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Review of Probiotics in Aquafeeds Mechanism of Action and Current Status

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ABSTRACT

The demand for cultured carp species has grown tremendously during the last decade due to their high market value. Probiotics use in aquaculture has gained attention as microbial candidates to maintain the health and the wellbeing of many aquaculture animals. Among the many microbial candidates, probiotic has sporulation capacity that makes them survive harsh environmental conditions, is non-pathogenic and non-toxic when fed to fish, and can produce antimicrobial substances making them more suitable candidates. Present review summarizes the results of probiotic administration on growth performance, gut physiology, intestinal microbiota, immune response and health status of different fin fish as well as shell fish species. Furthermore, this study tries to cover the gaps in existing knowledge and suggest issues that merit further investigations.

KEYWORDS: Dietary probiotic, Growth, Gut microbiota, Immune response.

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INTRODUCTION

The term "probiotics" was coined by Lilly and Stillwell (1965), literally meaning "for life" and they defined probiotics as "substances produced by one protozoan which stimulates the growth of another". Metchnikoffs (1907) defined probiotics as "microbes ingested with the aim of promoting good health". Furthermore, Parker (1974) modified the definition as "organism and substance which contribute to the intestinal microbial balance". Later, Fuller (1989) redefined probiotics as a "live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance". Tannock (1997) explained probiotic, as "living microbial cells administered as dietary supplements with the aim of improving the health of gastrointestinal tract". Finally, Gatesoupe (1999) defined probiotic as "a viable mono or mixed culture or mixed culture of the organism which when applied to the animal or man beneficially affects the host by improving the properties of indigenous flora". Considering the difference between environment in aquatic ecosystem and those terrestrial animals, a modified definition proposed for probiotics in aquaculture by Merrifield et al. (2010b) as, "a probiotic organism can be regarded as a live, dead or component of a microbial cell, which is administered via the feed or to the rearing water, benefiting the host by improving disease resistance, health status, growth performance, feed utilization, stress response or general vigor, which is achieved at least in part via improving the hostsmicrobial balance or the microbial balance of the ambient environment."

In the last decades, considerable efforts were made for the development of alternative strategies to improve the health condition of animals. Probiotics have attracted particular interest due to their broad application in larval and early fry stages where vaccines cannot be used. In such a situation, minor improvement of the non-specific immune system or the beneficial intestinal microbial flora may reduce mortality significantly.

Probiotic Strains

Probiotics selected for dietary application should have a beneficial effect on the host, and its evaluation must be carried out on the basis of criteria such as the ability of cells to produce metabolites and enzymes, colonization properties, factors affecting the strain survival, and interactions with host in terms of pathogenicity. Bacterial groups such as lactic acid bacteria (LAB) sp., *Bacillus sp*., and *Bifidobacterium sp*. as probiotics have been identified and are mostly used in aquaculture.

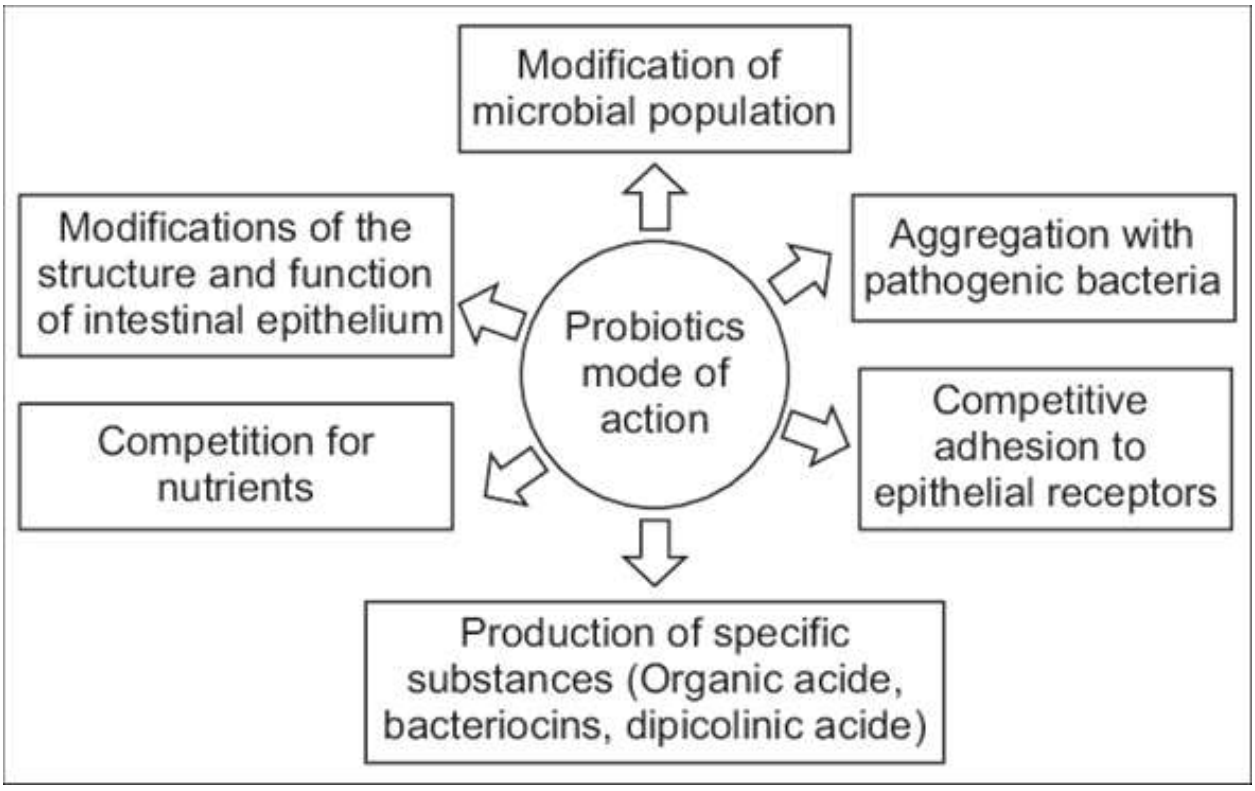
Characteristics of Potential Probiotic Strains

