BorneoScience
The Journal of Science & Technology
Volume 44 Issue 2 : September 2023
Online ISSN : 2231-9085 | ISSN : 1394-4339

CURRENT PROBIOTICS APPLICATION FOR AQUACULTURE FEED: A REVIEW

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Received 28th March 2023; accepted 12th September 2023
Available online 30th November 2023
Doi: https://doi.org/10.51200/bsj.v44i2.4703

ABSTRACT. *Probiotics are a helpful alternative that can be used to increase aquaculture output in a sustainable way. For probiotics to have the desired effects when applied to a certain aquaculture species, first, the proper strain and dosage must be chosen. The second, probiotics can be given intravenously, orally, or as feed additives, the last of which is frequently employed in aquaculture. Probiotics application in aquaculture production provides several advantages, including enhanced stress tolerance, enhanced disease resistance, enhanced immunological defence against infections, enhanced disease performance, and enhanced feed utilization. To improve the economic performance of the aquaculture species, probiotics can be used at the farm level. This review seeks to assess the current apprehension of probiotics as fish-feed supplements in aquaculture, types of probiotics, administration methods, mechanism of action, benefits, and effects. It also constrains the approach of contribution probiotics application to the aquaculture activities which enhance the fish health and growth based on the results of experimental studies.*

KEYWORDS. Aquaculture, benefits, effects, mechanism of action, probiotics

INTRODUCTION

Aquaculture activities have experienced significant growth worldwide, emerging as a crucial contributor to the global food production sector. Aquaculture is the term used to describe the farming of aquatic organisms in coastal and inland settings employing alterations to the growing process to boost productivity. According to the United Nations Food and Agriculture Organisation (FAO) (2020), from 25.7% in 2000 to 46% in 2018, aquaculture's share of the world's fish production increased. From 1961 to 2017, fish consumption increased on average by 3.1% annually, outpacing increases in consumption of all other animal protein foods (meat, dairy, milk, etc.) and the global population growth rate of 1.6%. In 2017, consumption of fish accounted for 7% of all proteins ingested and 17% of the animal protein intake of the entire world's population (FAO, 2020). All statement points to a huge increase in worldwide fish consumption over other sources of animal protein.

The aquaculture industry grapples with various challenges due to the expansion and commercialization of production in response to rising demand. These challenges encompass maintaining water quality, controlling diseases and epizootics, enhancing broodstock domestication and improvement, devising suitable feeding systems, and advancing hatchery and grow-out technologies. (FAO, 2022). Out of these, disease outbursts are presently the foremost obstacles to the aquaculture production for many species, which isolating both financial and social growth in many nations (Mugimba *et al*., 2021). Diseases outbreaks caused significant financial losses to global finfish aquaculture, which are projected to range from US$ 1.05 to US$ 9.58 billion annually (Tavares-Dias & Martins, 2017). The aquaculture industry's expansion is hindered by very high mortality rates caused by harmful pathogens. In intensive aquaculture, bacterial infections have been found to represent biological production bottlenecks, necessitating the use of medications and antibiotics in health management techniques (Okocha *et al*., 2018). Antibiotics have been used traditionally in aquaculture for a number of reasons, including the improvement of growth and feed conversion efficiency as well as the management and prevention of fish infections (Leong *et al*., 2022). Probiotics play a vital role in the aquaculture industry, preserving overall fishes' health. Numerous research has been carried out to investigate the presence of bacteria and their features, including antibiotic resistance, in a natural water environment, such as a river, because the problem of antibiotic-resistant bacteria has grown more serious in aquatic habitats (Leong *et al*., 2018). The unrestricted use of antibiotics causes an imbalance in the gut flora and the predominance of antibiotic-resistant microbes, which impairs the health of fish and leads to residue building up in the muscle, posing a possible health risk to consumers (Lihan *et al*., 2021). Probiotics are an alternate strategy to control fish health in the aquaculture business because of the uncertainty surrounding the use of antibiotics in aquaculture. Probiotics can be utilised in aquaculture to increase growth, feed utilisation, disease resistance, immunological response, and water quality (Subedi & Shrestha, 2020). Therefore, probiotic is a health-promoting alternative used in fish feed to help reduce the negative impact of antibiotics and enhance aquaculture production in a sustainable way (Hai, 2015). This review seeks to assess the current understanding of probiotics as fish-feed supplements in aquaculture, summarize the benefits and the effects.

