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A review on application of probiotic, prebiotic and synbiotic for sustainable development of aquaculture

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Abstract
Aquaculture is one of the fastest emerging food producing sectors of the world. World aquaculture has immensely grown during the last few years as well as becoming an economically significant zone. However in this culture, aquatic organisms are subjected to stress condition that weakens fish immune system as well as increases the susceptibility to diseases. Thus, affects both economic development and socio-economic status of the local people in many countries. Disease control in aquaculture industry has been achieved by following different methods using traditional ways, synthetic chemicals and antibiotics. Application of antimicrobial drugs and pesticides has steered to the evolution of resistant strains of bacteria. Therefore, alternative methods are much more essential to maintain a healthy microbial environment in the aquaculture systems. The present review summarizes and discusses the effects of probiotic, prebiotic or synbiotic administration on growth performance, stress tolerance, intestinal microbiota, immune response and health of aquatic organisms.

Keywords: Aquaculture, probiotic, prebiotic, synbiotic, aquaculture

1. Introduction
Aquaculture is one of the world's promising and fastest developing food-producing sectors with the largest potential to accomplish growing demand of aquatic food [1]. World aquaculture has immensely grown during the last few years as well as becoming an economically significant zone. With the rising commercialization and intensification of aquaculture production, diseases and deterioration of environmental conditions are major problems in fish farming and face massive economic losses [2]. For prevention and control of diseases, antibiotics used as traditional strategy during the last decades and also for fish growth as well as efficacy of feed conversion. However, the development of antimicrobial resistant pathogens were recognized but there is a huge risk of transmission of resistance bacteria from aquatic environment to human [1]. As per them, using antibiotic is harmful for aquatic animals because it kills beneficial microbiota in gastrointestinal system of animals and it also accumulates in fish products to be unsafe for human consumption. Considering these factors, the improvements of non-antibiotic agents are more suitable for health management in this culture [3]. Dietary supplement such as probiotic, prebiotic and synbiotic provide nonspecific disease protection and also act as growth promoting factors. Although various studies evaluating the effectiveness of probiotic, prebiotic and synbiotic in fish has received till present very little attention and available data are still insufficient. Therefore, the objective of this review is to compile the existing data on the use of probiotic, prebiotic and synbiotic in aquaculture, highlighting the most important properties demonstrated to date.

2. Probiotic
Probiotics shows a new dimension in disease resistance and improving water quality in aquaculture industry. The Greek word probiotic means "for life", was introduced by Parker [4]. According to him, probiotics are "Organisms and substance, which contribute to intestinal and microbial balance". Fuller, [5] defined probiotics as "live microbial feed supplement which beneficially affect the host animal by improving its intestinal microbial balance". Probiotics are used in aquaculture to improve growth performance [6], nutrition [7], decrease diseases [8] and develop immune system [9].

2.1 Micro Organisms of Probiotic
Now a days, Probiotics are commonly used as therapeutic, prophylactic and growth supplements in aquaculture as well as "functional food" for human health [1]. Probiotics include Gram-positive bacteria, Gram-negative bacteria and many other organisms like yeast, bacteriophages and unicellular algae [10]. Pandiyan *et al*, [1] also added that Lactic Acid Bacteria have been broadly used as probiotic strains which are usually present in the intestine of healthy fishes such as the *Lactobacilli* and *Bifidobacteria*. Some gram positive bacteria like *Bacillus*, *Enterococcus, Streptococcus* act as common probiotic strains which are the main gastrointestinal microbiota [11]. On the other hand, Gram negative facultative anaerobes are dominant in gastrointestinal tract of fishes and shellfishes, as well as, symbiotic anaerobes prevail in posterior part of digestive tract of tropical herbivorous fishes [1].

2.2 Types of Probiotics
Probiotics are classified into following two groups:

2.2.1 Feed Probiotics
Some bacterial, fungal strains can be blended with feeding pellets or by encapsulating into live feed stock or administered orally to feed rearing animals to prevent disease and enhance essential microbial flora of the gut [11, 12]. Viability of strains should be tested before feeding animals. Probiotics like lactic acid bacteria applied in the feed of fry of Atlantic cod, showed adequate growth, survival and immune response [13].