The major charecterstics include accurate taxonomic position, typical inhabitants of the targeted species, capable of survival, proliferation, and metabolic activity in the target site (which implies: resistance to gastric acid and bile, ability to persist, albeit for short periods, in the gastrointestinal tract, adherence potential preferred, ability to compete with the resident flora), production of antimicrobial substances, antagonism (*in vivo*) towards pathogenic bacteria, ability to modulate immune responses, ability to exert at least one clinically documented health benefit, genetically stable, amenability of the strain and stability of the desired characteristics during processing, storage, and delivery, viability in high populations and d esirable organoleptic and technological properties when included in fermentation processes

Mode of Action

Fuller (1989) and Verschuere et al. (2000b) described modes of action for probiotics. While considering the possible probiotic effect *in vivo*, one has to make a distinction between the intrinsic ability of the strain to positively influencing the host and its ability to reach and maintain itself in the location where the effect is to be exerted. Similarly, if the candidate probiotic is not capable of efficient proliferation in the gut after being ingested, it is improbable that it will exert strong effects unless it is added regularly through the diet (Fig.1).

Fig 1. Mechanism of probiotic action



Production of antimicrobial compounds

Probiotic microorganisms have the ability to release chemical substances with bactericidal or bacteriostatic effect on pathogenic bacteria that are in the intestine of the host, thus constituting a barrier against the proliferation of opportunistic pathogens. In general, the antibacterial effect is due to one or more of the following factors: production of antibiotics, bacteriocins, siderophores, enzymes (lysozymes, proteases) and/or hydrogen peroxide, as well as alteration of the intestinal pH due to the generation of organic acids (Verschuere et al. 2000). Taoka et al. (2006) showed that viable probiotics administered to tilapia *Oreochromis niloticus*, increased nonspecific immune response, determined by parameters such as lysozyme activity, neutrophile migration, and bactericidal activity, which improved the resistance of fish to infection by *Edwardsiella tarda*. In turn, Robertson et al. (2000) isolated a strain of *Carnobacterium* sp. from salmon bowel and administered alive to rainbow trout and Atlantic salmon, demonstrating *in vitro* antagonism against known fish pathogens: *Aeromonas hydrophila, A. salmonicida, Flavobacterium psychrophilum, Photobacterium damselae,* and *Vibrio* species. There is also evidence on the effect of dead probiotic cultures consisting on a mixture of *Vibrio fluvialis* A3-47S, *Aeromonas hydrophila* A3-51, and *Carnobacterium* BA211, in the control of furunculosis in rainbow trout. For this specific case, the number of leukocytes was greater than with live cells, in fact, the data suggest that cellular immunity more than humoral factors was involved in the benefits of these preparations of inactivated bacterial cells. In the case of shrimp, studies have focused on the evaluation of probiotics such as *Bacillus cereus, Paenibacillus polymyxa,* and *Pseudomonas* sp. PS-102 as biocontrol agents against pathogens of various *Vibrio* species. Probiotic strains isolated from the gastrointestinal tract of clownfish (*Amphiprion percula*) have been used to inactivate several pathogens such as *Aeromonas hydrophila* and *Vibrio alginolyticus* among others. The intestine of the host comprises particular antimicrobial substances, is thought to constitute a barrier against the proliferation of pathogens, and exerts its effects by the production of organic acids and the production of bacteriocins (Williams and Vickers, 1986).

