Review Article:

Advances in Probiotic-Assisted Nutritional Strategies for Sustainable Aquaculture: Mechanisms, Applications, and Future Perspectives

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Abstract
Aquaculture has become one of the fastest-growing food-producing sectors worldwide and plays a critical role in ensuring global food security by supplying high-quality animal protein. As production intensifies, sustainable nutritional strategies that enhance animal health while reducing environmental impacts have become increasingly important. Among these strategies, probiotics have emerged as promising functional feed additives due to their ability to improve growth performance, feed utilization, gut health, immune competence, and disease resistance in cultured aquatic organisms. Probiotics promote a balanced intestinal microbiota through competitive exclusion of pathogenic microorganisms, production of antimicrobial compounds, and stimulation of digestive enzyme activity, thereby enhancing nutrient digestion and absorption. Their efficacy is influenced by multiple factors, including microbial strain, host species, physiological status, dietary formulation, and farming conditions. In addition to improving growth and survival, selected probiotic strains have demonstrated beneficial effects on reproductive performance, including enhanced fecundity, gamete quality, hatchability, and larval viability. Recent advances also highlight the role of probiotics in reducing antibiotic dependence and supporting environmentally responsible aquaculture practices. Overall, probiotic-assisted nutritional strategies represent an effective and sustainable approach to improving aquaculture productivity, animal welfare, and production resilience, while contributing to the long-term sustainability of the aquaculture industry.

Keywords: Probiotics; Sustainable aquaculture; Functional feed additives; Fish nutrition; Gut microbiota; Disease resistance.

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Introduction

In developing countries aquaculture is the main source for production of animal protein as compared to the other sources of food production. In Asia, fish is the dominant source of protein so now recently the aquaculture industries are increasing tremendously to fulfill the need of fish growth for using as food source (FAO, 2019). In coming world for compensation of animal protein shortage, aquaculture plays an important role in their expansion. In the field of aquaculture, feed plays essential role for production outcomes. Sectors of feed are expanding day by day to meet their level of good health and growth of shrimp and fish also to reach the culturists potential requirements. Success in animals and humans feeding proved the efficiency of probiotics and it gains attention in aquaculture, in culturing of organism's probiotics show good effect on reducing stress, promoting growth and controlling disease spread (Ibrahem *et al.,* 2013).

Probiotics has now been used as a nutritional factor in aquaculture industry for enhancing function and physiology of gastrointestinal (Diplock *et al.,* 1999). This enhancement face many hurdles and also creates opportunities for scientists to develop new products, improve food quality and nutritional units for good health of host organisms. In aquaculture, probiotics administration seems to be advantageous for disease prevention, nutritional factors and biological control (Balcazar *et al*., 2006) (Table 1).

History

This term was first used by Parker (1974) to describe it as a microbial food supplement. He defines that it is an organism or substances that play role in microbial balance in intestine.

The modified term of probiotics was given by Lilly and Stillwell in 1965 from the word probiotika. The term was applied to explain that microorganisms produced substance that enhances the logarithmic growth phase of host animal. The use of probiotics was first applied in aquaculture by Kozasa (1986), and give information about advantageous role of probiotics on poultry and humans.

The first experimental attempt of the probiotic application in aquaculture was made by considering the beneficial effects of probiotics on humans and poultry (Kozasa, 1986) the swift development of probiotic use in aquaculture has been driven by the harmful consequences of widely applied antibiotics and broad-spectrum chemicals that destroy beneficial microbes along with disease causing bacteria in aquatic organism (Lakshmi *et al*., 2013). Additionally, probiotics also work through different mechanisms in aquaculture system to eliminate the organic wastes and pollutants, as a result of incorporation of bioremediation and biocontrol when dealing with the environmental problems. In this context, probiotics can play an effective role in aquaculture production by providing greater non-specific disease protection as well as pollution free water sources (Panigrahi *et al*., 2010; Nandi *et al*., 2018).

In 1986, first time the probiotics were applied to test their ability to enhance the hydrobionts growth. Further probiotics were used for controlling infections of bacteria and improving water quality. Nowadays it is documented that probiotics

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can improve reproductive efficacy, stress tolerance and nutrients digestibility. Recently new probiotics are producing commercially from different species of bacteria such as *Lactobacillus* sp., *Bacillus* sp., *Carnobacterium* sp., *Enterococcus* sp., and the yeast *Saccharomyces cerevisiae* and it is recommended that their use is regulated carefully (Martínez *et al.,* 2012).

Table 1: Probiotics and its utilization.