2.2.2 Water probiotics
According to Prasad *et al*, [11] water probiotics are applied to reduce organic pollutants and various contaminants in water by directly applying to rearing medium. These improve water quality by converting organic matter to smaller units. Breakdown of organic matters evolve simpler substances like glucose and amino acids that are used as food for beneficial bacteria which reduce the accumulation of organic pressure and provide congenial environment to farmed stock. Probiotic bacteria such as *Bacillus* sp. can convert organic matter to CO_2 so that organic effluent can be minimized in aquatic system. By using nitrifying bacteria, the quantity of nitrate, nitrite, ammonia are reduced to a large extent. These lead to purify the water in the hatchery enhancing larval survival and growth [14, 15].

2.3 Significance of Probiotics in Aquaculture
Probiotics use in aquaculture show great impact on aquatic organisms. Probiotics decrease accumulation of organic load and maintain water quality in an efficient way. A modern probiotic organism can easily fulfill the desires of sustainable aquaculture development because it can heighten two major key factors of growth performance and disease resistance [16]. Prasad *et al,* [11] reported that *Lactic Acid Bacteria*, a popular probiotic strain, can be applied to control bacterial pathogen. In addition, another well-known probiotic organism, *Bacillus* sp. is used to diminish metabolic waste in aquatic system. Many strains of *Aeromomas* sp., *Pseudomonas* sp., *Vibrio* sp. act against infectious hematopoietic necrosis virus to show antiviral activity [17]. These probiotic organisms may be used singly or in combination such as incorporation of individual or combined supplementation of *Lactobacillus rhamnosus* and *Lactobacillus sporogenes* enhance health and disease resistance of common carp [18, 19, 20, 21]. Probiotics do not cause water pollution because of their eco-friendly nature, thus more and more suitable for aquaculture system. They not only promote animal health but also maintain consumer health safety [11]. Uses of probiotics and their target aquatic organisms are briefly demonstrated in table 1.
There are some possible benefits linked to the administering of probiotics which have already been suggested as:

2.3.1 Improvement of Water Quality
According to Michael *et al,* [22] the contamination of various nitrogenous compounds such as ammonia, nitrite and nitrate has been a serious problem in aquaculture system. In high concentrations, these compounds may be tremendously harmful and cause mass mortality. Ma *et al,* [23] described that *Lactobacillus* sp. simultaneously eliminates nitrogen and pathogens from polluted shrimp farms. According to Stanier *et al,* [24] gram-positive bacteria are usually more efficient in transforming organic matter back to CO_2 than gram-negative bacteria, which would convert organic carbon to bacterial biomass or slime. The aerobic gram-positive bacteria, such as *Bacillus* sp. were associated with development of water quality, reduction of pathogenic population in culture environment, enhancement of survival and growth rate, and the better health condition of juvenile *Penaeus monodon* [25,26].

2.3.2 Control of Diseases
Probiotics or their products have been found useful for health benefits and disease prevention in fish culture system. These include microbial adjunct that stop pathogens from multiplying in the gastrointestinal tract, on the superficial surfaces and in culture environment of the culture species [27]. Administration of probiotic can reduce the use of antibiotics and synthetic chemicals in the fish feed [5]. The effect of probiotic organisms is achieved through improving the immune system of culture organism, enhancing their disease resistance potential or generating inhibitory-substance that prevent the pathogenic organisms from disease formation in the host [16]. As per Rengpipat *et al*, [28] the use of *Bacillus* sp. showed disease protection by initiating both cellular and humoral immune resistances in tiger shrimp (*P. monodon*).

2.3.3 Growth Promoters
Probiotics have been used in aquaculture to enhance the growth of cultivated species and yet no side effect on the host. Yassir *et al,* [29] used probiotic bacteria as growth promoter on tilapia (*Oreochromis niloticus*) in his work and the highest growth performance with *Micrococcus luteus* was noted and the best feed conversion ratio was seen with the same probiotic organism. So they mentioned *M. luteus* as a growth promoters in fish culture. Lactic acid bacteria also referred as growth promoters due to effect on the growth rate in juvenile carp [30].

2.3.4 Source of Nutrients and Enzymatic Contribution to Digestion
According to Michael *et al,* [22] various microorganisms have a valuable effect in the digestive system of aquatic organisms. It has also been stated that *Bacteroides* and *Clostridium* sp. have supplied nutrients like fatty acids and vitamins to the host in fish aquaculture. Some microorganisms such as *Agrobacterium* sp., *Pseudomonas* sp., *Microbacterium* sp. and *Staphylococcus* sp. may contribute to nutritional processes in Arctic charr (*Salvelinus alpines*) [31].