Competition for nutrients

The competition of nutrients has been considered among the mechanisms through which probiotics inhibit pathogens (Ringø et al., 2016). Previous study has reported that competition for iron is an essential element in marine bacteria (Verschuere et al., 2000). The majority of bacteria need Iron for their growth. However, there is limited available of iron in the tissues and body fluids of animals (Verschuere et al., 2000). The siderophores which are iron-binding agents, help bacteria to obtain the necessary amount of Iron for their growth. There is direct relation between production of siderophore and virulence of some pathogens (Gram et al., 1999). The beneficial effects of Gram-positive genus Bacillus on water quality in culture environment has been reported in previous studies (Rafiee and Saad, 2005; El-Haroun et al., 2006; Hai, 2015; Dawood and Koshio, 2016). It seems that genus Bacillus is more effectual for converting organic matter to CO2 as well as balancing phytoplankton production (Balcázar et al., 2006). It has been reported that supplemented F. vannamei feed with Bacillus sp., Saccharomyces cerevisiae, Nitrosomonas sp., and Nitrobacter sp. (a commercial product) could decrease the concent rations of inorganic nitrogen and phosphate from 3.74 to 1.79 mg/L and 0.1105 to 0.0364 mg/L, respectively (Li et al., 2006). In addition, probiotics also enhanced growth performance and feed utilization in aquatic animals through increasing digestive enzymes activity (Yu et al., 2009; Zokaeifar et al., 2012; Hoseinifar et al., 2017a). For example, Van Hai et al. reported that dietary probiotics (Pseudomonas aeruginosa and Ps. Synxantha) enhanced western king prawn growth performance (Van Hai et al., 2009; Hai et al., 2010). Recently research by Faturrahman et al. also revealed dietary probiotic (Vibrio Alg3.1RfR-Abn1.2RfR-enriched protein) improved growth rate of Haliotis asinine (Rohyati, 2015). The incease of digestive enzyme

activity and improvement of the digestive process following treatment with probiotic has been attributed to production of extracellular enzymes such as proteases, carbohydrolases and lipases (Arellano-Carbajal and Olmos-Soto, 2002; Leonel Ochoa-Solano and Olmos-Soto, 2006; Soleimani et al., 2012; Eshaghzadeh et al., 2015;Hoseinifar et al., 2015a,b). Furthermore, considering provision of vital nutrients like fatty acids, biotin and vitamins, probiotics might be a complementary food source (Verschuere et al., 2000). Competitive exclusion is preliminary based on the principle to reduce intestinal colonization by enteric pathogens sharing the common environment (Barrow, 1992). This competition may be for nutrients and energy. Verschuere et al. (2000b) reported that the aquatic environment is dominated by heterotrophs which compete for organic substrate, carbon, and energy sources. He also described the protective action of selected bacteria by competing with the pathogen for chemicals and available energy.

Verschuere et al. (1999) selected several strains with a positive effect on the survival and growth of artemia juveniles. It was suggested that the selected bacteria exerted their protective action by competing with the pathogen for chemicals and available energy. Reid et al. (1993) effectively correlated the ability of siderophores to scavenge iron from the environment and the reduction of the pathogenic Vibrio count in salmon. The requirement for iron is high for many pathogens, including *V. anguillarum*. In a challenge test with this bacterium, salmon mortality increased linearly with dietary iron content. Smith and Davey (1993) reported that fluorescent Pseudomonas F 19/3 is capable of inhibiting the growth of *Aeromonas salmonicida* in culture media due to competition for iron.

Competition for adhesion site

One possible mechanism for preventing colonization by pathogens is competition for adhesion sites on gut or other tissue surfaces. The ability to adhere to the enteric mucus and wall surface is necessary for bacteria to establish in the fish intestine. Further, bacterial adhesion that can be nonspecific to tissue surface is reported to have an important role during the initial stages of the pathogenic competition for adhesion receptors with pathogens. This was supported by Nurmi and Rantala (1973); Salminen and Wright (1993), described the probiotic effect might be due to the adhesion of the bacteria to the epithelial surface of the gut wall, and adhesion capacity also has been demonstrated for a fish pathogen like *V. anguillarum* and *Aeromonas hydrophila* in vitro and for candidates probiotic such as *Carnobacterium* strain K1 (Joborn et al. 1997) and *V. anguillarum* (Olsson et al. 1992; Tannock, 1997).

Competitive exclusion has been suggested as a mode of action of probiotic in prevention of pathogens (Mahdhi et al., 2012; Sorroza et al., 2012); achieved by colonization of probiotics in GI mucosal epithelium (Macey and Coyne, 2006; Merrifield et al., 2010a; Lazado et al., 2011; Korkeaaho et al., 2012). Different types of surface determinants suggested to be involved in probiotis interaction with intestinal epithelial cells and mucus which per se prevents pathogens colonization (so called competitive exclusion). The primary reason for this could be competitions for adhesion receptors (Montes and Pugh, 1993) which can antagonize pathogens (Luis-Villaseñor et al., 2011) and reduce their colonization (Chabrillón et al., 2005). This clearly shows the potential of probiotics administration as a substitute for antibiotics and other chemicals (Cheng et al., 2014). It has been reported that passive forces, electrostatic interactions, hydrophobic, steric forces, lipoteichoic acids were among the factors which affect adhesion of probiotics to attachment sites (Wilson et al., 2011).Westerdahl et al. (1991) stated that competition for attachment sites and nutrients following occupyingmucosal surfaces could be possible mode of action for protective effects of probiotic against pathogens.