Probiotics	Fish	Immune system	Nutrient utilization	References
Bacillus sabtilis	Asian seabass (*Lates calcarifer*)	Upregulationof immune-related genes (IL-1b, TNF-a)	Enhanced apparent digestibility of dry matter and protein	(Adorian *et al.,* 2019)
Lactobacillu s plantarum	Nile tilapia (*Oreochromis niloticus*)	Increased lysozyme and phagocytic activity	Improved protein efficiency ratio and feed conversion ratio	(Abou-El-Atta *et al.,* 2019)
	Common carp (*Cyprinus carpio*)	Increased lysozyme and phagocytic activity	Increased weight gain and performance, enhanced digestibility, improved feed conversion ratio	(Liu *et al.,* 2021)
Lactobacillu s rhamnosus	Rainbowtrout (*Oncorhynchus mykss*)	Enhanced immune cell activity, upregulation of immune-related genes, increased cytokine production, and improved lysozyme and complement activity	Enhanced protein utilization, improved liver function and detoxification, and improved feed conversion ratio	(Hajirezaee and Khan jani, 2023)
Lactobacillus acidophilus	Zebra fish (*Danio rerio*)	Increased serum lysozyme and complement activity	Improved growth performance and feed efficiency	(Hoseinifar *et al.,* 2015a)
Heat-killed Lactobacill us plantarum	Nile tilapia (*Oreochromis niloticus*)	Increased lysozyme activity, enhanced complement activity, and a boost in respiratory burst activity	Increased protein efficiency ratio and improved apparent digestibility of dry matter.	(Van Doan *et al.,* 2020)
Heat inactivated Bacillus coagulans	Common carp (*Cyprinus carpio*)	Increased serum lysozyme activity, higher complement C3 levels, and enhanced super oxide dismutase activity	Increased apparent digestibility of dry matter and crude protein, as well as enhanced intestinal protease and lipase activities.	(Xu *et al.,* 2016)

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Probiotics application is a vast concept used for the enhancement of species such as aquatic organisms, pet and farm animals guided by the success of probiotics in human's medicine. It is noticed that antimicrobial production and attachment by lactic acid bacteria are the important factor in removing pathogens (Munoz-Atienza *et al.,* 2013). Lactic acid bacteria attachment to the mucus layer may be the first defensive barrier against the bacteria that cause disease o infections bacteria (Ringo *et al.,* 1998).

The ability of pathogenic bacteria attachment is related to causing virulence which is considered as its first step (Bengmark, 1998). The capacity of probiotics attachment to the mucosal layer intestine of fish is reported in the study (Vine *et al.,* 2006). Rinkinen *et al.* (2003) determined that the efficiency or capacity of attachment depend on bacterial strain, they are not host specific.

Types of probiotics (Table 2; Fig. 1)

Table 2: Types of probiotics (Rao *et al*., 2016).

No.	Types	Description
1	Viable probiotics	Live form with guaranteed number of organisms has a protocol for counting and to be very steady and effective
2	Non-Viable Probiotics	Dead form of organism.
3	Fermentation probiotics	These are produced through fermentation.
4	Freeze-Dried Probiotics	These will expire speedily upon departure refrigeration

Selection criteria for probiotics

Numerous authors have described the necessary characteristics to qualify a microorganism as a probiotic. Necessary requirements for a probiotic to be effective and qualified as such are listed as follows (Kiron, 2015):

1- A suitable microorganism should be able to colonize the host and survive harsh conditions like bile, gastric juice, and host pH.

2- The probiotic candidate must be free of antibiotic-resistant genes and must not modify heritable traits of the host organism.

3- The microbe should benefit the host system by enhancing the growth and development of the immune system against pathogens. It also should have antimicrobial properties.

4- The probiotic candidate should not have harmful effects on the host. The evaluation of probiotics involves experiments conducted both outside and inside the living system in vitro or in vivo tests focus on resistance to bile and pH, adherence factors, anti-pathogenic effect, and non-antibiotic resistance. On the other hand, the in vivo evaluation of the probiotic candidate must show beneficial effects in the host (increasing the immune response, growth and absorption and utilization of food, modulation of intestinal microbiota, and reducing stress), not have harmful effects assessed by using a biosafety assay and improve the diseases resistance with an experimental challenge.