PROBIOTIC USED IN FISH FEED

The probiotic term means "for life," and it comes from the Greek words "pro" and "bios" (Pradhan *et al*., 2020). Hill *et al*. (2014) explain a probiotic as living microorganisms that, when given to a host in sufficient quantities, improve their health. Hoseinifar *et al*. (2018) described probiotics as microbial feed additives that modify the gut microbiota to confer host organism. Due to technological advancements, probiotics have become readily accessible in our everyday food products, and they are even integrated into animal feed as functional additives. (Manik *et al*., 2021). El-Saadony *et al*. (2021) claim that probiotics used in aquaculture contain bacteria that improve the quality of the water as well as the water itself or prevent diseases from growing in the water. The World Health Organisation (WHO) and the Food and Agriculture Organisation of the United Nations (FAO) both stated that living microbes are beneficial to the host's health when administered in the right quantities. Probiotics have recently been interpreted as "living or dead, or even a component of the microorganisms that work under multiple modes of action in delivering good benefits to the host or its environment" under an aquaculture understanding by Lazado & Caipang (2014). Probiotics must undergo a proper selection as the appropriate strains can have detrimental consequences on the host (Lingoh *et al*., 2020; Schuurman *et al*., 2022).

When choosing potential probiotics, it is ideal to look for strains with the following qualities: (i) enhanced growth or resistance to disease in the host animal; (ii) non-pathogenic and non-toxic; (iii) present as live cells, preferably in large numbers; and (iv) capacity to survive and metabolise in the gut environment; and (v) stability and ability to remain viable for longer periods under sterile conditions. Probiotics may be given orally, intravenously, or directly submerged in water (Anjana & Tiwari, 2022) which can be applied singly or in combination (Hai, 2015). By controlling intestinal microbial stability, secreting antibacterial compounds such as bacteriocins and carboxylic acids, fighting with pathogens to stop their attachment to the gut, pathogen survival, and producing an antitoxin impact, probiotics have an antimicrobial effect (Jahangiri & Esteban, 2018). In terrestrial animals, lactic acid-producing bacteria are frequently used, whereas a wide variety of microorganisms are used in aquaculture, where effective supply of both Gram-positive and Gram-negative bacteria (Hai, 2015). The concept of probiotic therapy has provided fresh perspectives on the importance of gut flora in disease prevention (Abd AL-Khaliq, 2019). Aquaculture frequently utilizes non-bacterial probiotics such as bacteriophages, microalgae, and yeasts. Basically, there are five groups of probiotics listed in Table 1. In aquaculture, probiotics can be provided in a variety of ways, including through feeding, injection, or direct submersion in water (Hai, 2015; Anjana & Tiwari, 2022). The methods of probiotic preparation are illustrated in Figure 1.

Table 1. Methods of probiotic preparation.

Types of probiotics	Descriptions
Freeze dried	Lyophilization process to remove moisture from the probiotics while preserving their viability and functionality.
Fermentation	Produced through fermentation process.
Viable	This is a live system that contains a certain number of organisms, has a routine for counting, and is extremely reliable and effective.
Non-viable	Dead organism

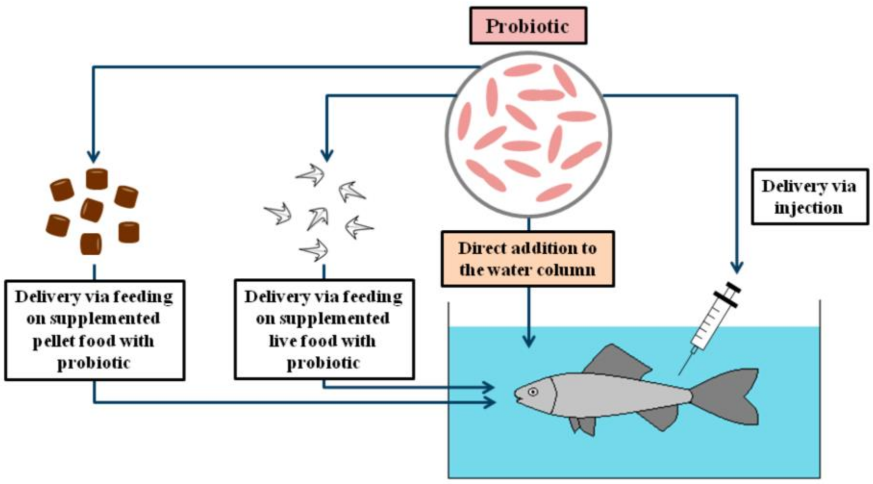

Figure 1. Different methods of probiotic administration (Jahangiri & Esteban, 2018).