Modification of microbial metabolism

Lactobacilli ferment lactose to lactic acid, thereby reducing the pH to a level that harmful bacteria cannot tolerate. Hydrogen peroxide is also pro-

duced, which inhibits the growth of gram negative bac-teria. It has also been reported that lactic acid producing bacteria of the *Streptococcus* and *Lactobacillus species* produce antibiotics (McDonald et al., 2002; Klose et al., 2010). The antagonistic effects of gut microbiota against pathogens and other organisms are due to competition for nutrients and adhesion sites, formation of metabolites such as organic acids and hydrogen peroxide and pro-duction of bacteriocins (Ringo et al., 2010). Probiotics also modify the metabolism of the microbial ecosystem in the large intestine to increase short chain fatty acid production and thereby increase sodium and water absorption and decrease colonic motility (Sakata et al., 1999).

Microorganisms can produce natural substances to prevent or inhibit pathogenic microbes (Rico et al. 2014). Microbes can display both specific resistance and multi-resistance. These resistance genes are inherited from generation to generation and can transfer to other bacterial species or strains through horizontal gene transfer. For instance, microbial pathogens such as *E. coli*, *Enterococcus* spp., and *Salmonella* spp. have been found to have resistant genes (Petersen and Dalsgaard, 2003). Several articles have reported that while probiotic strains, such as *Bacillus* spp., show resistance to penicillin and kanamycin, some LAB strains display multiple resistances against cefoxitin, chloramphenicol, penicillin, kanamycin, and oxacillin (Chantharasophon et al. 2011; Chemlal-Kherraz et al. 2012). It has therefore been suggested that probiotics should be free of plasmid-encoded antibiotic resistance genes.

Fuller (1989) displayed that increased or decreased enzyme activity are key factors for the modification of microbial metabolism. It may be caused due to a variety of clinical signs. Lactose intolerance can be altered using probiotic bacteria secreting enzymes â-galactosidase. Similarly, *L. acidophilus* in humans could suppress the activity of â-glucoronidase, nitroreductase and azoreductase. The activity of â-glucoronidase, nitroreductase and azoreductase can be suppressed using *L. acidophilus* (Fuller, 1989). Microbial metabolism can also be affected by pH changes due to the production of lactic acid by bacteria (De Bruin, 1976).

Modulation of immunity

The first defense line against infections is innate immune responses (or non-specific immune responses) which include different cells and mechanisms that protect host organism from infectious diseases. It has been reported that probiotics can affect the elements of non-specific immune system such as mono-nuclear phagocytes (monocytes, macrophages) and polymorphonuclear leukocytes (neutrophils), natural killer (NK) cells etc. Previous studies revealed increment of leucocytes (Korkea-aho et al., 2012), monocytes (Aly et al., 2008b), erythrocytes, granulocytes, macrophage, and lymphocytes in various fishes following treatment with probiotics (Kim and Austin, 2006a,b; Nayak et al., 2007; Kumar et al., 2008). For instance, rainbow trout fed Clostridium butyricum showed increased resistance against vibriosis through affecting phagocytic activity of leukocytes (Sakai et al., 1995). Furthermore, dietary Bacillus sp. S11 positively affected cellular and humoral immunity in tiger shrimp (Penaeus monodon) which resulted in protection against disease (Rengpipat et al., 2000). Also, combined administration of Bacillus and Vibrio sp. in young white shrimp showed beneficial effects on growth performance, survival as well as resistance against V. harveyi and white spot syndrome virus (Antony et al., 2011). The authors attributed the protection to elevation of phagocytosis and antibacterial activity; indeed immunomodulation. Beside these results on shrimps, dietary Lactobacillus rhamnosus (ATCC 53103) (105 CFU g”1) increased the respiratory burst in rainbow trout (Oncorhynchus mykiss) (Nikoskelainen et al., 2003). Therefore, probiotics are beneficial bacteria which not only capable of inhibiting pathogens, but also regulating the host immune system. Probiotics possess conserved microbe-associated molecular patterns (MAMPs), including peptidoglycan (PGN), lipoteichoic acids (LTA), S-layer protein A (SlpA),

exopolysaccharides (EPS), flagellin and microbial nucleic acids which can be recognized by certain pattern recognition receptors (PRRs), and induces a signaling cascade that can result in the production of cytokines, chemokines, and other effector molecules thus activating the immune response in the host (Bron et al., 2012; Remus et al., 2012). During past years, there was increasing interests toward determination of mode of action of probiotics on intestinal immune system. In this regard, the researchers evaluated the possible relationship between TLR signaling-mediated recognition of probiotics and activation of the intestinal immunity. For example, it has been reported that TLR2 signaling pathway was involved in recognition of probiotic Psychrobacter sp. SE6 and inducing subsequently immune responses in grouper Epinephelus coioides (Sun et al., 2014).