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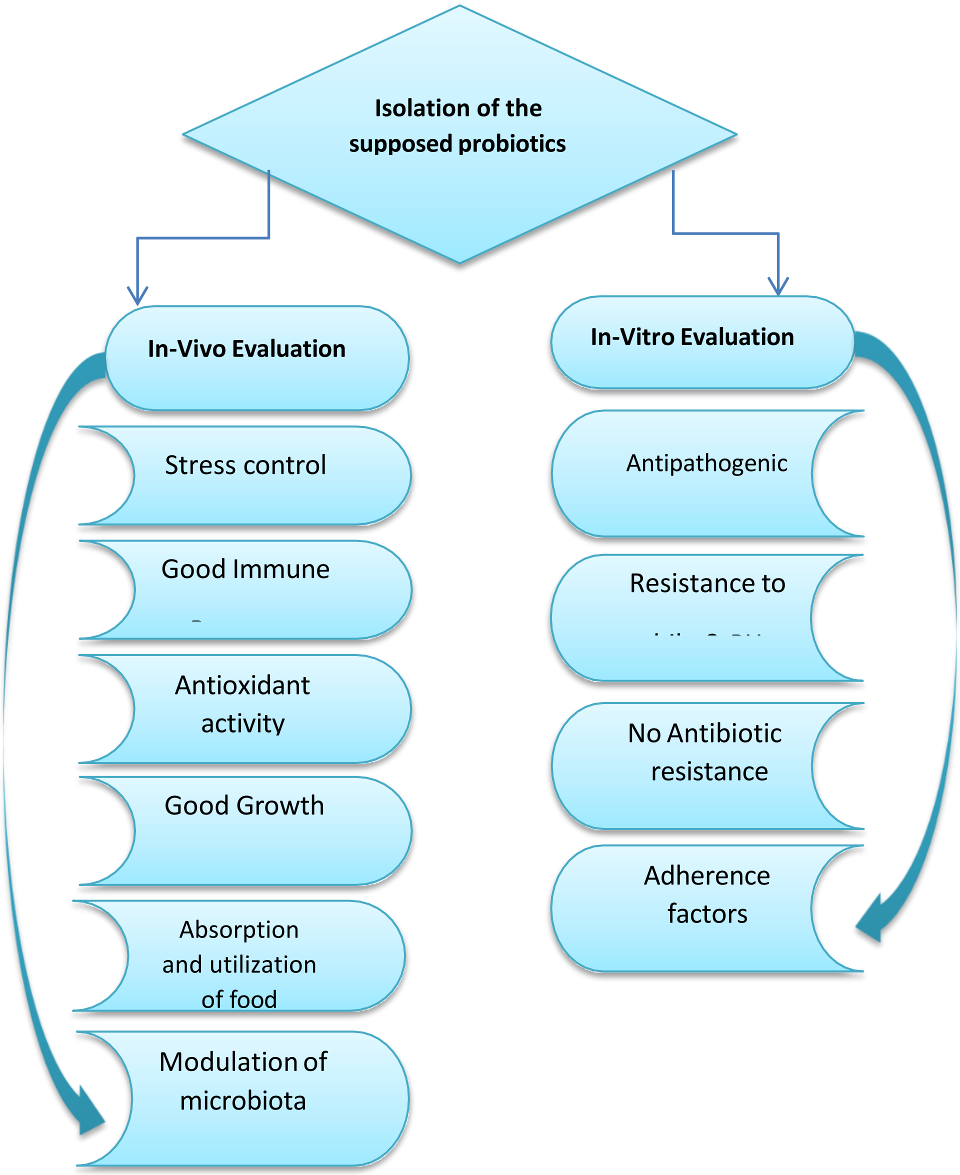

Figure 1: Probiotics types of flow-chart representation.

Application methods of probiotics

Application of probiotics based on its category, it is divided into two broad forms

1- Water probiotics are the bacterial species that proliferate in medium of water and reduce the growth of pathogenic bacteria by consuming all available nutrients and leaving no source of food for pathogenic bacteria

2- Gut probiotics are applied orally along with other feed ingredients to enhance gut microbial flora (Hasan and Banerjee, 2020) (Fig. 2).

Probiotics can be categories in several forms according to their mode of action (Hasan and Banerjee, 2020).

1. The type of probiotics called gut probiotics that can be applied orally with food of fish to enhance the efficiency of gut.
2. Type of probiotics that can be added in water medium, eliminate the pathogenic bacteria called water probiotics.

