quality (reduction of ammonia, nitrite, and nitrate) and stimulates growth (AW, WG SGS, FCR, and DEA).	(Zokaeifar *et al.*, 2014)
Post-larval	*Arthrobacter* sp.	*Litopenaeus vannamei*	Addition to water	Improves survival, growth speed, and the immunological state	(Xia *et al.*, 2014)
	Bacillus subtilis	*Litopenaeus vannamei*	Added to food	Improves survival and the immunological state	(Tseng *et al.*, 2009)
Larval	*Bacillus* spp. (isolates and commercial: Epicin® and Alibio^{MR})	*Litopenaeus vannamei*	Addition to water	Improves survival and growth speed	(Luis-Villaseñor *et al.*, 2011)
Post-larval	*Psychrobacter* sp.	*Litopenaeus vannamei*	Addition to water	Improves survival, stimulates growth (AW and length), and the immune system	(Franco *et al.*, 2016a)
Larval and post-larval	*Bacillus licheniformis* and EPICIN 3W (commercial)	*Litopenaeus vannamei*	Addition to water	Stimulates growth (AW and length), and the immune system	(Franco *et al.*, 2016b)
Larval and post-larval	*Bacillus* spp., yeasts (*Debaryomyces hansenii* and	*Litopenaeus vannamei*	Addition to water, micro and bio-encapsulation	Stimulates growth (WG, SGS, size), improves survival, water quality (reduces nitrite and ammonia concentrations, and regulates the	(Nimrat *et al.*, 2011)

	Rhodotorula sp.) and micro algi (*Chaetoceros* sp.)			pH), and increases TBH.	
Larval	*Bacillus* sp.	*Artemia franciscana*	Addition to water	Improves survival and stimulates the immune system	(Niu *et al.*, 2014)
Post-larval	Commercial product (*Rhodopseudomonas palustris, Lactobacillus plantarum, Lactobacillus casei* and *S. cerevisiae*)	*Litopenaeus vannamei*	Addition to water	Improves survival, stimulates growth (SGS, FCR), and reduces production time; improves water quality (reduction on nitrate concentration, regulation of pH, reduction of organic matter).	(Melgar Valdés *et al.*, 2013)
Post-larval	*Lactobacillus* spp.	*Marsupenaeus japonicus*	Added to food	Improves survival and stimulates the immune system	(Maeda *et al.*, 2013)
Post-larval	*Bacillus endophyticus and B. tequilensis;* Alibio (commercial product)	*Litopenaeus vannamei*	Addition to water	Improves survival and modulates the composition of intestinal microbiota	(Luis-Villasenor *et al.*, 2013)
	Bacillus subtilis	*Litopenaeus vannamei*	Addition to water	Improves stress tolerance (temperature, nitrite, and salinity), and stimulates the immune system	(Liu *et al.*, 2010)
	Bacillus subtilis	*Litopenaeus vannamei*	Added to food	Improves survival and stimulates the immune system	(Liu *et al.*, 2014)
Post-larval	Photosynthetic bacteria and *Bacillus* sp.	*Litopenaeus vannamei*	Added to food	Stimulates growth (average DEA, protease activity, lipase, amylase, and cellulase)	(Wang, 2007)
Post-larval	*Bacillus* sp., *Lactobacillus* sp. (EM Korea Co. Ltd., Korea) and *Rhodobactor* sp. (Doosan EcoBizNet Co., Ltd., Korea)	*Fennerpenaeus chinensis*	Addition to water	Stimulates the immune system and growth, reduces oxidative stress	(Kim *et al.*, 2015)
Larval and post-larval	*Bacillus* spp. (commercial product: Protexin Aquatech)	*Fenneropenaeus indicus*	Addition to food, addition to water, and bio-encapsulation	Improves survival and stimulates growth (DEA, AW, SGS, FCR)	(Ziaei-Nejad *et al.*, 2006)
Post-larval	*Bacillus licheniformis, B.*	*Litopenaeus vannamei*	Added to food	Antiviral activity, improves survival, stimulates growth and the immune system	(Sánchez-Ortiz *et al.*, 2016)

	subtilis and *B. subtilis subsp. subtilis*	

WG: weight gain; DEA: digestive enzymatic activity; AW: average weight increase; SGS: specific growth speed; FCR: food conversion rate; ID: time reduction for metamorphosis; THB: total heterophobic bacteria