2.3.5 Improvement of the Immune Response
As per Michael *et al,* [22] among the various beneficial effects of probiotics, modulation of immune system is one of the most important benefits of probiotics. They also reported that fish larvae, shrimps and other invertebrates have immune systems that are dependent mainly on non-specific immune responses for their resistance to infection. Sakai *et al*, [32] has been demonstrated that oral administration of *Clostridium butyricum* bacteria to rainbow trout improved their resistance to vibriosis by developing the phagocytosis of leucocytes.

2.3.6 In Aquaculture Management
These beneficial probiotic organisms can be administered through feeding, injection or immersion to improve aquaculture management [22].

3. Prebiotic
Prebiotics are non-digestible food ingredient that stimulate the growth or activity of beneficial gut commensal bacteria in host thus improves host health [33]. Gibson *et al*, [34] reported that a food ingredient which acts as prebiotic must possess the following criteria such as showing resistance to gastric acidity, hydrolysis by digestive enzyme, fermentation by gastrointestinal microflora and increase the abundance of intestinal bacteria related to health.

3.1 Prebiotic Organisms
Nowadays, carbohydrates use as most efficient prebiotics which can be classified on the basis of molecular size or step of polymerization [35]. According to Mahious and Ollevier [36], Fooks *et al*, [37] and Gibson *et al*, [34] various food substances such as non-digestible carbohydrates, some proteins and peptides, certain lipids act as prebiotic ingredient. Prebiotic compounds like fructooligosaccharides, mannanoligosaccharides, inulin or B-glucanare considered as the most effective prebiotics in aquaculture [33] (Table 2). Prebiotics are mainly fermented by *Bifidobacteria*, *Lactobacillus* and *Bacteriodes* [38].

3.2 Significance of Prebiotics in Aquaculture
Prebiotics are essential dietary supplement which enhance growth performance as well as microbial activities of digestive tract, also boost immune system and improve stress resistance that are discussed below.

3.2.1 Use of Prebiotics on Growth Parameters
A huge number of prebiotics are used as feed supplement to achieve better growth performance. Growth parameters vary on the basis of aquatic organisms as well as prebiotic supplementation. A diet containing 20 g kg^{-1} oligofructose, a fructooligosaccharide produced by partial enzymatic hydrolysis of inulin, resulted in increased growth of turbot larvae, but 20 g kg^{-1} inulin itself had no effect on growth [39].

3.2.2 Application of Prebiotics on Immune System
As per Yousefian and Amiri [38], last few decades, antibiotics were used to control bacterial diseases but this type of chemical substance is advisable to avoid in aquaculture. In recent years in the aquaculture industry, alternative strategies have been developed for disease control as well as reduction in the widespread use of antibiotics. Prebiotic is a well-known group of these strategies which enhances non-specific immune response. According to Bailey *et al*, [40] prebiotic can modify microbes of GI tract by increasing immune responses.

3.2.3 Application of Prebiotics on Microbes of Gastrointestinal Track
The gastrointestinal track of all invertebrates and vertebrates plays a vital role for providing habitat to different kinds of microorganisms [41]. Various prebiotic oligosaccharides such as inulin and oligofructose are fermented in the colon where they stimulate the growth of bacterial populations related with a well-functioning colon and this stimulation occurs because oligosaccharides are readily fermented by beneficial colonic bacteria and are not used effectively by pathogenic bacterial species [38].

4. Synbiotics
Synbiotic defines as nutritional supplements combining probiotics and prebiotics in the form of synergism therefore improving beneficial effects of individual probionts [42]. According to Gibson and Roberfroid [43] synbiotics was stated to 'characterise some colonic foods with interesting nutritional properties that make these compounds candidates for classification as health-enhancing functional ingredients'. Synbiotic positively affects the host by improving the survival and inserting of live microbial dietary supplements in the digestive tract by selectively stimulating the growth and/or by triggering the metabolism of one or a limited number of health-promoting bacteria, hence promoting the host "welfare" [42].

4.1 Significance of Synbiotics in Aquaculture
Synbiotics can be applied through supplementation or external bathing to develop growth performance, proper feed utilization, disease resistance, digestibility and stimulation of the immune system of aquatic organisms [44]. Application of synbiotics on different aquatic organisms in aquaculture system are given in table 3.