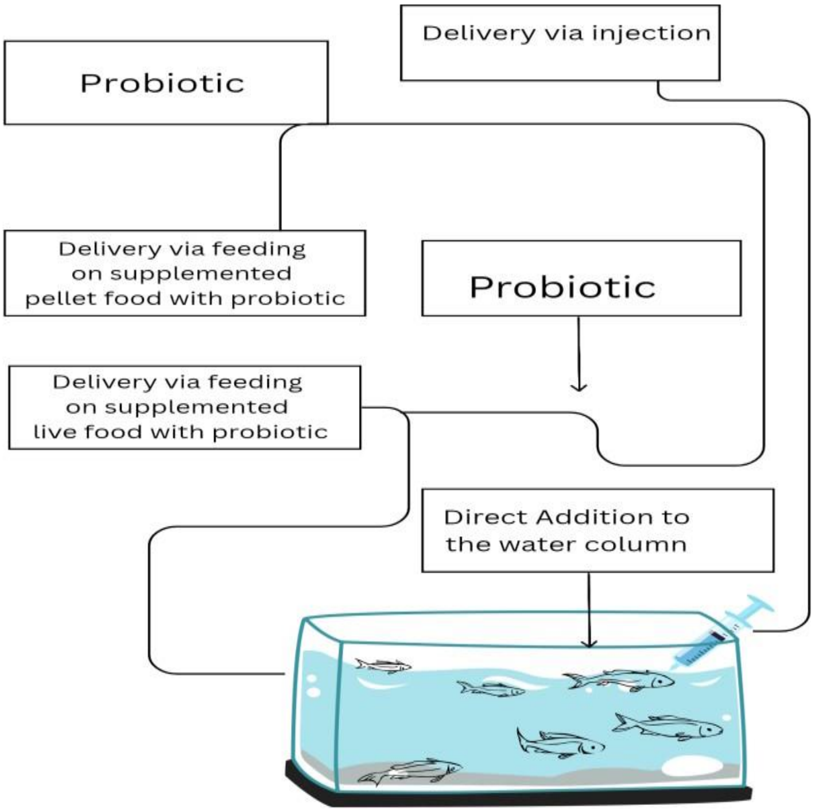

Figure 2: Probiotics application.

It is accepted widely that probiotics contain some important characteristics that increase the efficacy of agent at which it applied (Thakur *et al*., 2016; Priyodip *et al*., 2017).

Probiotic application

Probiotics can be applied in a several ways which was described (Ringo *et al*., 2018; Banerjee and Ray, 2017).

1. Add in the culture water and artificial diet.
2. Addition of probiotics through live feed.

At first, you need to understand the mode of action and proper method of application for taking good results in aquaculture practices. Gram-negative facultative symbiotic anaerobes such as Vibrio, Pseudomonas, Plesiomonas, and Aeromonas were also reported to be potential probiotic candidates present in the gastro-intestinal tract (GIT) of fish and shellfish (Lakshmi *et al*., 2013).

According to the food and agriculture organization [FAO] probiotics are recommended for improving aquatic environmental conditions by reducing morality and enhancing resistance to harmful pathogens furthermore probiotics candidates play an important role in enhancing the nutritional status of the host. The beneficial effects are temporal on occasion, depending on the time of application. There is a strong tendency to combine different photosynthetic bacteria, *Bacillu nitrifiers*, and denitrifiers together therefore, probiotics are often labeled as multifunctional and can be applied to various species under diverse culture conditions (Wang and Wang, 2008).

Probiotics are added constantly to fish cultures to maintain the health by enhancing the expression of several immunological factors, and to reduce the pathogen load to the gut mucus layer by

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occupying the physical space (Banerjee and Ray, 2017). Moreover, probiotic candidate also plays a crucial role in nutrient enhancement in host. Hamdan *et al*. (2016) have reported the enhancement of crude lipid, total protein, and body weight in Nile tilapia (*Oreochromis niloticus*) fed with probiotic strain of *Lactobacillus* sp. The most commonly used probiotic preparations in this purpose are the lactic acid bacteria (Ringø *et al*., 2018). These microorganisms are typically found in large population within the gut of healthy animals and are classified by the food and drugs administration It (FDA), is generally regarded as safe (GRAS status) (Giri *et al*., 2013). Additionally, *Lactobacillus brevis* and *Bacillus subtillis* have been reported to generate significant quantity of *phytase subtilis* are capable of producing higher amount of enzyme phytase (up to 1,354,906.6 U/mL) facilitating the breakdown and use of plants phytase, chemically known as myo-inositol *hexaphosphate* (Priyodip *et al*., 2017). Till date, several bacterial candidates (*Pseudomonas sp., Brevibacterium sp., Microbacterium sp., Agrobacterium sp., and Staphylococcus sp.*) have been reported to contribute in nutritional and metabolism physiology in Arctic charr (*Salvelinus alpines*) (Ringø *et al*., 2014).

Single and combination

Probiotics can be applied singly or in combination in different forms such as multistrain probiotics, probiotics with plant extract and probiotics with yeast extract (Shefat, 2018). The combination of *Bacillus megaterium* PTB 1.4 and *Pediococcus pentosaceus* E2211 administrated in feed showed best result than the single application of either probiotic in catfish *Clarias sp*. (Hamka *et al*., 2020). Combining probiotics often produces better outcomes than single-strain use, including higher survival rates in fish species such as Nile tilapia. (54.17%) and *Bacillus velezensis* (41.67%) (Van Doan *et al*., 2018). The combination of probiotic *Bacillus coagulans* and plant extract Mentha piperita showed the higher growth performance, nutrient retention and immunity in *Catla catla* than their single application (Bhatnagar *et al*., 2019).

Dose

The appropriate number of probiotics is influenced by the strain used, species of fish, their physiological state, farming conditions, and the objective of use. *Bifidobacterium* strains @ 1×107 CFU/g but not with 3×107 CFU/g (Sahandi *et al*., 2019). This suggests that more probiotics in feed does not necessarily result in more increase in growth. Dose is an important consideration while supplementing functional nutrients in aquafeeds., While a specific dose enhanced growth in rainbow trout, higher doses did not help and even decreased weight gain in pabda catfish *Ompok pabda* (Chowdhury *et al*., 2020) (Table 3).

