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 A. hydrophila, V. parahaemolyticus, V. aureus, 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) [28, 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; Rengippat et al. 2000) [7, 10, 33, 34, 52, 53, 64, 67, 78]. 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 ×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 immunomodulatory 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 Peyers' 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 Vadelstein, 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 × 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 to 10^10 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 leukocyte 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 Mitchthys 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) [79]. On the other hand, Son et al. (2009) [79], found best dose of probiotic for grouper (Epinephelus coioides) to be 10^6 CFU/kg of feed compared to 10^5 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) [10]. In another study, Son et al. (2009) [79] 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) [10] 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) [80]. 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, Rhodospseudomonas palustris) supplemented into water at the rate of 1× 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) [11] conducted the effect of probiotics on the Oreochromis niloticus at different stocking density ranging from 10 to 60 fish/m² and found best growth, haematological parameters and economic efficacy of probiotics within a stocking density of 30 fish/m². 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 edwaediellosis (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], lactococcosis (Brunt and Austin, 2005; Vendrell et al. 2008) [12, 79], streptococcus (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) [3]. Kumar et al. (2008) [41] found that *Bacillus subtilis* treated *Labeo rohita* at the rate of \(1 \times 10^6\) 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) [79] 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.

### References

1. Abd el-Hamid AM, Mehrim AI, El-Barbary MI, Ibrahim SM, Abd El-Wahab AI. Evaluation of a New Egyptian probiotic by African catfish fingerlings. Environ. Sci. Technol 2009;2:133-145.

2. Abraham TJ, Babu CHS, Mondal S, Banerjee T. Effects of dietary supplementation of commercial human probiotic and antibiotic on the growth rate and content of intestinal microflora in ornamental fishes. Bangladesh J Fish Res 2007;11(1):57-63.

3. Al-Dohail MA, Hashim R, Aliyu-Paiko M. Effects of the probiotic, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus*, Burchell 1822) fingerling. Aquac. Res 2009;40:1642-1652.

4. Allameh SK, Yusoff FM, Ringo E, Daud HM, Saad CR, Ideris A. Effects of dietary mono-and multiprobiotic strains on growth performance, gut bacteria and body composition of Javanese carp (*Puntius gonionotus*, Bleecker 1850). Aquaculture Nutrition 2016;22(2):367-373.

5. Aky SM, Ahmed YAG, Ghareeb AAA, Mohamed MF. Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of *Tilapia nilotica* (*Oreochromis niloticus*) to challenge infections. Fish Shellfish Immunology 2008;25:128-136.

6. Bakke-Mckellep AM, Froystad MK, Lilleeng E, Dapra F, Refstie S, Krogdahl A. Response to soy: T-cell-like reactivity in the intestine of Atlantic salmon, *Salmo salar* L. J Fish Dis 2007;30:13-25.

7. Balcazar JL, Vendrell D, Ruiz-Zarzuella I, Muzquiz JL. Probiotics: a tool for the future of fish and shellfish health management. J Aquac. Trop 2004;19:239-242.

8. Bandyopadhyay P, Mishra S, Sarkar B, Swain SK, Pal A, Tripathy PP et al. Dietary Saccharomyces cerevisiae boosts growth and immunity of IMC *Labeo rohita* (Ham.) juveniles. Indian Journal of Microbiology 2015;55(1):81-87.

9. Banerjee G, Ray AK. The advancement of probiotics research and its application in fish farming industries. Research in Veterinary Science 2017;115:66-77.

10. Biswas G, Korenaga H, Nagamine R, Takayama H, Kawahara S, Takeda S et al. Cytokine responses in the Japanese pufferfish (*Takifugu rubripes*) head kidney cells induced with heat killed probiotics isolated from the Mongolian dairy products. Fish Shellfish Immunol 2013;34(5):1170-1177.

11. Brogden G, Krimmling T, Adamek M, Naim HY, Steihagen D, Von Köckritz-Blikwede M. The effect of β-glucan on formation and functionality of neutrophil extracellular traps in carp (*Cyprinus carpio* L). Developmental and Comparative Immunology 2014;44(2):280-285.

12. Brunt J, Austin B. Use of a probiotic to control lactococcosis and streptococcosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis 2005;28:693-701.

