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REVIEW PAPER

EFFECTS OF PROBIOTIC *BACILLUS* SPECIES IN AQUACULTURE – AN OVERVIEW

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The ingestion of a large amount of certain types of beneficial bacteria can reduce the multiplication and development of pathogenic bacteria in the gut. A "probiotic" is a product that contains live microorganisms which positively influence the host intestinal microbiota by preventing the proliferation of pathogenic bacteria and promoting the growth and development of beneficial bacteria. *Bacillus* spp. are Gram-positive endospore-forming bacteria with beneficial effects in aquaculture industry. The dietary supplementation of *Bacillus* spp. in fish culture improved especially growth performance, immune response and the disease resistance of fish against pathogenic bacterial infections. The objective of the current paper is to review the recent published investigations reported in the scientific literature on the use of probiotic *Bacillus* spp. in aquaculture, focusing on their beneficial effects on the host. This review includes the main effects of *Bacillus* spp. administration in shrimp culture, carp culture, tilapia culture, and other fish culture.

Keywords: *Bacillus* spp., probiotics, aquaculture, beneficial effects

Probiotics

A probiotic is defined as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989). Verschuere *et al.* (2000) proposed a modified definition of the term "probiotic" as "a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment". Recently, the Food and Agriculture Organization of the United Nations (FAO), together with the World Health Organization (WHO), defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002).

This paper proposes a revised definition of the term "probiotic" as "a product that contains live microorganisms which positively influence the host intestinal microbiota by preventing the proliferation of pathogenic bacteria and promoting the growth and development of beneficial bacteria".

Probiotics are Gram-positive, lactic acid-producing bacteria that cause an increase in enzymatic activity, leading to a better feed digestibility. The criteria for lactic acid bacteria strains to be used as "probiotics" include (Sharma *et al.*, 2014): (i) exert a beneficial effect on the host; (ii) endure into a food product at high cell counts; (iii) survive during passage through the gastrointestinal tract; (iv) adhere to the intestinal epithelium; (v) produce antimicrobial substances; (vi) have antagonistic activity against pathogenic bacteria; (vii) stabilize the intestinal microflora; (viii) stable against bile, acid, enzyme and oxygen; and (ix) safety assessment (non-pathogenic, non-toxic and non-allergic). A probiotic organism must possess certain properties, such as (Kesarcodi-Watson *et al.*, 2008): (i) not be harmful to the host; (ii) be accepted by the host; (iii) reach the location where the effect is required to take place; (iv) work *in vivo* as opposed to *in vitro* findings; and (v) not contain virulence resistance genes.

Probiotics are used in: (i) human nutrition, as dietary supplements; (ii) animal feed, as growth promoters and competitive exclusion agents; and (iii) aquaculture systems, for enhancing the growth and disease-resistance (Dong *et al.*, 2009). Methods to select probiotic bacteria for use in the aquaculture industry might include the following steps (Gomez-Gil *et al.*, 2000; Sahu *et al.*, 2008): (i) collection of background information; (ii) acquisition of potential probiotics; (iii) evaluation of the ability of potential probiotics to out-compete pathogenic strains (*in vitro* evaluation); (iv) evaluation of pathogenicity and survival test; (v) evaluation of the effect of the potential probiotics in the host (*in vivo* evaluation); and (vi) economic cost/benefit analysis. The stability of probiotics is influenced by various factors, such as: species, strain biotype, water activity, temperature, hydrogen-ion concentration, osmotic pressure, mechanical friction and oxygen (Wang *et al.*, 2008). The common Gram-positive probiotic organisms used in aquaculture industry include: *Bacillus*, *Lactobacillus*, *Lactococcus*, *Micrococcus*, *Streptococcus*, *Enterococcus*, *Carnobacterium*, and *Weissella* species (Irianto & Austin, 2002; Nayak, 2010). Probiotics, such as *Bacillus* spp. (a Gram-positive endospore-forming bacteria), can be introduced into the culture environment to control and compete with pathogenic bacteria as well as to promote the growth of the cultured organisms. In addition, *Bacillus* spp. are non-pathogenic and non-toxic microorganisms without undesirable side-effects when administered to aquatic organisms (Farzanfar, 2006). Their levels of incorporation range between 10^7 and 10^8 CFU/g feed, and the application period between 2–4 weeks for enhanced immune response and improved host survival (Kiron *et al.*, 2012). Spore probiotics *Bacillus* spp. (such as *Bacillus subtilis*, *Bacillus clausii*, *Bacillus cereus*, *Bacillus coagulans* and *Bacillus licheniformis*) have a number of advantages over non-spore forming bacteria (such as *Lactobacillus* spp.), as follows (Cutting, 2011): (i) spores can be stored at room temperature in a desiccated form without any effect on viability; and (ii) spores are capable of surviving the low pH of the gastric barrier.