Immunostimulants are chemical compounds used for enhancing the immune system of animals against to infections by viruses, bacteria, fungi, and parasites. Sakai (1995) reported that bacterial compounds act as immunostimulants in fish and shrimp. The immune system in the larval stages of fish and prawn are poorly developed. Research trial shows that administration of lactic acid bacteria orally helps to increase resistance to enteric infections in warm-blooded animals. Norqvist et al. (1989) observed that vaccination of rainbow trout with attenuated *V. anguillarum* induces protection against *A. salmonicida*, and similar trends have been reported by Sakai et al. (1995) said that rainbow trout immersed in *V. anguillarum* bacterial solution showed increased security.

Enzymatic activity

It is well established that probiotics are beneficial to the intestinal health of host animals. Among the beneficial bacteria, *Agrobacterium* sp., *Brevibacterium* sp., *Clostridium* sp., *Microbacterium* sp., *Pseudomonas* sp., and *Staphylococcus* sp. can contribute to the host's nutrition, especially in supplying fatty acids and vitamins to host cells (Sakata, 1990; Ringø et al. 1995). Some fish gut microbiota may participate directly in the digestion processes of fish. Enzyme-producing microbiota such as *Bacillus* and *Enterobacteriacea* (*Acinetobacter* sp., *Aeromonas* sp., *Flavobacterium* sp., *Photobacterium* sp., *Pseudomonas* sp., *Vibrio* sp., *Microbacterium* sp., *Micrococcus* sp., *Staphylococcus* sp.), and some unidentified anaerobes and yeasts are potential contributors (Ray et al. 2012).

The metabolic and physiological roles of fish gut microbiota have been the subject of several studies. These microbiota are able to stimulate gut epithelial differentiation and proliferation, gut motility, protein uptake, nutrient metabolism, and innate immunity (Rawls et al. 2004, Rawls et al. 2006, Bates et al. 2006). However, these functional roles are mostly limited to the initial stage of fish fry. In bivalves and crustaceans, microbiota in the gut facilitate digestion by producing extracellular enzymes such as proteases and lipases, as well as providing necessary growth factors (Prieur et al. 1990, Wang et al. 2000). Despite numerous studies demonstrating that microbial activity in the digestive tract may be an important source of nutrients and enzymes to the host, it is difficult to attribute the exact contribution of gastrointestinal microbiota in exothermic animals because of the complexity and variable ecologies of the digestive tracts of different fish species (Ray et al. 2012).

The enzymes produced from the probiotic bacteria promote the digestion of feeds and detoxify the injurious metabolites liberated by the flora. Tovar et al. (2002) observed that feeding of live yeast *Debaryomyces hansenii* to sea bass (*Dicentrarchus labrax*) larvae leads to an increase in amylase and trypsin secretion. Further, Yanbo and Zirong (2006) evaluated alteration in digestive enzyme activities by dietary supplementation of *Bacillus sp.* and a mixture of probiotic bacteria in common carp. The *Bacillus sp*. isolated from *Cyprinus carpio* has considerable extracellular amylolytic, cellulolytic, proteolytic, and lipolytic activities.

Current status of probiotics in aquaculture

Farmed fish and shellfish are strongly influenced by the microorganisms of the surrounding water

(Verschuere et al. 2000; Defoirdt et al. 2011) because they are in constant contact with the water and continuously ingest it. The aquaculture ecosystem supports eukaryotes and commensal bacteria, and opportunistic pathogens can reach high densities in this favorable environment (Moriarty, 1998). Opportunistic pathogens such as *Vibrio* spp invade the host through the gut and invade fish through the gills and skin (Weber et al. 2010). Some of the most studied probiotic candidates belong to the *Firmicutes* phylum, namely LAB (lactic acid-producing bacteria) and *Bacillus* spp (Amoah et al. 2019; Azad et al. 2019; Balc´azar et al. 2008; Carnevali et al. 2004; Venkat et al. 2004; Araújo et al. 2016). Although they are not adapted to or common in the marine environment, LAB can tolerate acidic pH and bile salts, which enable them to survive in gut systems (Merrifield et al. 2010; Bentzon-Tilia et al. 2016). These bacteria can colonize the intestinal mucus, where they assist in the processing and uptake of feed, promoting the growth of the fish (Ringø et al. 2010; Vieco-Saiz et al. 2019). *Pediococcus acidilactici* was isolated from the gut of rainbow trout larvae (*Oncorhynchus mykiss*) and their feed (Araújo et al. 2016; Araújo et al. 2015). The strains were bioactive against common fish pathogens due to bacteriocin production. They performed well in safety assessments as they did not display antibiotic resistance, produce hemolysins, or degrade gastric mucin (Araújo et al. 2016). Other LAB bacteria such as *Carnobacterium maltaromaticum*, *L. curvatus*, *L. sakei*, *L. plantarum*, *L. lactis*, and *Leuconostoc mesenteroides* have also been isolated from the intestines of salmonids (Balca´zar et al. 2007). Some of these strains such as *L. lactis* CLFP 101, *L. plantarum* CLFP 238, and *L. fermentum* CLFP 242 were tested for their antibacterial effect and ability to inhibit adhesion of *Aeromonas hydrophila*, *A. salmonicida*, *Yersinia ruckeri*, and *V. anguillarum* to intestinal mucus from rainbow trout (*in vitro*) (Balca´zar et al., 2008). Despite its pathogenicity to some finfish and shellfish (Ben et al., 2009; Cao et al., 2018; Go´mez-Leo´n et al., 2005), the addition of *V. alginolyticus* to the culture water could reduce the occurrence of *V. parahaemolyticus* and increase the survival of white leg shrimp (*Litopenaeus vannamei*). This study suggested that the probiotic properties came from antagonism towards the target pathogens. *Shewanella putrefaciens* Pdp11 isolated from the skin of healthy gilthead seabream (*Sparus aurata*) (Chabrillo´n et al., 2005) was able to colonize the mucus and reduce adhesion of the pathogens *V. harveyi* and *Photobacterium damselae* subsp *piscicida* in gilthead seabream and Senegalese sole (*Solea senegalensis*) (Chabrillo´n et al., 2005). Further studies have revealed that *S. putrefaciens* Pdp11 can improve growth when added to the feed of juveniles of both fish species (SA´Enz de RodrigA´N˜Ez et al. 2009; Varela et al., 2010). This probiotic strain can also modulate the intestinal microbiota and expression of immune-related genes (Varela et al., 2010; Tapia-Paniagua et al., 2014) during high-stocking induced stress (Varela et al., 2010: Cordero et al., 2016). Altogether, this indicates that *S. putrefaciens* Pdp11can have multiple mechanisms, which act together to protect and improve the health of animals reared in aquaculture.