Stress tolerance

Adding probiotics greatly improves the ability of aquaculture species to tolerate stress. Fish given *Lactobacillus plantarum* exhibited a smaller rise in cortisol under ammonia stress than those on a control diet, demonstrating decreased stress levels. The use of *Aspergillus oryzae* as a probiotic in Nile tilapia significantly improves the defense against hypoxia stress (Dawood *et*

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al., 2019). Supplementation of dietary commercial probiotics at 0.2% has significantly higher saline water stress tolerance than higher and lower doses in pabda catfish (Chowdhury *et al*., 2020).

Table: 3 Dosage of probiotics.

Fish Specie	Probiotics	Dose	Duration	Beneficial effects	References
O.niloticus	*B. subtilis and B. licheniformis (Biogen)*	3g Kg-1	14 weeks	Significant improvement in growth parameters (weightga in, AWG, ADG, SGR, and FCR) and haematological parameters (haemoglobin, RBC count, PCV, blood platelets, and WBC count, plasma proteins)	(Mehrim, 2009)
O.niloticus	*B. subtilisand S. cerevisae*	1.5gkg-1	6 weeks	Improved growth performance (weight gain, feed conversion ratio, and protein efficiency ratio)	(Marzouk *et al*., 2008)
Oncorhynchus mykiss	*Bacillus species and Aeromonas sobria*	2×10^{8}	2 weeks	Enhancedprotection againstmultiplepathogens (*Aeromonas salmonicida*, *Streptoc occus iniae*,*Yersinia ruckeri* , *Vibrio ordalii*, *Vibrio anguillarum*, and *Lactococcus garvieae*)	(Brunt *et al.,* 2007)
O.niloticus	*B. subtilisand L.plantarum*	10^{7} CFUg-1	60 days	Improved immunity (phagocytic, lysozyme, phenol oxidase, phosphatase activities and immunoglobulin)	(El-*Ezabi et al.,* 2011)
Labeo rohita	*B. subtilis and Lactobacillus lactis/ B. subtilis, L. lactis and S. cerevisiae*	10^{11} CFUg-1	60 days	Improved growth parameters (growth, protein efficiency ratio, nutrient retention and digestibility, lower feed conversion ratio), and micro ecology of gut	(Mohapatra *et al.,* 2014)
O.niloticus	*B. subtilisand B. licheniformis*	3g kg^{-1}, 5g kg^{-1}, 7g kg^{-1} and 10g kg^{-1}	4 weeks	Enhanced immunity (lysozyme, protease, antiprotease, SOD, immunoglobulin M level in both serum and skin mucus, upregulationofC-lysozyme, HSP-70, b-defensin, transforming growthfactor-binmid-intestinesandheadkidney)	(Abarike *et al*., 2018)

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Probiotics and different types of food in aquaculture

Probiotic enriched formulated artificial balanced diet is beneficial for the health of fish furthermore its application is easy. Additionally, farmers have to be attentive of three main constraints (Vine *et al*., 2006; Vílchez *et al*., 2015) viz.,

1- Leaching of feed which reduces the availability of probiotic to the host. Thus, dose standardization and regular monitoring is required.
2- Probiotic candidates confer beneficial effects to the host only when they are alive and functional under different appropriate environmental conditions, which farmers must carefully consider.
3- Nature of various ponds differs depending on the physicochemical parameters and natural feeds (zooplankton and phytoplankton). So, application, types, and dose of probiotics will be varied accordingly.

As a result of all of this, a microorganism must meet the following criteria before it can be considered as a probiotic, being nonpathogenic, both for the host species and aquatic animals as a whole, as well as for human consumers; lacking antibiotic resistance genes encoded by plasmids; and tolerant of low to high pH as well as bile salt concentrations (>2.5%). Alternatively, key advantageous features include strong adherence successful colonization of epithelial surface and efficient growth. They should be appropriate for feed supplementation demonstrate favorable growth characteristics and process varying degree of anti-pathogenic activity. additionally this may produce beneficial enzymes or vitamins originates from the host and its environment being native to both the host and the culturing environment; being robust enough to survive industrial processes and being viable under normal storage conditions; possessing better sensorial properties, possessing action of fermentation, a tolerance to freeze-drying and viability in feed during packaging and storage; promoting fish growth, boosting immunity and protecting fish from disease-causing microorganisms (Simon *et al.*, 2021). or live systems. Moreover, they must tolerate bile, gastric fluids, and host pH while maintaining the ability to grow and attach. Probiotic assessment is conducted through both in vitro and in vivo A probiotic is more likely to be effective if it meets several of these characteristics, though rarely all. Candidates are typically selected based on their ability to inhibit pathogens in laboratory methods (Amenyogbe *et al.,* 2022).