The benefits of probiotics in aquaculture farming include several effects, such as (Nayak, 2010; Mohapatra *et al.*, 2013): (i) modulation of immune system; (ii) immunity stimulation under *in vitro* and *in vivo* conditions; (iii) feed conversion improvement on the organism through microbial colonization of the digestive tract; and (iv) disease control.

The mechanisms of action regarding the beneficial effect of probiotics on the host are as follows (Verschuere *et al.*, 2000; Balcázar *et al.*, 2006; Tinh *et al.*, 2008; Sahu *et al.*, 2008; Mohapatra *et al.*, 2013; Pandiyan *et al.*, 2013; De *et al.*, 2014): (i) production of inhibitory compounds; (ii) competition for chemicals or available energy; (iii) competition for adhesion sites; (iv) competition for nutrients; (v) improvement of water quality (vi) enzymatic contribution to digestion; (vii) modulation of host immune responses; and (viii) antagonistic activity (antibacterial, antiviral and antifungal activity).

Effects of *Bacillus* spp. in shrimp culture

According to Zokaeifar *et al.* (2014), the administration of *Bacillus subtilis* at a dose of 10^8 CFU/mL in the rearing water of shrimp (*Litopenaeus vannamei*) for 8 weeks confers beneficial effects for shrimp culture, such as: (i) water quality; (ii) growth performance; (iii) digestive enzyme activity; (iv) immune response; and (v) disease resistance. In addition, administration of *Bacillus subtilis* strains increased total protein, protease and amylase activity of treated shrimp after the colonization in the gastro-intestinal tract. Ziaei-Nejad *et al.* (2006) investigated the effects of commercial *Bacillus* probiotic strains (*Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus polymyxa*, *Bacillus laterosporus* and *Bacillus circulans*) on the digestive enzyme activity, survival and growth of white shrimp (*Fenneropenaeus indicus*).

The results showed that the administration of *Bacillus* bacteria to shrimp resulted in an increase of lipase, protease and amylase activity in the shrimp's digestive tract. Furthermore, adding the probiotic during the hatchery stages and then continuing throughout the farming stages is necessary to maximize survival of the shrimp at 11–17% higher compared to the control (about 71.5%) and wet weight at 8–22% higher compared to the control (about 12.26 g). Additionally, Seenivasan *et al.* (2012) found that *Bacillus subtilis* can be used as a probiotic at 3% inclusion in the experimental diet of *Macrobrachium rosenbergii* culture (a species of freshwater shrimp) to enhance the survival, growth, nutritional indices, tissue biochemical components and energy utilization performance. On the other hand, Luis-Villaseñor *et al.* (2011) showed antagonistic activity of four *Bacillus* strains isolated from the guts of healthy wild adult shrimp using a daily concentration of 10^5 CFU/mL against pathogenic strains of *Vibrio*, and the potential and efficiency of these probiotics to improve the rate of development, reaching a final index of development of 7.00 compared to the control (about 5.76), and a rate of survival reaching 67% compared to the control (about 4.9%) of shrimp *Litopenaeus vannamei* larvae. Balcázar *et al.* (2007) investigated the effect of four probiotic bacterial strains (*Bacillus subtilis* UTM 126, *Vibrio alginolyticus* UTM 102, *Roseobacter gallaeciensis* SLV03, and *Pseudomonas aestumarina* SLV22), isolated from the gastrointestinal tract of pacific white shrimp *Litopenaeus vannamei*, against the shrimp-pathogenic bacterium *Vibrio parahaemolyticus* PS-017. The mortality of the shrimp supplemented with probiotics was between 17%