Mechanism of action

Hancz (2022) described that there has been a lot of discussion about using probiotics in the creation of microbial management strategies and reducing the usage of medicinal drugs and antibiotics to achieve a more ecologically friendly and sustainable aquaculture. The probiotics' mechanisms of action are well defined and include creating pathogen-unfriendly surroundings by producing an inhibitor (hydrogen peroxide, lysozymes, proteases, and bacteriocins), competing with adherence sites for essential nutrients, enhancing host immune responses, supplying extra essential nutrients, vitamins, and enzymes, and dissolving direct absorption (Sankar *et al*., 2016; Plaza-Diaz *et al*., 2019; Kouhounde *et al*., 2022; Tegegne & Kebede, 2022; Zhang *et al*., 2023). Multiple screening steps are required to confirm the viability of probiotic bacteria for field application. Stringent screening steps are usually followed by molecular identification to confirm the identity of the good bacteria. world.

The conflict between microbial species over food sources and a territory is known as microbial antagonism. Thus, one organism outcompetes another, the organism that didn't succeed in the environment is inhibited. *In vitro* antagonistic screening against several strains of pathogenic bacteria can be used to assess the competitive exclusion by putative probiotic bacteria. Environmental factors that encourage the growth of bacterial species, such as culture water and abiotic or biotic factors, have a significant effect on the composition of microbial communities. The metabolic and physiological roles of the gut microbiota have been the subject of several investigations. According to Ray *et al.* (2012), attribution of the precise contribution of the gastrointestinal microbiota in exothermic animals is challenging due to the complexity and variable ecologies of different fish species' digestive tracts. This is true even though numerous studies have suggested that microbial activity in the gastrointestinal system may be an essential nutrient and enzyme for the host. The non-specific immune system has been demonstrated to benefit from probiotics in several investigations using rats and in vitro models (Shi *et al*., 2016). Besides, the similar immune response system is stimulated by phagocytosis and antibacterial activity as shown in the Chinese mitten crab (*Eriocheir sinensis*), which is largely responsible for this protection (Wang *et al*., 2019). Abdel-Latif *et al*. (2023) described that supplementing fish with probiotics, either mono-species or multi species mixtures, can improve their immune response. Probiotics could be an alternative used to control the occurrence of viral infection prevention in aquaculture. According to Abomughaid (2020) and Dauda *et al*. (2013), probiotics with antiviral action against infectious IHNV (Infectious Hematopoietic Necrosis Virus) includes *Pseudomonas* sp., *Vibrio* sp., *Aeromonas* sp., and *Coryneform* bacteria isolated from salmonid hatcheries, with a plaque reduction of more than 50%. One of the variables is water quality which is linked to farm-based fish disease outbreaks. The jumble of organic and nitrogenous wastes such as ammonia and even nitrite, is a great concern especially in fish breeding. Probiotics, including *Bacillus* sp., can enhance pond water quality by breaking down organic matter and releasing CO_2, which is especially advantageous during intensive production (Hlordzi *et al*., 2020). In commercial shrimp farms in Malaysia, photosynthetic bacteria and *Bacillus* sp. can enhance water quality, juvenile shrimp survival rates, and growth rates.

