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Review on probiotics as a functional feed additive in aquaculture

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Abstract
Aquaculture is emerging as one of the most viable and promising enterprises in providing nutritional and food security to humans. Intensification of the aquaculture practices has led to the increase in the stress level in the animal as well as in the environment. This has led to the outbreak of disease, causing huge loss in the aquaculture sector every year. Conventionally, the disease control in aquaculture has relied on the use of chemical compounds and antibiotics. However, the abuse of antimicrobial drugs, pesticides and disinfectants in aquaculture disease prevention and growth promotion has led to the evolution of resistant strains of bacteria. Hence the use of probiotics might be a good option to reduce the risk of disease and enhance the productivity. Probiotics thus are opening a new era in the health management strategy from human to aquatic species including fish and shellfish. Probiotics are beneficial bacteria that help in maintaining the well-being of the host animal. They directly or indirectly protect the host animal against harmful bacterial pathogens. This review summarizes and evaluates brief information regarding the use of probiotics in aquaculture.

Keywords: pangasius, hybrid, digestive system, histology

Introduction
Aquaculture is one of the fastest growing food-producing sectors in the world. The global fish production is estimated to have reached about 179 million tonnes in 2018 (FAO, 2020) [23]. The highest production of finfish is recorded in Asian countries followed by America, Europe and Africa. Aquatic animals maintain a close relationship with their external environment, which enhance the risk of diseases susceptibility. Furthermore, high stocking density, water pollution and unscientific feeding enhance the risk of bacterial, fungal, and viral diseases in cultured animals (Banerjee and Ray, 2017) [9]. The use of antibiotics in aquaculture as a preventive measure has resulted in the evolution and spread of several resistant strains of pathogens like *Aeromonas* sp. *Escherichia tarda*, *Escherichia coli*, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, and many more (Allameh *et al*. 2016; Brogden *et al*. 2014) [4, 11]. There is a risk associated with the transmission of resistant bacteria from aquaculture environments to humans, and risk associated with the introduction in the human environment of non - pathogenic bacteria, containing antimicrobial resistance genes, and the subsequent transfer of such genes to human pathogens (FAO, 2005) [22]. Hence, the use of certain antibiotics in aquaculture industry has been restricted in several countries like the USA and Canada. The development of non-antibiotic and environment friendly agents is one of the key factors for health management in aquaculture. Hence the dietary supplementation of probiotics is considered as an efficient strategy to combat pathogenic agents (Bandyopadhyay *et al*. 2015; Wu *et al*. 2015) [8, 84]. The benefits of such supplements include improved feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, anti - mutagenic and anti-carcinogenic activity, and increased immune response (Priyadarshini *et al*, 2013) [61]. This review summarizes and evaluates the broader knowledge about the probiotics.

Probiotics
The term probiotic was originated from the Greek words “pro” and “bios” which means “for life” (Gismondo *et al*. 1999) and are often called as promoter of life that help in a natural way to improve the overall health status of the host organism.

The word ''probiotic'' was pioneered by Parker (1974) [56], who described probiotics as organisms and substances that contribute to the intestinal microbial balance. Fuller (1989) [24] revised the definition of probiotics as 'a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.' Probiotics, according to the currently adopted definition of the Food and Agricultural Organization and World Health Organization, are live microorganisms that when administered in adequate amounts confer a health benefit on the host (FAO, 2001) [21]. Over the years, various strategies to modulate the composition of the gut microbiota for better growth, digestion, immunity and disease resistance of the host have been investigated in various kinds of livestock as well as in humans (Burr *et al*. 2007) [15]. Probiotics may act as a microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving the nutritional and microbial balance in the intestinal tract (Villamil *et al*. 2002) [80].

General mechanism of probiotic action

Probiotics modulate the growth of intestinal microbiota, suppress potentially harmful bacteria and reinforce the body's natural defense mechanisms (Giorgio *et al*. 2010) [32], thus improving resistance against infectious diseases (Gildberg *et al*. 1997) [30]. Bacterial probiotics do not have a mode of action but act on species - specific or even strain-specific and immune responses of the animal, and their interaction with intestinal bacterial communities plays a key role (Simon, 2010) [73]. Probiotics produce inhibitory substances that may be antagonistic to the growth of pathogens in the intestine. The ability of some probiotics to adhere to the intestinal mucus may block the intestinal infection route common to many pathogens (Gatesoupe, 1999; Ringo *et al*. 2010) [29, 65]. They can also stimulate the appetite and improve nutrition by the production of vitamins, detoxification of compounds in the diet and breakdown of indigestible components (Abdel-Hamid *et al*. 2009) [1].