Table 1 shows the studies using probiotics in aquaculture. Aquaculture is mainly focused on increasing the production level in a limited area with minimal inputs. However, the occurrence of disease-causing agents is more prone to these practices, as commercialization of vaccination against infections is less practice in India. Ringo and Gatesoupe, 1998) reported that probiotics deserve much attention and possess a large scale of demands for cost-effective and sustainable aquaculture systems (Nayak and Savan, 1999).

Table 1. Studies using probiotics in aquaculture

Species (weight in g)	Probiotic	Doses and duration of administration	Observation	References
In fish/shrimp larvae/spawn stage				
Koi carp (0.26 g)	*L. acidophilus* and/or *S. cerevisiae*	10^6 CFU g^{-1} (45 days)	(↑) WG, SGR, FCR, FI, NPU	Dhanaraj et al. (2010)
Common carp (0.329 ± 0.01g)	*B. coagulans* (MTCC 9872), *B. licheniformis* (MTCC 6824) and *Paenibacillus polymyxa* (MTCC 122)	10^9 CFU g^{-1} (80 days)	(↑) SUR, FNW, SGR, FCR, PER, LZY, RBA, MPC, DR	Gupta et al. (2014)
Indian major carp species, rohu Egg size 38 day size (0.15 g) 68 day size (0.95 g)	*Lactobacillus* (*L. acidophilus; L. bulgaricus; L. casei; L. plantarum*) + *Bifidobacterium bifidum* + *S. cerevisiae* + *S. faecium* + yeast Torulopsis + *Aspergillus oryzae*	1, 1.5, and 1 doses (g kg^{-1} diet)	(↑) SUR, FNW, SGR	Jha et al. (2015)
In fish/shrimp fry stage				
Grass carp (2.10 ± 0.09 g)	*B. coagulans*, *Rhodopseudomonas palustris* and *L. acidophilus*	10^6 CFU g^{-1} (60 days)	(↑) FNW, WG (↔) PA, CEL, AMY	Wang (2011)
Javanese carp (4.5 ± 0.2 g)	*E. faecalis, L. fermentum* and *L. mesenteroides*	10^7 CFU g^{-1} diet (6 weeks)	(↑)WG, FCR, SGR, PER, LAB (↓)GNB (↔)CC	Allameh et al. (2015)
In fish/shrimp juveniles and adults stage				
Indian major carp species, catla (6.48 ± 0.43 g)	*B. circulans* PB7	2×10^5 cells 100 g^{-1} feed (60 days)	(↑) WG, FCR, PER, CCCP, CCL, PA, PHR, PHI, LEU, ACP, ALP, GOT, GPT, DR (↔)AMY	Bandyopadhyay & Mohapatra (2009)
Common carp (50–60 g)	*A. veronii, V.lentus,* And *F. sasangense*	1×10^8 cell g^{-1} (28 days)	(↑) LZY, C3, TSP, ALB, GLB, RBS, PHA, BLEU, 1L-1b, TNF-α, DR	Chi et al. (2014)
Indian major carp species, Catla (25–30 g)	*B. amyloliquefaciens* FPTB16	1×10^7, 1×10^8, and 1×10^9 (8 weeks)	(↑)SOD, MPC, LZY, TSP, DR	Das et al. (2013)