4.1.1 Application of Synbiotics on Fish Survival Rate, Growth Parameters and Feed Utilization
In rainbow trout, administration of *Enterococcus faecalis* and mannanoligosaccharides/polyhydroxybutyrate acid for 12 weeks not affects the survival rate of fish as well as the experimental fish was in good condition and there was no mortality during the feeding trial [42]. According to Ai *et al*, [45] and Geng *et al*, [46] administration of *Bacillus subtillis*/fructooligosaccharides in yellow croaker or *Bacillus subtillis*/chitosan in cobia, did not affect the survival rate, showing no changes among different dietary treatments. In case of Japanese flounder nourishing with *Bacillus clausii* and mannanoligosaccharides/fructooligosaccharides, in which fish retained active ingestion, showed proper growth and survival for all time [47].

In case of growth parameters, almost all references stated a positive effect of synbiotics application. In case of rainbow trout, dietary mannanoligosaccharides combined with *Enterococcus faecalis* significantly enhanced growth performance and nutrient utilization of fish, on the other hand, combination of *Enterococcus faecalis* and polyhydroxybutyrate acid developed growth performance of rainbow trout to some extent [42].

4.1.2 Uses of Synbiotics on Fish Digestive Enzyme Activity
Administration of synbiotics may effect on enzymes of fish digestive system. The increase in digestive enzyme activities would permit the host degrades more nutrients, enhancing digestion and promoting a probable increase in the weight gain rate and/or feed efficiency [42].

4.1.3 Uses of Synbiotics on Fish Immune System

As per Cerezeula *et al*, [42] synbiotics significantly effect on fish immune system considering a number of activities such as lysozyme activity, phagocytic activity, alternative complement pathway, respiratory burst, mucus production and superoxide dismutase activity. Lysozyme is the vital bactericidal enzymes of innate immunity, and shows acrucialdefense mechanism against various pathogens in fish [48].

4.1.4 Application of Synbiotics on Fish Disease Resistance

The efficiency of synbiotic therapy in terms of protection against infectious agents could be assessed by a challenge test because of its controlling power on pathogens and disease resistance capability [42].

Table 1: Uses of Probiotic in aquaculture system (Cruz *et al*. [49])

Uses of Probiotic	Probiotic Species	Gram Positive/negative Bacteria	Target aquatic species	Reference
Water quality	*Bacillus* sp.	+ve	*Penaeus monodon*	[50]
	Vibrio sp. NE 17	-ve	*Macrobrachium rosenbergii*	[51]
	Lactobacillus acidophilus	+ve	*Clarias gariepinus*	[52]
Control of diseases	*Enterococcus faecium* SF 68	+ve	*Anguilla Anguilla*	[53]
	Pseudomonas fluorescens	-ve	*Oncorhynchus mykiss*	[54]
	Lactococcus lactis	+ve	*Epinephelus coioides*	[55]
	Pseudomonas sp.	-ve	*Oncorhynchus mykiss*	[56]
	Bacillus sp.	+ve	Penaeids	[14]
	Vibrio alginolyticus	-ve	Salmonids	[57]
Growth promoter	*Lactobacillus lactis* AR21	+ve	*Brachionus plicatilis*	[58]
	Bacillus sp.	+ve	Catfish	[59]
	Streptococcus thermophiles	+ve	*Scophthalmus maximus*	[60]
	Bacillus coagulans	+ve	*Cyprinus carpio koi*	[61]
	Bacillus NL 110	+ve	*M. rosenbergii*	[62]
Digestion	*Lactobacillus acidophilus*	+ve	*Clarias gariepinus*	[52]
	Vibrio NE 17	+ve	*M. rosenbergii*	[51]
	Lactobacillus helveticus	+ve	*Scophthalmus maximus*	[60]
Improvement of immune response	*Clostridium butyricum*	+ve	*Rainbow trout*	[32]
	L. casei	+ve	*Poecilopsis gracilis*	[63]
	L. acidophilus	+ve	*Paralichthys olivaceus*	[64]

Table 2: Different Prebiotic substances in aquaculture (Song *et al*. [65])

Prebiotic substances	Subtype	Aquatic Organisms	Reference
Oligosaccharide	Fructooligosaccharides (FOS) Mannanoligosaccharide (MOS) Galactooligosaccharide (GOS) Arabinoxylan-oligosaccharide	*Salmo salar L.*	[66]
		Megalobrama terminalis	[67]
		Paralichthys olivaceus	[47]
		Acipenser stellatus	[68]
		Oreochromis niloticus	[69]
		Dicentrarchus labrax	[70]
		Panulirus ornatus	[71]
		Sciaenops ocellatus	[72]
		Atlantic Salmon	[66]
		Siberian sturgeon	[73]
Polyoligosaccharide	Inulin	Nile tilapia	[74]
		Huso huso	[75]
		Pseudoplatystoma sp.	[76]