Probiotics and fish immunity

Various studies revealed that probiotic bacteria, commercial probiotics, their supplementation in feed or any sort of inclusion can boost the cellular and humoral component of the innate immune system in several types of fish and shellfish including salmonids and shrimps (Biswas *et al*. 2013; Cerezuela *et al*. 2013; Goncalves *et al*. 2011; Pais *et al*. 2008; Rodriguez *et al*. 2007; Song *et al*. 2006; Panigrahi *et al*. 2004; Balcazar *et al*. 2004; Gullian *et al*. 2004; Rengpipat *et al*. 2000) [7, 10, 33, 34, 52, 53, 64, 67, 76]. Perusal of available literature indicates that several probiotics either individually or in combination can enhance both systemic as well as local immunity in fish.

Effect of probiotics on systemic immunity

Probiotics interact with the immune cells such as mononuclear phagocytic cells (monocytes, macrophages) and polymorphonuclear leucocytes (neutrophils) and natural killer cells to enhance innate immune responses. Like higher vertebrates, certain probiotics can enhance the number of erythrocytes, granulocytes, macrophages and lymphocytes in fishes (Irianto and Austin, 2002; Kumar *et al*. 2008) [36, 41]. Elevation of immunoglobulin level by probiotics supplementation is reported in many animals including fish (Al-Dohail *et al*. 2009; Nayak *et al*. 2007; Panigrahi *et al*. 2004) [3, 48, 53]. Different lactic acid bacteria (LAB) group of probiotics either in viable or non-viable form can elevate immunoglobulin level in fish (Panigrahi *et al*. 2005) [54]. Even one-week supplementation of probiotic like *Lactobacillus rhamnosus* at 2.8 $\times 10^8$ CFU/g feed was found to significantly increase the immunoglobulin level in *Oncorhynchus mykiss* (Nikoskelainen *et al*. 2003) [51].

Effect of probiotics on gut immunity

The gut is the organ where probiotics not only establish but also execute their functions including immunostimulatory activity. Therefore, the cross talk between probiotics, epithelial cells and gut immune system warrants high consideration. The immune system of the gut is referred to as gut associated lymphoid tissue (GALT) and the piscine gut immune system is quite different from mammals. Unlike mammals, fish lack Peyer's patches, secretory IgA and antigen-transporting M cells in the gut (Buddington *et al*. 1997) [14]. However, many diffusely organized lymphoid cells, macrophages, granulocytes and mucus IgM found in the intestine of fish constitutes the immune function (Uran *et al*. 2008; Bakke *et al*. 2007; Inami *et al*. 2009; Joosten *et al*. 1996; Picchietti *et al*. 1997; Rombout *et al*. 1993) [6, 35, 37, 57, 78]. It is believed that probiotics and/or their components/ products interact with GALT to induce immune response.

The effect of probiotics in stimulating the systemic immune responses are now well documented in several fish species but that of local gut immunity is lacking. Few studies that were conducted in recent times indicate that probiotics can stimulate the piscine gut immune system with marked increase in the number of Ig^+ cells and acidophilic granulocytes (AGs) (Picchietti *et al*. 2009; Salinas *et al*. 2008; Picchietti *et al*. 2007; Picchietti *et al*. 2008) [58-60, 70]. The presence of T-cells in the GALT has been documented in many fish (Picchietti *et al*. 1997; Romano *et al*. 2007; Scapigliati *et al*. 2000) [57, 68, 71] and probiotics can lead to a significant increase in T-cells in fish.

Factors affecting the immunomodulating potency of probiotics

Modulation of host immunity is one of the most purported benefits of probiotics consumption (Medina *et al*. 2007) [43] and fish is no exception. However, the mechanisms by which probiotics affect the immune system of host are unknown (Galdeano and Perdigon, 2006; Corr *et al*. 2009) [18, 25]. While factors such as adhesion properties, attachment site, stress factors, diet and environmental conditions determine the colonization of probiotics in the gut of host (Skjermo and Vadstein, 1999) [74], probiotics often exert host specific (Madsen, 2006) [42] and strain specific differences in their modes of action (Zekri *et al*. 2003) [85]. Nevertheless, the origin and source of probiotics (Sharifuzzaman and Austin, 2009) [72], viability (Gill *et al*. 2001) [31], dose (Donnet-Hughes *et al*. 1999) [20] and duration of supplementation (Vollstad *et al*. 2006) [81] can regulate their activities. There is no doubt that probiotics can stimulate piscine immune system like other animals but inappropriate dose and/or duration of probiotics supplementation can cause undesirable results (Vollstad *et al*. 2006) [81]. Therefore, the type of probiotics, dose kinetics, and method of administration with respect to fish are critical factors that can regulate immune responses in fish.