Common carp (152.3 mg)	*Streptococcus faecium, L. acidophilus* and *S. cerevisiae*	0.1% (9 weeks)	(↑) SUR, FNW, WG, SGR, PER, NPU, CCCP, CCL, CCA	Faramarzi et al. (2011)
Indian major carp species, Rohu (60 g)	*L. plantarum* VSG3	10^6, 10^8, 10^{10} CFU g^{-1} (60 days)	(↑) SGR, FCR, LZY, ACP, PHA, RBA, SOD, IgM, DR	Giri et al. (2013)
Gibel carp (7.55 g)	*S. cerevisiae*	1.5×10^{10} CFU (56 days)	(↑) FNW, WG, SGR, FCR (↔) LZY, C3	He et al. (2003)
Common carp (11.90 ± 0.13 g)	*S. cerevisiae* and/or amyloliquefaciens	10^{10} CFU S. cerevisiae + 1.2×10^{10} CFU B. amyloliquefaciens (8 weeks)	(↑) PIM, HSP70 gene expression (↔) FNW,WG, FCR, SUR, FI	Huang et al. (2015)
Indian major carp species, Rohu (15 ± 2 g)	*B. subtilis*	0.5×10^7, 1×10^7, 1.5×10^7 CFU g^{-1} (2 weeks)	(↑) RBA, SBA, DR	Kumar et al. (2008)
Indian major carp species, rohu (15 ± 2 g)	*B. subtilis*	0.5×10^7, 1×10^7, 1.5×10^7 CFU g^{-1} (2 weeks)	(↑) SUR, WG, LEU, Hb, TSP, GLB (↔) ALP, AST, ALT	Kumar et al. (2008)
Indian major carp species, rohu (6.0 ± 0.06 g)	*B. subtilis, Lactococcus lactis* and *S. cerevisiae*	(60 days)	(↑) WG, SGR, FCR, PER, ADCDM, ADC Protein, ADC Lipid, PA, LI, CCCP, CCL, THC	Mohapatra et al. (2012)
Indian major carp species, Rohu (60 ± 0.19 g)	*B. subtilis*	10^8 CFU g^{-1} (60 days)	(↑) TSP, GLB, ANTI, DR	Nayak et al. (2007)
Grass carp (45 g)	*B. subtilis* and *B. licheniformis*	1×10^8 CFU g^{-1} (4 weeks)	(↑) GLB, IgM, LZY, C3, MPC, SOD, T-AOC, GSH	Weifen et al. (2012)
Grass carp (35 g ± 5 g)	*S. xiamenensis* A-1, *S. xiamenensis* A-2, and *A. veronii* A-7	1×10^8 cells g^{-1} (28 days)	(↑) RBA, PHA, LZY, C3, TSP, ALB, GLB, DR, gene expression of (1 L-8, 1 L-1β, LZY-C, TNF-α)	Wu et al. (2015)
Grass carp (50 ± 2.5 g)	B. subtilis Ch9	1×10^9, 3×10^9 and 5×10^9 CFU kg^{-1} (56 days)	(↑) SGR, FCR, PA, AMY, LI, THC	Wu et al. (2012)
Common carp (5.9–7.1 g)	Bacillus sp.	10^{10} and 10^{11} CFU g^{-1} (60 days)	(↑) FNW,WG, FCR, PA, AMY, LI	Yanbo & Zirong (2006)

Larval stages

After hatching, the larvae come in direct contact with a mass of microorganisms residing in the aquatic environment. The fish eggs usually possess dense non-pathogenic and diverse adherent microbiota, which helps in protective tackles against pathogen colony formation. Gatesoupe (1997) finding displayed a better survival rate and resistance of turbot larvae to pathogenic *Vibrio*. Similar kinds of trends were reported in turbot larvae fed with lactic acid bacteria. Moreover, Douillet and Langdon (1993) also found an increment in the growth of *Crassostrea gigas* larvae, fed with bacterial strain CA2 (marine bacteria). Maeda and Liao (1992) also reported an improved growth rate of shrimp larvae compared to the control group using PM-4 strain (*Thalassobacter utilis*). Supplementation of *Lactobacillus* in the diet helps to increase the survival rate in turbot larvae during challenge study with pathogenic *Vibrio species* (Gatesoupe, 1997). A study of Harzevili et al. (1998) used Lactococcus lactis AR21, showed improvement in the growth of rotifers and inhibited the growth of *V. anguillarum*.

Fish juveniles and adults

An application of *Carnobacterium divergence* in the feed enhanced disease in Atlantic cod fry. In this line. Metaillier et al. (1993) displayed a significant growth rate in European sea bass (*Dicentrarchus labrax*) fed with a mixture of probiotic supplement diet. Similar trends were followed by Swain et al. (1996) and Hirata et al. (1998); results showed improved performance of rotifer *Brachionus plicatilis* in water by supplementation of mixed cultures consisting mainly of *Bacillus species*. Furthermore, Kennedy et al. (1998) reported improvement in larval survival rate, increased enzyme activity, and registered maximum growth in common snook, *Centropomus undecimalis* (Bloch), fed with Bacillus in the diet.

