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Harnessing Probiotics to Bolster Disease Resistance and Immunological Vigor in Fish: An Overview

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ABSTRACT: This review explores the potential of probiotics as a proactive strategy to enhance disease resistance and immunological vigor in fish. With aquaculture facing significant challenges related to disease outbreaks, the utilization of probiotics has gained prominence as a promising intervention. The paper synthesizes current research, delving into the diverse mechanisms by which probiotics modulate the immune responses of fish. From the specific strains employed to the ecological implications of their application, the review navigates through the nuanced landscape of probiotic interventions. By elucidating both direct and indirect impacts on immune parameters, the paper provides valuable insights for researchers, aquaculturists, and policymakers, offering a foundation for optimizing disease management strategies and fostering sustainable aquaculture practices.

Keywords: Aquaculture, Aquatic organisms, Disease, Immune effects, Probiotic efficiency.

INTRODUCTION

Probiotics are microbial feed additives that confer resistance to host organism through modification of intestinal microbiota; the phrase derives from the Greek words "pro" and "bios," both of which imply "for life". The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) both define probiotics as live bacteria that are consumed and have demonstrable health benefits for the host (Hotel and Córdoba, 2001). The probiotics used in aquaculture as a feed supplement or normal water treatment include various types of bacteria, bacteriophages, microalgae, and yeast (Llewellyn *et al*., 2014). The epithelial barrier is strengthened, there is greater adherence to the intestinal mucosa, harmful bacteria are competitively excluded, and anti-microorganism chemicals are produced by probiotics, among other major modes of action (Bermudez-Brito *et al*., 2012) as shown in the Fig. 1.

Incorporating probiotic mixes into feed is the most popular technique for administering them (92.8%), followed by directly incorporating them into water (4.8%) and live food (1.6%) (Melo *et al*., 2020). Probiotics come in a variety of forms, including multistrain probiotics, probiotics with plant extract, and probiotics with yeast extract, and can be used separately or in combination. The majority of research on probiotics in aquaculture has concentrated on the use of a single probiotic, whereas probiotic combinations are more advantageous. The efficient characteristics and forms of probiotic are depicted in Fig. 2.

Probiotic bacteria can immediately absorb or break down harmful or organic material in the water, enhancing the water quality. Probiotics can improve the nutrient cycle that maintains a healthy water quality habitat for cultured animals by decomposing the excreta of fish or prawns, leftover food, plankton remains, and other organic materials to CO_2, nitrate, and phosphate (Rao, 2010). Probiotic microorganisms have the ability to release compounds, such as Bacitracin and polymyxin generated by *Bacillus* sp., that have a bactericidal or bacteriostatic effect on pathogenic bacteria that are in the intestine of the host, improving disease resistance (Rao, 2010: Cruz *et al*., 2012). The numerous immune indices of aquaculture animals can be improved by probiotics such as activation of complement receptor expression (Balcazar *et al*., 2007), lysozyme and peroxidase activity, serum lysozyme, serum peroxidase, alternative complement, phagocytosis and respiratory brust activities in nile tilapia (Doan *et al*., 2018), increased red blood cells, white blood cells and hematocrit as well as total serum antibody level ad protease (Dawood *et al*., 2020).

EFFECT OF PROBIOTICS ON DISEASE RESISTANCE AND IMMUNOLOGICAL PARAMETERS IN FISH

Newaj-Fyzul *et al*. (2007) studied the effect of *Bacillus subtilis* AB1 against *Aeromonas* sp., which was pathogenic to rainbow trout. Immune parameters were enhanced by AB1, particularly the populations of lymphocytes, respiratory burst, serum and gut lysozyme, peroxidase, and phagocytic killing. In comparison to the non-probiotic control fish, which had a survival rate of 5% to 15%, the probiotic-fed fish had a range of survival rates after challenge from 65% to 100%. *Aeromonas* infection was more difficult to control when doses of viable AB1 were lower and higher than 10^7 cells per gram of feed. After incubating the pathogen with macrophages taken from the head kidney at a dosage of 4.4 × 10^7 cells per millilitre, the bactericidal activity of macrophages from AB1-fed fish was considerably higher than that of control. Additionally, there were statistically significant changes between the blood macrophages from fish that received probiotics and the controls in terms of their respiratory burst activity. After 60 minutes, the serum lysozyme activity for fish treated with AB1 and control fish was measured as 1269±134 and 438±75 U ml^{-1}, respectively. Also the fish fed with probiotics had much higher intestinal mucus lysozyme activity than controls, according to this research.