Nutrient competition

Nutrients are essential for bacterial growth. The use of similar nutrients gives rise to hostile competition among species (Wuertz *et al*., 2021). The utilizing nutrients present in the environment probiotics reduces accessibility to pathogenic microorganism (Kuebutornye *et al*., 2020). In fact, this restriction resulting from competition for nutrients is one of the main mechanisms used by probiotics to inhibit pathogens (Hoseinifar *et al*., 2018). Iron is essential for harmful bacteria, as it contributes not only to their growth but also to their virulence. For instance, *Bacillus spp*. can synthesize siderophores and show greater carbon utilization (Khan *et al.,* 2017). The absence of iron and carbon limits microbes'

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pathogenic functions. Furthermore, probiotics have the capacity to release a wide range of digestive enzymes. Thus, an increase in digestive enzymes can lead to the degradation of nutrients (Assan *et al.,* 2022).

Dawood *et al*. (2019) Research indicates that *Lactobacillus plantarum* significantly boosts digestive enzymes like amylase, lipase, and protease, with similar benefits observed from other probiotic strains and mixtures (Jang *et al*., 2019). *Lactobacillus casei* combined with b-glucan and mannan oligosaccharide significantly increased the production of these enzymes and proteases in carp (*Cyprinus carpio*), *Citrobacter, Lactobacillus bulgaricus*, and *Lactobacillus acidophilus* also increase rainbow trout amylase, trypsin, and alkaline phosphatase (Mohammadian *et al*., 2019). A study by Tarkhani *et al*. (2020) reported an increase in intestinal digestive enzyme activity in Caspian roaches (*Rutilus caspicus*) following the administration of *E. faecium*. Amenyogbe *et al*. (2022) also reported increased intestinal digestive enzyme activity in juvenile cobia (*Rachycentron canadum*) after supplementing indigenous isolates *Pantoea agglomerans* RCS2, indigenous isolates *Bacillus sp*. RCS1 and *Bacillus cereus* RCS3, and a mixture of *P. agglomerans* RCS2, *Bacillus sp*. RCS1 and *Bacillus cereus* RCS3. Even though these enzymes are linked to fish metabolism, their exact contribution remains unclear.

Prompt of decolonization (pathogenic bacteria)

For preventing colonization by pathogen, the possible mechanism is to compete physically for sites of attachment on host 's gut mucosal layer. It is called as ability to adhere to mucus and surface of wall is important for bacteria to become immerged in intestine of fish (Roeselers *et al*., 2011; Martínez *et al*., 2012). Adhesion of bacteria to the surface of tissue is effective in the early stage of infection of pathogens, the first effect of probiotics might be the competition for adhesion receptors with infectious pathogens (Chabrillón *et al*., 2006).

Uses

Probiotics minimize, partially or completely, the growth of pathogenic bacteria (Akayli *et al*., 2016). Xia *et al*. (2020) determined that it, also increase the response of immune system and enhance production of mucosal enzymes to promote the growth of host animal without causing any secondary pollution problem. Blood parameter variations of fish are a good indicator of water quality, health and nutrition (Ahmed *et al*., 2020). El-Haroun *et al.* (2006) observed that probiotics start consumption of carbohydrates after their settlement in intestine and then produce wide range of digestive enzymes as protease, lipase and amylase which increase digestibility that directly influence the growth rate due to the free intestinal disorder and increase of pre-digestion secondary compounds. The commonly used probiotic in aquaculture belongs to yeast (*Debaryomyces, Phafia* and *Saccharomyces*), microalgae (*Tetraselmis*) and gram-negative bacteria (*Aeromonas*, *Al-teromonas, Photorhodobacterium, Pseudomonas and Vibrio*), gram positive (*Bacillus, Lactococcus, Micrococcus, Carnobacterium, Enterococcus, Lactobacillus, Streptococcus, Weissella*)

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(Nayak 2013; Pandiyan *et al.,* 2013).

Bacillus subtilis and *Saccharomyces cerevisiae* are widely used probiotics in aquaculture due to their low cost and efficiency that can increase the feed utilization, and growth also they survive in the intestinal tract by colonizing in the gut of fish (Van, 2015; Allameh *et al.,* 2016; Hassaan *et al.,* 2018).

Probiotics enhance the larval nutrition and broodstock by establishing their presence in the gut of fish and providing enzymes (Protease, Amalyse, and lipase) and essential nutrients (fatty acids and protein). Fatty acids and protein are essential constituents of yolk that are considered as diet for maturation, development of oocytes and good rate of vitellogenesis (Dahlgren, 1980).

Lysozyme is considered as essential bactericidal enzymes having indispensable tool of innate immunity for fighting against pathogenic agents in fish. In serum, mucosal membrane of intestine and skin lysozyme is present. Single or combined probiotics are used to enhance the level of lysozyme in teleost (Adel *et al*., 2017).