Advantages and disadvantages of using probiotics

Systems for growing aquatic life in fresh, brackish, and marine waters are all examples of aquaculture systems. However, the disease's occurrence is utmost impediment to its long-term viability. Probiotics are frequently used in aquaculture systems to control bacterial pathogens. *Streptococcus thermophilus, Lactobacillus, Bifidobacterium, Pediococcus* sp., *Carnobacterium* sp., *Flavobacterium* sp., *Bacillus* sp., *Cytophaga, Pseudomonas* sp., *Alteromonas* sp., *Aeromonas* sp., *Enterococcus* sp., *Nitrobacter* sp., *Nitrosomonas* sp., and *Vibrio* sp. are examples of marketable probiotics. The detrimental impacts of probiotics on aquaculture systems and the environment, however, have largely gone unstudied. Due to temperature-related stress, the benefits of utilising probiotics include *Lactobacillus sporogenes, Bacillus subtilis, Saccharomyces cerevisiae*, and *Lactococcus lactis* could increase the production of red blood cells and immunology system in fish (Mohapatra *et al*., 2014). Besides, *Lactobacillus delbrueckii* has positive effects on European sea bass growth and body weight (Pérez-Pascual *et al*., 2020). Resistance to *Aeromonas* sp. and *Salmonicida* sp. has improved by using *Lactobacillus rhamnosus* (Nikoskelainen *et al*., 2001). Because nutrients and other microbes are also involved, probiotics have drawbacks of their own. The mode of action is still unknown; further research into the processes of bacterial competition is required; and it is also necessary to determine the ecological significance of the various in-situ processes. Aquatic species' intestinal contents contain substantially higher microbial populations than the surrounding water when those populations are cultured. As a result, there is a chance that aquaculture conditions could spread resistant germs to people. According to article published in International Magazine For Animal Feed & Additives Industry (2023), there is a possibility of the introduction of non-native microbes into the aquatic environment, which might change the ecology and have an impact on native species; a risk that pathogenic microbes which has been used as probiotic may mutate and contaminate aquaculture products; probiotic overuse; lastly the high production cost which will be the burden for small scale aquaculture farm.

Effects of probiotics on fish

According to Rahman *et al.* (2021), “For life” was the phrase introduced by Parker from the Greek word. Probiotics are chemicals and living organisms that support intestine and microbial balance. A probiotic organism needs to be resistant to the stomach's acidic condition, bile, and pancreatic enzymes, access to the intestinal mucosa layers, and the ability to colonise for an extended period in order to function well inside the host. Probiotic effects on fish performance include growth, feed conversion ratio, protein efficiency ratio, digestibility, body composition, immune system response, and pathogenicity and challenge capacities, according to Allameh *et al.* (2017). According to the article, how probiotic bacteria affect the growth performance as it depends on what type of probiotic bacteria was used to feed in fish. Some have an effect on reducing the stress factors in fish. Some have effect inside the digestive tract. The probiotics that were provided to the fish diets could improve their protein efficiency ratio. Next, it also has a part in increasing the functions of the immune feedback in fish. One of the instances is the utilization of lactic acid bacteria (LAB) to boost immunity and control populations of possible infections. Fish immune systems, both specialised and generalised, can be stimulated by probiotics. Probiotic bacteria can also improve fish's immune systems by increasing their acidophilic granulocytes and immunoglobulin cells. Probiotics can also lower the risk of disease outbreaks or lower the frequency of illness.

Improve feed utilization

Most of the research has proved that probiotic has the ability to alter enzymes production and hence improve the fish's feed utilization. *Lactobacillus pentosus* supplementation increased the feed utilisation in white prawns (*Litopenaeus vannamei*), according to research by Zheng & Wang (2017). The addition of heat-killed *Lactobacillus plantarum* at 50, 100, or 1000 mg/kg over 12 weeks significantly improved the amylase, lipase, and protease activities of Nile tilapia (Dawood *et al*., 2019) and thin clawed crayfish (Valipour *et al*., 2019). Besides, feed efficiency increased with increasing probiotic dosage as shown by most researchers. Some of the significant results were discussed as Muchlisin *et al.* (2017) stated that the optimal probiotic dosage for keureling fish (*Tor tambra*) fry was 10 ml kg -1 with feed conversion rate of 2.37. Compared to prebiotic supplementation, probiotic-based diets have a greater favourable impact on the feed conversion rate (1.43-1.8), protein efficiency rate (1.33-1.71) and survival (100%) of *Channa striata* fingerlings (Munir *et al*., 2016). Application of multi-strain probiotics for 12 weeks in cage cultured striped catfish (*Pangasianodon hypophthalmus*) in Bangladesh has proved that feed efficiency increased (from 2.69 to 3.03 protein efficiency) (Chowdhury & Roy, 2020). Probiotic such as *Saccharomyces cerevisiae* obtained the best performance at concentration of 4 g kg^{-1} while *Bacillus subtilis* required higher dose of 10 g kg^{-1} in order to present with better feed conversion ratio (1.61) and significantly higher protein level (86-89%) (Opiyo *et al.*, 2019).