and 22% compared with the mortality of infected shrimp not treated with probiotics (33%), while the mean mass of the surviving shrimp after 28 days was 3.98 ± 0.1 g in comparison with control groups (3.46 ± 0.2 g). Furthermore, a study of Li *et al.* (2007) revealed that *Bacillus licheniformis* administered to white shrimp *Litopenaeus vannamei* water culture at 10^5 CFU/mL inhibited *Vibrio* species by competitive exclusion, and improved shrimp immunity by increasing their total haemocyte count activity, superoxide dismutase activity and phenoloxidase activity. Liu *et al.* (2010) showed that *Bacillus subtilis* E20 (isolated from the human health food, natto) has probiotic potential for white shrimp *Litopenaeus vannamei* larval breeding by adding the probiotic to larval rearing water at a level of 10^9 CFU/L, thus improving the larval survival rate, development, stress resistance, and immune status. Moreover, Nimrat *et al.* (2012) showed that the administration of mixed *Bacillus* probiotics (isolated from *Penaeus monodon* intestine) improved growth rate (with average values between 19.5% and 19.6%) and survival rate (with values between 83.9% and 84.7%) of 22-day-old postlarval white shrimp (*Litopenaeus vannamei*), increased beneficial bacteria in shrimp and culture water and enhanced water quality parameters (especially pH, ammonia and nitrite). On the other hand, Gullian *et al.* (2004) investigated the effect of competitive exclusion by administering live probiotic bacteria (*Bacillus* P64) to wild adult shrimp (*Penaeus vannamei*) challenged with pathogenic bacteria (*Vibrio harveyi*).

Results demonstrated that isolated strain *Bacillus* P64 showed both probiotic and immunostimulatory effects in the prevention of wild shrimp diseases by competitive exclusion or by stimulation of a defence reaction in the host. The colonization percentage reached by *Bacillus* P64 was 58% while the inhibition against *Vibrio harveyi* was 34%. Furthermore, Guo *et al.* (2009) investigated the effect of three probiotics (*Bacillus foraminis*, *Bacillus cereus biovar toyoi* and *Bacillus fusiformis*), isolated from hydrogen-producing fermented solution, against pathogenic bacteria in shrimp larviculture. Results showed that a daily addition of *Bacillus fusiformis* at an optimum concentration of 10^5 CFU/mL can improve the survival and accelerate the metamorphosis of the larval shrimp *Penaeus monodon* and *Litopenaeus vannamei* by competitive exclusion effect against pathogens.

Effects of *Bacillus* spp. in carp culture

Li *et al.* (2012) indicated that *Bacillus* preparations isolated from the pond of grass carp (*Ctenopharyngodon idellus*) and added into the culture water (10^8 CFU/m^3 *Bacillus subtilis* (with denitrifying function) and *Bacillus licheniformis*) and feed (0.5% *Bacillus subtilis* mixture, containing 10^8 CFU/kg diet) per 7 days can increase the immunity activity (serum immunoglobulin levels and most of non-specific immune parameters content) and enhance the antioxidant ability of grass carp. Wang & Xu (2006) found that the addition of probiotics (1 g/kg photosynthetic bacteria and 1 g/kg *Bacillus* spp. isolated from the pond of common carp *Cyprinus carpio*) in carp basal diets improved growth performances, feed conversion ratio and digestive enzyme activities. Moreover, Wang (2007) showed that the probiotic-supplemented diets, consisting of 5 g/kg photosynthetic bacteria

(*Rhodobacter sphaeroides*) and 5 g/kg *Bacillus* spp. (*Bacillus coagulans*) isolated from a carp (*Cyprinus carpio*) pond, resulted in an increase of growth performance (final weight, daily weight gain and relative gain rate) of shrimp *Penaeus vannamei*. Additionally, Das *et al.* (2013) suggest that *Bacillus amyloliquefaciens* FPTB16 is a potential probiotic, in terms of improving immunity and disease resistance of Indian carp species catla (*Catla catla*) against pathogenic bacteria *Edwardsiella tarda* infection, with an optimal dietary supplementation of 10^9 CFU/g.