Intestine health

As a feed supplement probiotics play a key role in improving enzymatic contribution in intestine, value of feed intake, enhance anticarcinogenic and antimutagenic activity, reduce the growth of infectious microorganisms, increase immunity and growth promoting factor (Harikrishnan *et al*., 2010). A study revealed that using probiotics strains can produce extracellular enzymes (lipase, protease, amylase) which show efficient result on digestive mechanisms of aquatic organisms and also produce growth factors e.g. fatty acids, amino acids and vitamins (Balcazar *et al*., 2006). The enhancement of intestine morphology includes intestine width, intestine length, lamina propria, goblet cells and area of villi were observed in fish that was treated with probiotics containing diet. Liver of probiotics treated fish show good result in regular nuclei shape an also decrease distance between liver tissues. Number of nuclei increase, diameter of hyperplastic muscles and muscle fiber were also observed while using multi strain species of probiotics in the feed of fish in comparison to control group (Hossain *et al*., 2022). Probiotics are live microorganisms which are when used in balanced quantity can enhance health of host organism (Giannenas *et al*., 2015; Egerton *et al.,* 2018). They have capacity to survive in the intestinal tract of animals. The advantageous effect of probiotics on fish is in controlling disease spread (Sharifuzzaman and Austin, 2017), survival and growth promoter (Kathia *et al*., 2018). contribution of enzymes for digestion and feed efficiency (Egerton *et al*., 2018; Mehrabi *et al*., 2018), enhancement of immune system (Bagherzadeh *et al*., 2018). Due to usage of probiotics the use of chemicals and antibiotics has reduced. Many of the probiotics applied through encapsulation, feeding, injection and immersion (Chizhayeva, et al.,2019).

Digestive enzymes

Taoka *et al.* (2007) determined that applying direct probiotics (*Clostridium butyricum*, *Bacillus subtilis*, *S. cerevisiae* and *Lactobacillus acidophilus*) or using it in feed can enhance the digestive enzymes production in tilapia. Metchnikoff (2004) was the first who determined the efficient

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role of some good bacteria while testing on farmers who consumed milk having pathogenic bacteria reliance on gut microbes for food makes it possible to take steps to change the flora of our bodies and to replace harmful microbes by beneficial microbes.

Increase growth and survival rates

Probiotics increase the appetite of organism which enhances survival and growth rate. It is reported that using *Bacillus spp*. single or combine with other spp. of bacteria in Tilapia can efficiently improve survival and growth performance (weight gain, fresh weight, specific growth rate, average weight gain, feed efficiency, feed conversion ratio) (Gobi *et al.,* 2018; Galagarza *et al.,* 2018). Results have been reported in other similar article on microbial species such as *Micrococcus luteus* (Abd El-Rhman *et al*., 2009), *Psychrobacter namhaensis* SO89 (Makled *et al*., 2017), *Lactobacillus* spp. (Vílchez *et al.,* 2014; Xia, 2018), *E. faecium* (Mehisan *et al*., 2015), *Aspergillus oryzae* **(**Dawood *et al.,* 2019**)** and *Pedio- coccus acidilactici* (Ferguson *et al*., 2010).

Effects of probiotics on the reproductive performance and larval quality of fish

Nutritional effects are also known for efficient reproductive performance and gonadal development of fish. It is reported that diet which have low level of protein, lipid, vitamin E and C, fatty acids then carotenoids produced poor quality of eggs as compared to the diet which have balanced level of formulated diet for fish (Watanabe *et al.,* 1995). The reproductive health of fish is important for their offsprings. Quality of gametes in fish reproduction is crucial for intensive culturing of fish species and fish farming industries. The most effective impairment in captive fish farming is to regulate the quality of gametes for the commercial fish farming development (Aydın and Çek-Yalnız, 2019). Some probiotics (good bactetia) not only enhance fecundity and ovulation but also promote quality of gametes, survival of larva and embryo, rate of hatchability (Vílchez *et al*., 2014; Ahmadnia- Motlang *et al*., 2017). In aquaculture the use of antibiotics is as preventive measure against various resistant pathogens of humans e.g *Escherichia tarda*, *Vibrio vulnificus*, *Aeromonas sp*., *Vibrio parahaemolyticus*, *Escherichia coli*, *Vibrio cholerae*, and many more (Allameh *et al*., 2016). Sai Gopal and Lakshmi Vishwanath (2013) reported about the development of resistivity against pathogens of aquatic environment under long term antibiotic pressure. So, the application of antibiotics is banned/restricted in many countries, then use probiotics in supplementary diet as an alternate which show benefical results to control pathogens (Bandyopadhyay *et al*., 2015; Wu *et al*., 2015). In the field of aquaculture, the concept of probiotics application is so different from using in terrestrial animals. Now it is well known that application of probiotics could enhance the efficiency of guts by causing variation in community of microbes (Nayak, 2010).