Dose of probiotics

Dose of probiotics could be limiting factor for achieving optimum beneficial effects in any host (Minelli and Benini, 2008; Kishi *et al*. 1996) [40, 45]. The optimum concentration of probiotics is not only required for establishment and subsequent proliferation in gut but also need to exert various beneficial effects including immunostimulatory activity. Different *in vitro* and *in vivo* studies indicate that immune response of fish varies with the concentration of probiotics. The dose of probiotics is usually selected based on their ability to enhance the growth and protection in host. For instance, Brunt *et al*. (2007) [13] determined the effective dose of the probiotic strain belong to *Bacillus* species to be 2 $\times 10^8$ cells at which they have recorded least percentage mortality in *O. mykiss* during challenge study.

In aquaculture, the dose of probiotics usually varies from 10^{6-10} CFU/g feed. The optimum dose of a probiotics can vary with respect to host and also type of immune parameters. Panigrahi *et al*. (2004) [53] recorded high serum lysozyme, phagocytic activity of head kidney leucocyte and complement activities in *O. mykiss* fed for 30 days with *Lactobacillus rhamnosus* strain at 10^{11} CFU/g feed but not at a dose of 10^9 CFU/g feed. Furthermore, stimulation of a particular immune response with respect to different tissue/organ also varies with dose. For instance, elevation of lysozyme activity in serum and skin in *Miichthys miiuy* is reported at two different doses i.e. 10^7 and 10^9 CFU of *Clostridium butyricum*/g of feed, respectively (Song *et al*. 2006) [76]. On the other hand, Son *et al*. (2009) [75], found best dose of probiotic for grouper (*Epinephelus coioides*) to be 10^8 CFU/kg of feed compared to 10^6 and 10^{10} CFU/kg of *Lactobacillus plantarum* in terms of growth, immune enhancement and protection. Therefore, lower dose can fail to stimulate the piscine immune system while high dose can exert deleterious effects (Nikoskelainen *et al*. 2001) [50]. In another study, Son *et al*. (2009) [75] found higher dose (i.e. 10^{10} CFU/kg feed) of *Lactobacillus plantarum* failed to protect fish on challenge study despite enhancement of certain immune parameters at the particular dose. Earlier, Nikoskelainen *et al*. (2001) [50] also recorded higher percentage of mortality in *Oncorhynchus mykiss* fed at high dose of *Lactobacillus rhamnosus* (10^{12} CFU/g feed) compared to lower dose (10^9 CFU/g feed). Therefore, the dose of the individual probiotics needs to be determined for a particular host.

Mode of supplementation

Although probiotics are used as dietary supplements, Moriarty (1998) [46] proposed to extend the definition of probiotics in aquaculture to microbial ‘‘water additives’’ and several probiotics are also directly used as water additives with documented health and environmental benefits (Zhou *et al*. 2010) [86]. In fish, probiotics are applied in different methods like bath, suspension and feed. However, supplementation of probiotics as feed additive is best method for successful colonization and establishment in gut (Moriarty, 1998; Gildberg *et al*. 1997; Rengpipat *et al*. 1998; Robertson *et al*. 2000) [30, 46, 63, 66]. Oral administration of probiotics is more effective in enhancing immunity as well as subsequent protection as compared to water supplementation (Taoka *et al*. 2006) [77]. Likewise, suspension or bio encapsulation of probiotics is usually adopted for fish larvae (Gatesoupe, 1991; Robertson *et al*. 2000; Gatesoupe, 1993; Keskin *et al*. 1994; Munro *et al*. 1999; Nicolas *et al*. 1989) [27, 38, 47, 49, 66]. Probiotics like *Lactobacillus delbrueckii* strain when supplemented through live carriers like rotifers and artemia succeeded in stimulating local immunity in larvae of *Dicentrarchus labrax* (Picchietti *et al*. 2009) [60].

Apart from dietary supplementation, water borne uptake of probiotics can also modulate the piscine immune system with elevation of several immune parameters (Taoka *et al*. 2006; Wang *et al*. 2008b; Zhou *et al*. 2009) [77, 83]. In a study, Zhou *et al*. (2009) found that among three probiotics (*Bacillus subtilis*, *Bacillus coagulans*, *Rhodopseudomonas palustris*) supplemented into water at the rate of 1$\times$ 10^7 CFU/ml for every 2 days during 40 days, *B. coagulans* and *R. palustris*, showed promising result with improved growth, immunity and health status of *Oreochromis niloticus*.