Table 3: Application of synbiotic on different aquatic organisms (Cerezeula *et al*. [42])

Synbiotic (Probiotic/Prebiotic)	Aquatic Organisms	Reference
Enterococcus faecalis/MOS, PHB	*Oncorhynchus mykiss*	[77]
Bacillus clausii /MOS, FOS	*Paralichthys olivaceus*	[47]
Bacillus subtilis/ Chitosan	*Rachycentron canadum*	[46]
Bacillus subtilis/ FOS	*Larimichthys crocea*	[45]

[MOS-Manna oligosaccharide, FOS- fructooligosaccharide, PHB- plyhydroxybutyrate acid]

5. Constraints in the use of probiotic, prebiotic and synbiotic:

The use of probiotic, prebiotic and synbiotic receive excessive attention as a beneficial approach in aquaculture system but sometime due to insufficient information on their modes of action, the results of these application were hampered [78]. In human, probiotics may be responsible for four types of side effects in susceptible individuals: systemic infections, deleterious metabolic activities, excessive immune stimulation and gene transfer. There are few reports of bacteremia in humans, where separation of probiotic bacteria from infections seems to be the consequence of an opportunistic infection caused by skin lesions, cancer, chronic illness, or a drug induced abnormality. These conditions lead to a decreased intestinal wall that promotes the route of the bacteria through the mucosal epithelium and ultimately that may progress to septicemia [49]. Though, no evidence has been found in the fishes. According to Verschuere *et al.*, [79] the use of probiotics as biological control agents should be treated as a kind of risk insurance that may not provide any remarkable advantage when the culture is executed under optimal conditions and in the absence of pathogens. When culturing the aquatic species, microbial populations in the intestinal contents are much higher than those in the surrounding water [80]. Since, there is a chance of transmission of resistant bacteria from aquaculture environments to humans. The probiotics are destroyed by any other chemical or drug which generally interferes with the establishment of useful microbes [78]. Injecting probiotic into aquatic animals has been used to stimulate fish immune response against bacterial pathogenic infection [81, 82]. However, it is tough to inoculate probiotics into cultured fish, especially into small animals, and to treat huge numbers of fish in this way [83]. According to Hoseinifar *et al*, [84] the administration of *Lactobacillus lactis* on haematological parameters of *Acipenser persicus* demonstrated that the number of blood lymphocytes decreased whereas both the granulocytes increased.

Evidences on the effect of prebiotic and synbiotics on fish immunomodulation is limited. In case of beluga juveniles (*Huso huso*), prebiotic like dietary oligofructose didn’t show any significant effect on serum total protein, serum glucose, RBC counts, mean cell haemoglobin concentration, mean corpuscular volume or different enzyme activities like alkaline phosphatase, serum lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase [85]. The effects of fructooligosaccharide on haemato-immunological parameters of stellate sturgeon juvenile were studied by Akrami *et al*, [68], after 11 weeks of administration, they revealed that haemato-immunological parameters (RBC, WBC, haemoglobin, haematocrit and lymphocyte levels etc.) were significantly developed, while respiratory burst activity of leucocytes was not significantly improved by the dietary supplementations.Very limited studies have been conducted on constraints in the use of probiotic, prebiotic and synbiotic in aquaculture systems.

6. Conclusion

In recent years, probiotics, prebiotics as well as synbiotics have become an essential parts of the aquaculture practices for improving the growth performance and disease resistance. Probiotics play an important role in feed conversion, growth rates, weight gain, immune response and disease resistance of fish. On the other hand, prebiotics also have various beneficial effects mainly in disease resistance and nutrient availability of fish. Synbiotics, the combined application of probiotics and prebiotics, which improve the survival and establishment of the live microbial dietary supplement in the gastrointestinal tract of the host. The use of synbiotics confirm greater benefits than the application of individual probionts. Administration of a commercial synbiotics show an increase in growth performances, survival rate, serum protein and albumin content and enhanced feeding efficiency. These biotic applications are essential for developing the health status and production of fish, in place of more traditional immunological and disease control methods such as vaccinations, antimicrobials and immunostimulants. The significant variation in growth, feed utilization as well as health benefits with the dietary use of these biotic is possibly dependent on the fish species, duration of feeding and supplement dose.

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