13. Brunt J, Newaj-Fyzul A, Austin B. The development of probiotics for the control of multiple bacterial diseases of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis 2007;30:573-579.

14. Buddington RK, Krogdahl A, Bakke-McKellep AM. The intestines of carnivorous fish: structure and functions and the relations with diet. Acta. Physiol. Scand. Suppl 1997;638:67-80.

15. Burr G, Gatlin D, Ricke S. Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in fish farm aquaculture. J World Aquac. Soc 2007;36:425-436.

16. Cerezuella R, Fumanal M, Tapia-Paniagua ST, Meseguer J, Morinigo MA, Esteban MA. Changes in intestinal morphology and microbiota caused by dietary administration of inulin and *Bacillus subtilis* in gilthead sea bream (*Sparus aurata*). Fish Shellfish Immunol 2013;34(5):1063-1070.

17. Chang CI, Liu WY. An evaluation of two probiotic bacterial strains, *Enterococcus faecium* SF68 and *Bacillus toyoii*, for reducing edwardsiellosis in cultured European eel, *Anguilla anguilla* L. J Fish Dis 2002;25:311-315.

18. Corr SC, Hill C, Gahan CGM. Understanding the mechanisms by which probiotics inhibit gastrointestinal pathogens. Adv. Food Nutr. Res 2009;56:1-15.

19. Das S, Ward LR, Burke C. Prospects of using marine actinobacteria as probiotics in aquaculture. Appl. Microbiol. Biotechnol 2008;81:419-429.

20. Donnet-Hughes A, Rochat F, Serrat P, Aeschlimann JM, Schiffrin EJ. Modulation of nonspecific mechanisms of defense by lactic acid bacteria: effective dose. J Dairy Sci 1999;82:863-869.
21. Food and Agriculture Organization of The United Nations (FAO). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, in the joint FAO/WHO expert consultation report on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. FAO Headquarters, Rome 2001.

22. FAO. In: Serrano PH, ed. Responsible Use of Antibiotics in Aquaculture. Rome: FAO. FAO Fisheries Technical Paper 469 2005:98.

23. FAO. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome 2020.

24. Fuller R. Probiotics in man and animals: a review. J Appl. Bacteriol 1989;68:365-378.

25. Galdeano CM, Perdigon G. The probiotic bacterium *Lactobacillus casei* induces activation of the gut mucosal immune system through innate immunity. Clin. Vacc. Immunol 2006;13:219-226.

26. Gatesoupe FJ. The effect of three strains of lactic bacteria on the production rate of rotifers, *Brachionus plicatilis*, and their dietary value for larval turbot, *Scophthalmus maximus*. Aquaculture 1991;96:335-342.

27. Gatesoupe FJ. *Bacillus* sp. spores as food additive for the rotifer *Brachionus plicatilis*: improvement of their bacterial environment and their dietary value for larval turbot, *Scophthalmus maximus*. 4th Int. Symp. Fish. Nutr. Feeding 1993, 24-27.

28. Gatesoupe FJ. Lactic acid bacteria increase the resistance of turbot larvae, *Scophthalmus maximus*, against pathogenic Vibrio. Aquat. Living Resour 1994;7:277-282.

29. Gatesoupe FJ. The use of probiotic in aquaculture. Aquaculture 1999;180:147-165.

30. Gildeberg A, Mikkelsen H, Sandaker H, Ringo E. Probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod (*Gadus morhua*). Hydrobiologia 1997;352:279-285.

31. Gill H, Rutherford K, Cross M. Dietary probiotic supplementation enhances natural killer cell activity in the elderly: an investigation of age-related immunological changes. J Clin. Immunol 2001;21(4):264-271.

32. Giorgio G, Nina C, Yantyati W. Importance of *Lactobacilli* in food and feed biotechnology. Res. Microbiol 2010;161:480-487.

33. Gonzalves AT, Maita M, Futami K, Endo M, Katagiri T. Effects of a probiotic bacterial *Lactobacillus rhamnosus* dietary supplement on the crowding stress response of juvenile Nile tilapia (*Oreochromis niloticus*). Fish. Sci 2011;77:633-642.