In numerous investigations, it has been shown that feeding *Bacillus* and *Lactobacillus* supplemented feeds to *Oreochromis niloticus, Rachycentron canadum, Oncorhynchus mykiss, Paralichthys olivaceus, Litopenaeus vannamei*, and *Labeo rohita* significantly stimulates serum lysozyme and phagocytic activities (Rahman *et al*., 2012).By significantly increasing the amounts of IL-1 and TNF mRNA in the kidneys, research showed that in *Oreochromis niloticus* innate immunity and disease resistance are enhanced when *Bacillus amyloliquefaciens* is added to its diet. When challenged with Yersinia *ruckeri* or *Clostridium perfringens*, fish that had been given *Bacillus amyloliquefaciens* fared improved relative survival rates (Selim and Reda 2015). They also appeared to increase in vitro serum bactericidal activity against *Aeromonas hydrophila.* In another investigation, two months of dietary *Bacillus* (B47b) supplementation led to noticeably improved survival rates against *A. hydrophila* FW52. According to this data, Bacillus species are quite successful at combating *S. agalactiae* and *A. hydrophila* (Mohapatra *et al*., 2013).

The effects of a synbiotic supplement on growth performance, haematological parameters, and resistance to *Saprolegnia parasitica* in fingerlings of rainbow trout, *Oncorhynchus mykiss* was examined (Firouzbakhsh *et al*., 2014). Fish were fed three levels of dietary synbiotics, at 0.5, 1.0, and 1.5 g kg^{-1} each, three times per day. Following 60 days of feeding, the fingerlings were exposed to *Saprolegnia parasitica*, and up to 15 days of mortality were noted. The synbiotic-fed groups had noticeably greater survival rates following challenges with *Saprolegnia parasitica* in comparison to the control group. According to these findings, feeding rainbow trout fingerlings a nutritional synbiotic for 60 days at a dose of 1.0 g per kg increases growth performance, survival rate, and feeding efficiency while also making the fish more tolerant to parasite infection by *Saprolegnia*. Dahiya *et al*. (2012) evaluated the elimination of *Aeromonas hydrophila* by the use of single pro-biotics; Probiotic 1 (*Lactobacillus sporogenes*), Probiotic 2 (*Saccharomyces boulardii*) or mixture of probiotics; Probiotic 3 (*Nitromonas* spp., *Rhodococcus* spp., *Bacilus megaterium*, *Lecheni formis*, *Desulphovibrio sulphuricum*, *Psuedomonas* spp., *Chromatium* spp., *Chloro-bium* spp., *Thiobacillus* spp., *Thioxidants* spp., *Thiobacilus ferroxidant*, *Methylomonas methyanica*, *Glucon acetobactor*, *Azospirillum* spp., *Trichoderma* spp., *Shizophyllum commune* and *Sclertium gluconicum*). The results indicated that the number of viable *A. hydrophila* in fish dramatically decreased by the use of probiotic cultures. Over a four-week period, probiotic 1 followed by probiotic 3 and probiotic 2 catfish were found to be more effective. Al-Hassani and Mustafa (2022) evaluated the efficiency of synbiotic consisting of *Saccharomyces serevisiae*, *Bacillus subtilus*, Lactic acid bacteria and ß-glucan, on the survival rate and health status in common carp challenged with *Saprolegnia* spp. A total of hundred *C. carpio* fingerlings, each weighing 49.55–50.50 g, were divided into five treatment groups at random and fed various concentrations in each group. Compared to the control group's (75%), the therapy supplemented with 2% of synbiotics had the highest survival rate (100%), indicating that the high amount (2%) improves growth rate and survival rate. Six fish from each treatment group and the control group (C) + were chosen at random at the conclusion of the experiment to participate in a challenge test using a viable suspension of *Saprolegnia* spp. The mean values of WBCs have seen significant modifications. When compared to the C+ group, respiratory burst activity was significantly higher in all synbiotic diet groups. These findings can be viewed as a helpful diet for increasing the common carp's immunological response.