Effects on phytoplankton

Probiotic bacteria play vital role in controlling algal growth, particularly of red tide plankton (Qi *et al*., 2009). Bacteria antagonistic toward algae will be undesirable in green water larval rearing

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technique in hatchery where unicellular algae are cultured but will be advantageous when undesired algae species are developed in the culture pond.

Bacteriostatic effects of probiotics

Bacterial populations of probiotics are able to secrete diverse chemical substances with bactericidal or bacteriostatic activity against Gram-negative as well as Gram-positive bacteria. These inhibitory compounds arise from different categories,including proteins (like lysozyme and proteases), chemical elements such as hydrogen peroxide, and iron-binding compounds known as siderophores (lysozyme and different kind of proteases), chemical (hydrogen peroxides), and iron-chelating compound like siderophores (Giri *et al*., 2013).

Augmentation in the immune system

Probiotics play the beneficial role as immunostimulatory to assist in the protection of aquatic cultured species by reducing the impact of diseases and entrance of pathogens (Hai, 2015; Dawood and Koshio, 2016) (Table 4).

Table 4: Effectiveness of probiotics against pathogen.

Probiotics	Fish	Pathogenic agent	Effectiveness	References
Bacillus subtilis	Red hybrid tilapia	*Streptococcus agalactiae*	Reduced mortalities	(Ng *et al*.,2014)
Lactobacillus plantarum	Catfish	Viral hemorrhagic septicemia virus	Improves survival rate	(Hai, 2015)
Bacillus subtilis,	Nile tilapia	*Streptococcosis*	Competitive exclusion, immune enhancement	(Aly *et al*., 2008)
Flavobacterium sasangense	Common carp	*A.hydrophila*	Enhance immune response and disease resistance	(Chi *et al*., 2014)
Bacillus subtilis	Tilapia	Spring viremia of carp virus	Enhance immune response	(Tang *et al*., 2021)
Bacillus licheniformis	Tilapia	Streptococcus iniae	Improved the disease resistance	(Han *et al.,*2015)
Lactobacillus acidophilus	Nile tilapia	Pseudomonas fluorescens, Streptococcus iniae	Improve immune function and disease resistance	(Al-Dohail *et al*.,2011)

Probiotic candidates, including *Pseudomonas and Vibrio* species, have been proven effective in combating infectious *hematopoietic necrosis virus* (IHNV) (Sahu *et al*., 2008). Furthermore, Paralychthys olivaceus fed with Sporolac (*Lactobacillu*s sp.) supplemented food develop resistance against lymphocystis disease virus (LCDV) (Harikrishnan *et al*., 2010). Similar experiments have also proved the enhanced virus resistance power in grouper fish fed with probiotic strain of *Bacillus subtilis* E20 (Liu *et al*., 2012)

Effect on reproduction

Few studies have examined how probiotics affect reproduction in aquaculture, although strains such as *Bacillus subtilis* and *Lactobacillus species* are commonly used

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Lactobacillus casei. It is well researched that reproduction in different factors like fertilization, gonadosomatic index, fecundity, and production fry from the females is influenced by probiotics (Abasali and Mohammad, 2011). Recent study also reported that probiotics increase the daily numbers of ovulated eggs compared to control levels with higher hatching rate and faster embryonic development in zebrafish (Gioacchini *et al*., 2013). Additionally, it is now profoundly accepted that probiotics reduce the concentration of the stress hormone cortisol and activate the expression of antioxidative enzymes (superoxide dismutase, catalase, and glutathione peroxidase) to increase the stress tolerance (Zolotukhin *et al*., 2018). Probiotics are now gaining considerable global attention for both scientific research and commercial applications in aquaculture (Hoseinifar et al., 2016; Banerjee and Ray, 2017)

Conclusion

Probiotics represent a well-established and sustainable strategy for enhancing aquaculture productivity and the health of cultured species, particularly in light of the rapid expansion of the industry and the increasing demand for environmentally responsible disease management practices. Accumulating evidence indicates that the efficacy of probiotics is contingent upon factors such as strain specificity, dosage, mode of administration, and host species, with multi-strain formulations frequently demonstrating synergistic interactions. Both experimental and field studies have consistently shown that probiotics contribute to the maintenance of gut microbial homeostasis, suppression of pathogenic microorganisms, and modulation of host immune responses, thereby enhancing disease resistance. In addition, probiotic application has been associated with improved water quality and a reduced reliance on antibiotics, which is critical for limiting the development and spread of antimicrobial resistance in aquaculture systems. Overall, current findings support the strategic and sustained use of probiotics as an effective and eco-friendly approach for improving growth performance, physiological health, and disease resilience in aquaculture species.

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