34. Gullian M, Thompson F, Rodriguez J. Selection of probiotic bacteria and study of their immunostimulatory effect in *Penaeus vannamei*. Aquaculture 2004;233:1-14.

35. Irani M, Taverne-Thiele AJ, Schroder MB, Kiron V, Rombout JHWM. Immunological differences in intestine and rectum of Atlantic cod (*Gadus morhua* L.). Fish. Shellfish Immunol 2009;26:751-759.

36. Irianto A, Austin B. Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis 2002;25:333-342.

37. Joosten PHM, Kruijer WJ, Rombout JHWM. Anal immunisation of carp and rainbow trout with different fractions of a *Vibrio anguillarum* bacterin. Fish Shellfish Immunol 1996;6:541-551.

38. Keskin M, Keskin M, Rosenthal H. Pathways of bacterial contamination during egg incubation and larval rearing of turbot, *Scophthalmus maximus*. J Appl. Ichthyol 1994;10:1-9.

39. Kim DH, Austin B. Innate immune responses in rainbow trout (*Oncorhynchus mykiss*, Walbaum) induced by probiotics. Fish Shellfish Immunol 2006;21:513-524.

40. Kishi A, Uno K, Matsubara Y, Okuda C, Kishida T. Effect of the oral administration of *Lactobacillus brevis* sub sp *coagulans* on interferon alpha producing capacity in humans. J Am. Coll. Nutr 1996;15:408-412.

41. Kumar R, Mukherjee SC, Ranjan R, Nayak SK. Enhanced innate immune parameters in *Laboe rohita* (Ham.) following oral administration of *Bacillus subtilis*. Fish Shellfish Immunol 2008;24:168-172.

42. Madsen K. Probiotics and the immune response. J Clin. Gastroenterol 2006;40(3):232-234.

43. Medina M, Izquierdo E, Emahar S, Sanz Y. Differential immunomodulatory properties of *Bifidobacterium logum* strains: relevance to probiotic selection and clinical applications. Clin. Exp. Immunol 2007;150:531-538.

44. Mehrim AI. Effect of dietary supplementation of *Biogen*® (Commercial probiotic) on mono-sex Nile tilapia *Oreochromis niloticus* under different stocking densities. J Fisher. Aquat. Sci 2009;4(6):261-273.

45. Minelli EB, Benini A. Relationship between number of bacteria and their probiotic effects. Microb. Ecol. Health Dis 2009;20:180-183.

46. Moriarty DJW. Control of luminous *Vibrios* sp. in penaeid aquaculture ponds. Aquaculture 2009;164:357-358.

47. Munro PD, Henderson RJ, Barbour A, Birkbeck TH. Partial decontamination of rotifers with ultraviolet radiation: the effect of changes in the bacterial load and flora of rotifers on mortalities in start-feeding larval turbot. Aquaculture 1999;170:229-244.

48. Nayak SK, Swain P, Mukherjee SC. Effect of dietary supplementation of probiotic and vitamin C on the immune response of Indian major carp, *Laboe rohita* (Ham.). Fish Shellfish Immunol 2007;23:892-896.

49. Nicolas JL, Robic E, Ansquer D. Bacterial flora associated with a trophic chain consisting of microalgae, rotifers and turbot larvae: influence of bacteria on larval survival. Aquaculture 1989;83:237-248.

50. Nikoskelainen S, Ouwehand A, Bylund G, Salminen S. Protection of rainbow trout (*Oncorhynchus mykiss*) from furunculosis by *Lactobacillus rhamnosus*. Aquaculture 2001;198:229-236.

51. Nikoskelainen S, Ouwehand AC, Bylund G, Salminen S, Lilius E. Immune enhancement in rainbow trout (*Oncorhynchus mykiss*) by potential probiotic bacteria (*Lactobacillus rhamnosus*). Fish Shellfish Immunol 2003;15:443-452.

52. Pais R, Khushiramani R, Karunasagar I. Effect of immunostimulants on the haemolymph of tiger shrimp (*Penaeus monodon*). Aquac. Res 2008;39:1339-1345.

53. Panighrahi A, Kiron V, Kobayashi T, Puuangaew J, Satoh S, Sugita H. Immune responses in rainbow trout (*Oncorhynchus mykiss*) induced by a potential probiotic bacteria *Lactobacillus rhamnosus* JCM 1136. Vet. Immunol. Immunopathol 2004;102:379-388.