The effectiveness of synbiotic on haematological, histological alterations, and resistance against *Saprolegnia* spp. in *Cyprinus carpio* was evaluated by Salih and Mustafa in 2017. 100 *C. carpio*, each weighing between 49.55 and 50 g, were divided into five treatment groups at random. All of the treatment groups were put to the test in a *Saprolegnia* spp. viable fungal suspension at the conclusion of the feeding trail. The differential leucocyte count showed significant alterations, and the percentage of lymphocytes and monocytes considerably decreased in the T4 group compared to the C+ group. When compared to the C+ group, the percentage of neutrophils in T4 was considerably higher. Although dietary synbiotics at all levels considerably boost resistance to the *Saprolegnia* challenge, T4 had the best survival rate (83%) followed by T3 and T2 (66%), T1 (50%) and C- (16%). The findings showed that adding dietary synbiotics at a rate of 2.0% to the diet increased resistance to infection by *Saprolegnia* spp. The various probiotics tested in finfish and crustaceans that altered the pathogenic characteristics of bacteria are tabulated in Table 1 and 2 respectively. In addition to this the probiotics that produced the immune responses in fish are given in Table 3.

Table 1: Probiotics altering pathogenic characteristics of finfish bacteria.

Sr. No.	Pathogenic Bacteria	Species	Probiotics	References
1.	*Yersinia ruckeri*	*O. mykiss*	*B. subtilis* and *B. licheniformis*	Raida *et al*. (2003)
2.	*Vibrio anguillarum*	*O. mykiss*	Kocuria SM1	Sharifuzzaman & Austin (2009)
3.	*Aeromonas* salmonicida	*O. mykiss*	*Lactobacillus rhamnosus*	Nikoskelainen *et al*. (2001)
4.	*A. salmonicida* and *Yersinia ruckeri*	*O. mykiss*	*Carnobacterium maltaromaticum*	Kim and Austin (2006)
5.	*V. anguillarum, V. ordalii, Lactococcus garvieae, A. salmonicida, Streptococcus iniae* and *Yersinia ruckeri*	*O. mykiss*	*Bacillus* JB-1 or *Aeromonas sobria* GC2	Brunt *et al*. (2007)
6.	*Aeromonas bestiarum* and *Ichthyophthirius multifiliis*	*O. mykiss*	*Aeromonas sobria* GC2	Brunt and Austin (2005)
7.	*A. hydrophila*	*Labeo rohita* (Rohu)	*Bacillus circulans* PB7	Bandyopadhyay & Das Mohapatra (2009)
8.	*A. hydrophila*	*Labeo rohita*	*B. subtilis*	Kumar *et al*. (2006)
9.	*P. fluorescens* and *S. iniae*	Nile tilapia	*Lactobacillus acidophilus*	Aly *et al*. (2008a)
10.	*A. salmonicida*	Rainbow trout	*Lactobacillus rhamnosus* ATCC 53101	Nikoskelainen *et al*. (2001)
11.	*V. anguillarum*	Sea bass	*Vagococcus fluvialis*	Sorroza *et al*. (2012)
12.	*Aeromonas salmonicida*	Brown trout	*Leuconostoc mesenteroides*	Balcázar *et al*. (2009)
13.	*A. hydrophila*	Indian major carp	*Bacillus subtilis*	Kumar *et al*. (2006)
14.	*A. salmonicida*	Rainbow trout	*Micrococcus luteus*	Irianto and Austin (2002)
15.	*F. psychrophilum*	Rainbow trout	*Pseudomonas* sp.	Korkea-aho *et al*. (2011)
16.	*A. hydrophila*	Grass carp	*Shewanella xiamenensis*	Wu *et al*. (2015)
17.	*Yersinia ruckeri*	Rainbow trout	*Enterobacter cloacae*	Capkin and Altinok 2006)
18.	*Aeromonas* infection	*O. mykiss*	*B. subtilis* AB1	Newaj-Fyzul *et al*. (2007)
19.	*Yersinia ruckeri*	*O. mykiss*	*B. subtilis* and *B. licheniformis*	Raida *et al*. (2003)

Table 2: Probiotics altering pathogenic characteristics of shellfish bacteria.

Sr. No.	Pathogenic Bacteria inhibited or immune response produced	Species	Probiotics	References
1.	*V. harveyi*	*P. monodon*	*Streptococcus phocae* P180	Swain *et al*. (2009)
2.	Bacterial pathogens	Penaeids	*Microaglae Tetraselmis suecica*	Austin and Day (1990)
3.	Vibriosis	Penaeids	Yeasts (*Phaffia rhodozyma, Saccharomyces cerevisiae* and *Saccharomyces exiguous*)	Scholz *et al*. (1999)
4.	*Vibrio harveyi*	*L. vannamei*	*B. subtilis*	McIntosh *et al*. (2000)
5.	Increased survival rate	*L. vannamei*	*B. subtilis*	Xue *et al*. (2016)
6.	Increased weight gain	*M. malcolmsonii*	*B. subtilis*	John *et al*. (2018)
7.	Provided better survival and immune response	*P. monodon*	*Bacillus* sp. Strain DDKRC1	De *et al*. (2018)
8.	*Vibrio* spp	*Penaeus monodon*	*B. subtilis*	Vaseeharan *et al*. (2004)
9.	*Vibrio harveyi*	*L. vannamei*	*B. subtilis*	McIntosh *et al*. (2000)

Table 3: Probiotics producing enhanced immune response in finfishes.