Environmental conditions

The effectiveness of probiotics is dependent on the successful establishment of the probiotics in the gut. Several factors that influence the establishment and stability of probiotics and subsequent action include water quality, hardness, dissolved oxygen, temperature, pH, osmotic pressure and mechanical friction (Das *et al*. 2008) [19]. Apart from these, stress due to high stocking density can affect the performance of the probiotics. Mehrim (2009) [1] conducted the effect of probiotics on the *Oreochromis niloticus* at different stocking density ranging from 10 to 60 fish/m^3 and found best growth, haematological parameters and economic efficacy of probiotics within a stocking density of 30 fish/m^3. However, in aquaculture it is a neglected aspect and no systematic attempt has been made to correlate effect of probiotics on the immunity of fish at various environmental conditions. Temperature could be crucial since a probiotic would be most effective when used in its optimum temperature range matches that of fish which is identical with surrounding environment (Panigrahi *et al*. 2007) [55]. Panigrahi *et al*. (2007) [55] found better immune-efficacy of *Enterococcus faecium* in comparison to *Lactobacillus rhamnosus* and *Bacillus subtilis* due to its mesophilic and more psychrotolerant nature.

Probiotics and disease protection

Probiotic therapy offers a suitable alternative for controlling pathogens thereby overcoming the adverse consequences of antibiotics and chemotherapeutic agents. In fish culture, probiotics either in diet or bioencapsulation help in achieving natural resistance and high survivability of larvae and post larvae of fishes (Robertson *et al*. 2000; Abraham *et al*. 2007) [2, 66]. Significant increase in the mean weight and natural survival rate of larvae of *Scophthalmus maximus* was documented when fed with the rotifers enriched in lactic acid bacteria. High protection against a pathogenic Vibrio species was also recorded (Gatesoupe, 1994) [28]. Furthermore, the effectiveness of probiotics in terms of protection against infectious pathogens is often attributed to the elevated immunity. Protection against edwadsiellosis (Nayak *et al*. 2007; Taoka *et al*. 2006; Chang and Liu, 2002) [17, 48, 77], enteric red mouth disease (Kim and Austin, 2006; Raida *et al*. 2003) [39, 62], furunculosis (Irianto and Austin, 2002; Nikoskelainen *et al*. 2003; Nikoskelainen *et al*. 2001) [36, 50, 51], lactococossis (Brunt and Austin, 2005; Vendrell *et al*. 2008) [12, 79], streptococcosis (Brunt and Austin, 2005) [12] are successfully accomplished through probiotics feeding. *Bacillus subtilis* in combination with *Lactobacillus acidophilus* when fed to *Oreochromis niloticus* at the rate of 10^7 CFU/g of feed for a period of 2 weeks made them resistant to pathogens like *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Streptococcus iniae* (Aly *et al*. 2008) [5]. Kumar *et al*. (2008) [41] found that *Bacillus subtilis* treated *Labeo rohita* at the rate of 1×10^7 CFU/g of feed for 15 days made them resistant to *Aeromonas hydrophila* infection. *Oreochromis mossambicus* fed with lactic acid bacteria diet for 25 days at the rate of 10^6 CFU/g made them *Aeromonas hydrophila* pathogen resistant (Vijayabaskar and Somasundaram, 2008) [82].

Furthermore, probiotics treatment leads to better protection of fish from multiple diseases (Aly *et al*. 2008; Brunt *et al*. 2007; Robertson *et al*. 2000) [5, 13, 66]. Apart from protection against bacterial pathogens, probiotics can protect against viral and protozoan infections as well. Recently, successful control of Ichthyophthiriasis (*Ichthyophthirius multifiliis*, Ich) by *Aeromonas sobria* in *Onchorynchus mykiss* (Pieters *et al*. 2008) and iridovirus of grouper *Epinephelus coioides* by *Lactobacillus plantarum* (Son *et al*. 2009) [75] is achieved.

Conclusion

The beneficial effects of dietary supplements like probiotics have been recorded in a wide range of animal models including fish. The concept of probiotics has already been established in aquaculture practices especially as a promising alternative to chemicals and antibiotics. Over the years several candidate probiotics strains belonging to gram positive and gram-negative groups of bacteria are introduced into culture practices. Looking into the fact that most of the probiotics can exert immunomodulatory effect in fish, a complete understanding of the interactions between gut microbes, the intestinal epithelium, and the gut immune system is also necessary so that proper strategy can be developed for stimulating the local as well as systemic immunity through manipulation of gut microbiota with suitable probiotics without altering the intestinal homeostasis.

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