54. Panighrahi A, Kiron V, Puuangaew J, Kobayashi T, Satoh S, Sugita H. The viability of probiotic bacteria as a factor influencing the immune response in rainbow trout
Oncorhynchus mykiss. Aquaculture 2005;243:241-254.
55. Panigrahi A, Kiron V, Satoh S, Hirono I, Kobayashi T, Sugita H. Immune modulation and expression of cytokine genes in rainbow trout Oncorhynchus mykiss upon probiotic feeding. Dev. Comp. Immunol 2007;31:372-382.
56. Parker RB. Probiotics, the other half of the antibiotic story. Anim. Nutr. Health 1974;29:4-8.
57. Picchiotti S, Terribili FR, Mastrolia L, Scapigliati G, Abelli L. Expression of lymphocyte antigenic determinants in developing GALT of the seabass Dicentrarchus labrax (L.). Anat. Embryol 1997;196:457-463.
58. Picchiotti S, Mazzini M, Taddei AR, Renna R, Fausto AM, Mulero V. Effects of administration of probiotic strains on GALT of larval gilthead seabream: immunohistochemical and ultrastructural studies. Fish Shellfish Immunol 2007;22:57-67.
59. Picchiotti S, Guerra L, Selleri L, Buonocore F, Abelli L, Scapigliati G. Compartmentalisation of T cells expressing CD8a and TCR b in developing thymus of sea bass Dicentrarchus labrax (L.). Dev. Comp. Immunol 2008;32:92-99.
60. Picchiotti S, Fausto AM, Randelli E, Carnevali O, Taddei AR, Buonocore F. Early treatment with Lactobacillus delbrueckii strain induces an increase in intestinal T-cells and granulocytes and modulates immune related genes of larval Dicentrarchus labrax (L.). Fish Shellfish Immunol 2009;26:368-376.
61. Priyadarshini P, Deivasigamani B, Rajasekar T, Edward GJG, Kumanan S, Sakthivel M et al. Probiotics in aquaculture. Drug invention today 2013;5:55-59.
62. Raida MK, Larsen JL, Nielsen ME, Buchmann K. Enhanced resistance of rainbow trout, Oncorhynchus mykiss (Walbaum), against Yersinia ruckeri challenge following oral administration of Bacillus subtilis and B. licheniformis (BioPlus2B). J Fish Dis 2003;26:495-498.
63. Rengpipat S, Phianphak W, Piyatratitvorakul S, Menasaveta P. Effects of a probiotic bacterium in black tiger shrimp Penaeus monodon survival and growth. J Aquaculture 1998;167:301-313.
64. Rengpipat S, Ruksapatanporn S, Piyatratitvorakul S, Menasaveta P. Immunity enhancement in black tiger shrimp (Penaeus monodon) by a probiotic bacterium, Bacillus s11. Aquaculture 2000;191:271-288.
65. Ringo E, Lovmo L, Kristiansen M, Bakken Y, Salinas I, Myklebust R et al. Lactic acid bacteria vs. pathogens in the gastro-intestine of fish (a review). Aquac. Res 2010;41:451-467.
66. Robertson PAW, O'dowd C, Burrells C, Williams P, Austin B. Use of Carnobacterium sp. as a probiotic for Atlantic salmon (Salmo salar L.) and rainbow trout (Oncorhynchus mykiss Walbaum). Aquaculture 2000;185:235-243.
67. Rodriguez J, Espinosa Y, Echeverria F, Cardenas G, Roman R, Stern S. Exposure to probiotics and b-1, 3/1, 6-glucans in larviculture modifies the immune response of Penaeus vannamei juveniles and both the survival to white spot syndrome virus challenge and pond culture. Aquaculture 2007;273:405-415.
68. Romano N, Rossi F, Abelli L, Caccia E, Piergentili R, Mastrolia L. Majority of TCR β+ T-lymphocytes located in thymus and midgut of the bony fish, Dicentrarchus labrax (L.). Cell. Tissue Res 2007;329:479-489.
69. Rombout JHWM, Taverne-Thiele AJ, Villena MI. The gut - associated lymphoid tissue (GALT) of carp (Cyprinus carpio L.): an immunocytochemical analysis. Dev. Comp. Immunol 1993;17:55-66.