Effects of *Bacillus* spp. in tilapia culture

According to Aly *et al.* (2008a), two gram-positive bacteria (*Bacillus pumilus* and *Bacillus firmus*) isolated from tilapia (*Oreochromis niloticus*) showed probiotic activity and *in vitro* inhibitory effects against pathogenic bacterium *Aeromonas hydrophila*, and did not cause disease sings or mortalities when injected into the fish. Zhou *et al.* (2010) found that the treatment with probiotics (such as *Bacillus coagulans* B16) as water additives at a final concentration of 10^7 CFU/mL every 2 days could be used to enhance immune response and growth performance of tilapia. Moreover, Aly *et al.* (2008b) evaluated the effect of two probiotic bacteria (*Bacillus subtilis* at 0.5×10^7 bacteria/g diet and *Lactobacillus acidophilus* at 0.5×10^7 bacteria/g diet) on the immune response, resistance and *in vitro* inhibitory activity of Nile tilapia (*Oreochromis niloticus*) against challenge infections. The results showed that potential probiotics can be used to enhance the immune and health status (especially *Bacillus subtilis* which inhibited the development of *Pseudomonas fluorescens*), improving the disease resistance and enhancing the growth performance of Nile tilapia. On the other hand, He *et al.* (2013) evaluated the effects of low doses of dietary *Bacillus subtilis* C-3102 on the production, intestinal cytokine expression and adhesive bacteria of hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*). The results showed that the administration of low-level (10^5 CFU/g) *Bacillus subtilis* C-3102 for at least 28 days ensured the expression of beneficial host genes and healthy intestinal microbiota.

Effects of *Bacillus* spp. in other fish culture

Avella *et al.* (2010) showed the benefit of *Bacillus* probiotic mixture administration (composed by *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilus*), in terms of stress response and growth, as a valuable feed additive in sea bream larviculture. In fact, results showed that the *Bacillus* mixture increased both body weight and standard length in sea bream larvae (47 days post hatch) and juveniles (75 days post hatch). Furthermore, Cha *et al.* (2013) investigated the efficacy of *Bacillus subtilis*, *Bacillus pumilus*, and *Bacillus licheniformis*, as dietary supplements against *Streptococcus iniae* in olive flounder (*Paralichthys olivaceus*), and as water additives to increase fish survival. The results demonstrated that *Bacillus subtilis* has beneficial effects on growth performance, non-specific immune response and disease resistance of olive flounder (at a level of 0.5%) and on the quality of the rearing water. On the other hand, Liu *et al.* (2012) found that dietary *Bacillus subtilis* E20 mixture administration at 10^8 CFU/g for 28 days of feeding colonized the intestines of grouper *Epinephelus coioides* (commercial mariculture fish), improved the feed efficiency and growth rate, and enhance immunity and resistance against *Streptococcus* species and grouper iridovirus. Furthermore, Zhao *et al.* (2012) showed that the potential probiotic *Bacillus subtilis* T13 (isolated from healthy sea cucumber intestine) can improve growth performance, and up-regulate innate immunity together with increased resistance to skin ulceration disease caused by *Vibrio splendidus* infection in juvenile sea cucumbers *Apostichopus japonicus*, at dosage of 10^9 CFU/g diet. Touraki *et al.* (2012) evaluated the effect of two potential probiotics (*Bacillus subtilis* and *Lactobacillus plantarum*), in terms of survival and protection against vibriosis.

Results showed that *Bacillus subtilis* could be used as probiotic bacteria, administered at 10^9 CFU/mL of culture, through the live fish feed *Artemia franciscana* nauplii, to protect European sea bass larvae (*Dicentrarchus labrax*, L.) against pathogen *Vibrio anguillarum* infection. Moreover, Ramos *et al.* (2013) showed that dietary supplementation at 1.5 g/kg diet with multi-species probiotic (*Bacillus*, *Pediococcus*, *Enterococcus*, and *Lactobacillus* species) modulated gut microbiota and improved growth performance of juvenile rainbow trout (*Oncorhynchus mykiss*).

Conclusions

Probiotics are Gram-positive, lactic acid-producing bacteria that cause an increase in enzymatic activity, leading to a better feed digestibility. The mode of action of probiotics consists primarily in the development, maintenance or restoration of beneficial intestinal bacteria by exclusion of pathogenic bacteria. The purpose of this study was to evaluate the beneficial effects of probiotic *Bacillus* spp. administration in aquaculture industry. The administration of *Bacillus* spp. in fish culture increased lipase, protease and amylase activity of fish after the colonization in the gastro-intestinal tract. Furthermore, *Bacillus* spp. can be introduced into the culture environment to control and compete with pathogenic bacteria. The administration of probiotic *Bacillus* spp. improved growth performance, immune response, disease resistance and the survival of fish in the aquaculture system.

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