Sr. No.	Pathogenic Bacteria	Species	Probiotics	References
1.	Stimulation of lysozyme activity	Rainbow trout	*Carnobacterium divergence* and *Lactobacillus rhamnosus*	Panigrahi *et al*. (2004)
2.	Stimulation of lysozyme activity	Chinese drum	*Clostridium butyricum*	Pan *et al*. (2008)
3.	Stimulation of lysozyme activity	Grouper	*L. plantarum*	Son *et al*. (2009)
4.	Enhanced survival rate	Olive flounder	*Lactococcus lactis*	Heo *et al*. (2013)
5.	Enhance immune and improved disease resistance	Tilapia	*Bacillus pumilus*	Aly *et al*. (2008b)
6.	Enhanced immune response	*Catla catla*	*Bacillus circulans*	Bandyopadhyay & Das Mohapatra (2009)
7.	Enhanced phagocytic activity of leucocytes	Chinese drum	*Clostridium butyricum*	Pan *et al*. (2008)
8.	Improved innate immune response	Olive flounder	*Zooshikella* sp.	Kim *et al*. (2010)
9.	Enhanced immune response	Common carp	*Flavobacterium sasangense*	Chi *et al*. (2014)

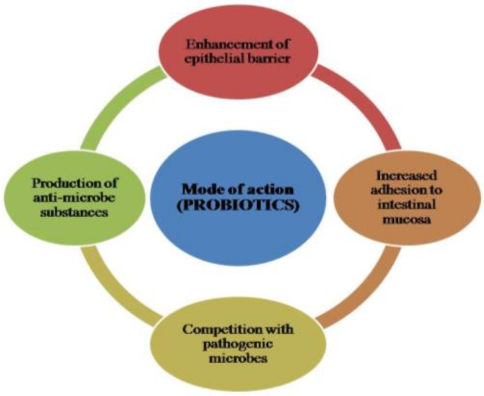
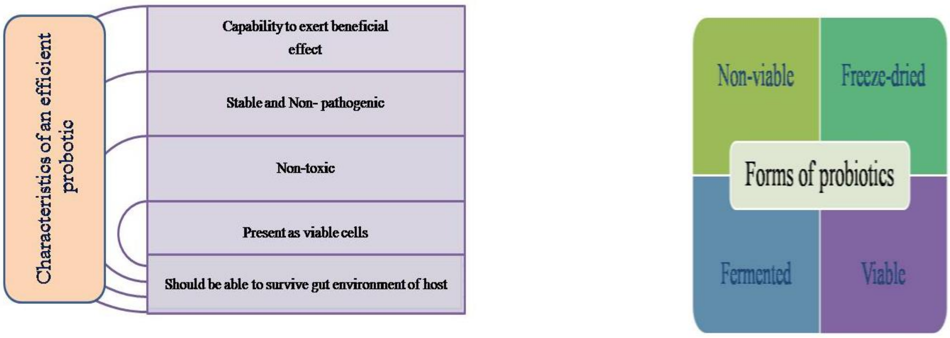


Fig. 1. Different modes of action by probiotics in fish (Bermudez-Brito *et al*., 2012).



A

B

Fig. 2. A. Characteristics of an efficient probiotic (Michael, 2014), **B.** Different forms of probiotic (Rao, 2010).

CONCLUSIONS

Aquaculture has developed into one of the industries with the fastest growth because it offers superior quality animal protein for dietary demands and food security. This escalating, intensified aquaculture production is constrained by a number of factors, such as disease outbreaks, high levels of stress, a scarcity of fish meal as a source of protein, etc. In the past, these problems have been treated with antibiotics and chemical disinfectants, but as a result, concerns about the safety of human and aquatic animal food have been raised, and as a result, environmental contamination has occurred. Aquaculture animals can thrive in the perfect habitat created by probiotics, which will also benefit the health of the animals.

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