70. Salinas I, Abelli L, Bertoni F, Picchiotti S, Roque A, Furones D. Monospecies and multispecies probiotic formulations produce different systemic and local immunostimulatory effects in the gilthead sea bream (Sparus aurata L.). Fish Shellfish Immunol 2008;25:114-123.
71. Scapigliati G, Romano N, Abelli L, Meloni S, Ficca AG, Buonocore F. Immunopurification of T-cells from sea bass Dicentrarchus labrax (L.). Fish Shellfish Immunol 2000;10:329-341.
72. Sharifuzzaman SM, Austin B. Influence of probiotic feeding duration on disease resistance and immune parameters in rainbow trout. Fish Shellfish Immunol 2009;27:440-445.
73. Simon O. An interdisciplinary study on the mode of action of probiotics in pigs. J Anim. Feed Sci 2010;19:230-243.
74. Skjermo J, Vadstein O. Techniques for microbial control in the intensive rearing of marine larvae. Aquaculture 1999;177:333-343.
75. Son VM, Chang CC, Wu MC, Guu YK, Chiu CH, Cheng W. Dietary administration of the probiotic, Lactobacillus plantarum, enhanced the growth, innate immune responses, and disease resistance of the grouper Epinephelus coioides. Fish Shellfish Immunol 2009;26:691-698.
76. Song Z, Wu T, Cai L, Zhang L, Zheng X. Effects of dietary supplementation with Clostridium butyricum on the growth performance and humoral immune response in Mitchys miyui. J Zhejiang Univ. Sci. 2006;7(7):596-602.
77. Taoka Y, Maeda H, Jo JY, Kim SM, Park S, Yoshikawa T. Use of live and dead probiotic cells in tilapia Oreochromis niloticus. Fisher. Sci 2006;72:755-766.
78. Ura PA, Aydin R, Schrama JW, Verreth JAJ, Rombout JHWM. Soybean meal induced uptake block in the distal enterocytes of Atlantic salmon (Salmo salar L.). J Fish. Biol 2008;73:2571-2579.
79. Vendrell D, Balcazar JL, De Blas I, Ruiz-Zarzuela I, Girones O, Muzquiz JL. Protection of rainbow trout (Oncorhynchus mykiss) from lactococcosis by probiotic bacteria. Comp. Immunol. Microbiol. Infect. Dis 2008;31:337-345.
80. Villamil L, Tafalla C, Figueras A, Novoa B. Evaluation of immunomodulatory effects of lactic acid bacteria in turbor (Scophthalmus maximus). J Clin. Diagn. Lab. Immunol 2002;9:1318-1323.
81. Vollstad D, Bogwald J, Gaserod O, Dalmo RA. Influence of high-M alginate on the growth and survival of Atlantic cod (Gadus morhua L.) and spotted wolffish (Anarhichas minor Olafsen) fry. Fish Shellfish Immunol 2006;20:548-561.
82. Vijayabaskar P, Somasundaram ST. Isolation of bacteriocin producing Lactic acid bacteria from fish gut and probiotic activity against common freshwater fish pathogen Aeromonas hydrophila. Biotechnology 2008;7(1):124-128.
83. Wang YB, Tian ZQ, Yao JT, Li WF. Effect of probiotics, Enterococcus faecium, on tilapia (Oreochromis niloticus) growth performance and immune response. Aquaculture 2008;277:203-207.
84. Wu ZQ, Jiang C, Ling F, Wang GX. Effects of dietary supplementation of intestinal autochthonous bacteria on the innate immunity and disease resistance of grass carp (Ctenopharyngodon idellus). Aquaculture 2015;438:105-114.

85. Zekri IN, Blum S, Schiffrin EJ, Von Der Weid T. Divergent patterns of colonization and immune response elicited from two intestinal Lactobacillus strains that display similar properties in vitro. Infect. Immun 2003;71:428-436.

86. Zhou X, Tian Z, Wang Y, Li W. Effect of treatment with probiotics as water additives on tilapia (Oreochromis niloticus) growth performance and immune response. Fish Physiol. Biochem 2010;36(3):501-509.