Probiotics function as digestion and growth promoter

Fuller (1989) stated dietary application of dietary probiotics in aquaculture would help reduce the application of antibiotics and synthetic chemicals. Several studies have proved the remarkable application of probiotics ineffective nutrient utilization, enhanced digestive enzymes activities, higher growth, and prevention of intestinal disorders. Probiotics are known to colonize the host, improve the intestinal microbial balance and affect the digestive process.

Several microorganisms, such as *Agrobacterium sp*., *Pseudomonas spp*., *Brevibacterium spp*., *Microbacterium spp*., and *Staphylococcus spp*., may contribute to nutritional processes in Arctic charr (*Salvelinus alpinus*),, may serve as a supplementary source of food so that fatty acids, vitamins (Sakata, 1990) and essential amino acids. Practically, a wide variety of microorganisms and their substrates have been reported to have a stimulatory function as a probiotic in specific growth rate, feed digestibility, and utilization efficiencies and survival of the aquatic animal species. In the case of enzymes, secreting proteases break peptide bonds and produce free amino acids that can then be absorbed by the host. The effectiveness of probiotics in the culturing of aquatic animals depends on factors such as hydrobiont species, body temperature, enzyme level, and genetic resistance of the host and water quality.

Probiotics function as immune system promoters

The larval fish, shrimps and other invertebrates are comprised of relatively less developed immune systems than the adult stages. Hence larvae are typically more prone to infection and primarily dependent on nonspecific immune responses. Bacterial load in the feed determines the colonization rate of bacteria in the digestive tracts. Consequently, beneficial bacteria stimulate the effect of pro-inflammatory cytokines on the activity of immune cells, antibodies, acid phosphatase, lysozymes, complement, and antimicrobial peptides in response to invasive pathogens. The study of some teleosts such as *Sparus aurata*, *Paralichthys dentatus*, *Scophthalmus maximus* and *Salmo salar* shows dramatically reduction in bacterial activity through the dietary supplementation of probiotics by live food and culture water. The rod-shape beneficial

bacteria, lactic acid bacteria (strains MM1 and MM4) were reported along with the secretion of hydrogen peroxide and bacteriocin-like substances, which have strong inhibitory activities against the pathogens of gram-negative *Vibrio metschnikovii* and *V. harveyi*, and gram-positive *Staphylococcus aureus* that infects orange-spotted grouper (*E. coioides*) (Sun et al. 2012). Similar trends were also reported by Nikoskelainen et al. (2003) showed that administration of lactic acid bacterium *Lactobacillus rhamnosus* (strain ATCC 53103) at a level of about 105 cfu g-1 feed stimulated the respiratory burst in rainbow trout (*O. mykiss*).

In general, shrimp possess an innate immune response, unlike higher vertebrates, which have an acquired immune response. Rengpipat et al. (2000) determined that the use of *Bacillus sp*. (strain S11) provided immune protection by activating both cellular and humoral immune defenses in tiger shrimp (*P. monodon*). In another study, dietary supplementation of a mixture of bacterial strains (*Bacillus spp.* and *Vibrios spp*.) in white shrimp juveniles have shown better growth performance and survival rate and gave a protective effect against the pathogens *V. harveyi* and white spot syndrome virus. This protection was attributed to the stimulation of the immune system by increasing phagocytosis and antibacterial activity (Balcazar, 2006). Bacillus spp. was used to improve and control the *Vibrio spp*. infection to penaeid shrimp.

Improvement in water qualities

According to Venkateswara (2007), probiotics have been reported to regulate micro flora, control pathogenic ones, enhances the decomposition of the undesirable organic substance, improve ecological environment by minimizing the toxic gasses like NH_3, N_20, H_2O_2, Methane etc, increases population of food organism in the water, increases nutritional level of the aquatic host and improve their immunity in the culture water. In several studies, improved water quality has been recorded during the addition of the probiotics especially with *Bacillus sp*. (Verschuere et al, 2000). The rationale is that Gram-positive *Bacillus sp*. are generally more effective in converting organic matter back to CO_2 than G-negative bacteria which could convert a greater percentage of organic carbon to bacterial biomass or slime.

Conclusion and future prospective

Administration of therapeutics and feed additives in aquatic environments has its limitations in comparison to terrestrial habitations; therefore the strategies employed for this area have been identified for several studies related to aquaculture. Among these finding, the most effective and affordable approach is utilization of probiotics as an alternative to chemicals and antibiotics have proven to be effective in promoting successful aquaculture, as they have the potential to improve water quality, increase tolerance to stress, generate high-quality livestock etc. Regarding all of these benefits, the routes of probiotic administration need to be more investigated. This review suggests that the best administration method should be selected according to age and size of fish, aquaculture system, and all other contributing factors. Although the direct addition of probiotics to the water has been shown to be effective in different studies, it cannot be proposed as the best way in all cases where probiotics are used. In conclusion, further research in the field of probiotic administration through water needs to be conducted in order to develop economically acceptable treatment practices for intensive production, always taking into account that the results may vary according to the different probiotics used.

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