Growth stimulator

The direct impact of bacterial probiotics on fish development performance is one of the anticipated outcomes of their use, either by increasing nutrition intake directly or by delivering nutrients (Roque Joel *et al*., 2020). Feeding with probiotics will enhance gut microflora health, increase in the release of digestive enzymes, stimulation of hunger, creation of vitamins, degradation of indigestible components, and increase the release of digestive enzymes (Lingoh *et al*., 2020). Supplementation of *Bacillus subtilis* in feed at 10^7 and 10^9 CFU/kg food for five weeks significantly increased the growth of Litopenaeus vannamei, a Pacific white shrimp (Kewcharoen & Srisapoome, 2019). The mud crab, *Scylla paramamosain*, gained weight and had a much faster rate of specific growth after receiving dietary supplements of *Enterococcus faecalis* and *Pediococcus acidilacti* (Ray *et al*., 2012). Ahmadifar *et al.* (2020) shown that feeding *Pediococcus acidilactici* to zebra fish (*Danio rerio*) improved their growth performance. Probiotic supplementation of 0.2% in striped catfish in Bangladesh resulted in impressive growth (29.66%) and economic performance (66.37%) as showed by (Chowdhury & Roy, 2020). Different probiotic are shown with different level of performance, for example *Saccharomyces cerevisiae* and *Bacillus subtilis* required different concentration in order to present with higher final weight (255.31g) and specific growth rates (SGR) (0.77%) (Opiyo *et al.*, 2019). Furthermore, application of probiotics in fin fishes which significantly increased their growth rate were proven as *Bacillus pumilus* in juvenile golden pompano, *Paenibacillus ehimensis* (Lin *et al*., 2022); *Bacillus circulans* PB7 in South Asian carp, Catla (Bandyopadhyay & Das Mohapatra, 2009), *Bacillus megaterium* and *Pediococcus pentosaceus* in catfish (*Clarias* sp.) (Hamka & Widanarni, 2020); *Pediococcus pentosaceus* in grass carp (Gong *et al*., 2019); *Lactobacillus plantarum* used in Nile tilapia (*Oerochromis niloticus*) (Gewaily *et al*., 2021) are well documented.

Disease resistance

The ability of probiotic microorganisms to secrete substances like bacitracin and polymyxin, which have a bactericidal or bacteriostatic effect on pathogenic bacteria in the intestine of the host, boosts the host's resistance to disease. Disease resistance is shown by supplementation of *Enterobacter* sp. in rainbow trout which enhanced the disease protection against *Flavobacterium psychrophilum* (LaPatra *et al*., 2014); The majority of people are focusing on *Aeromonas* sp. either as pretreated pathogen probiotic or as fish pathogen because different species of *Aeromonas* bacteria can inhibit the growth of each other. Previously Irianto and Austin (2002) have observed that consuming *Aeromonas hydrophila* as a probiotic reduces the mortality caused by other *Aeromonas* pathogen such as *Aeromonas salmonicida* in rainbow trout. Besides, Irianto *et al.* (2003) showed that feeding goldfish (*Carassius auratus*) with formalin-inactivated *A. hydrophila* A3-51 boosted resistance against *A. salmonicida*. Additionally, Pieters *et al*. (2008) showed that feeding rainbow trout with 10^8 cells/ g of *A. sobria* GC2 and 10^{10} cells/ g of *Brochothrix thermosphacta* BA211 increased their resistance to the causative agent of fin rot, *A. bestiarum*. However, *A. hydrophila* is causing disease in most carp fish, thus application of other Aeromonas sp. may be able to stop the disease. This is shown by Chi *et al*. (2021) proved that common carp's disease resistance to *Aeromonas hydrophila* was improved by applying *A. veronii* and *Flavobacterium sasangense* in farming; It has been demonstrated that *Lactococcus garvieae*, which is present in raw cow’s milk, increases Nile tilapia's resistance to *Staphylococcus aureus*. (Abdelfatah & Mahboub, 2018); *Cromileptes altivelis* resistance to *Vibrio harveyi* was improved by *Lactococcus lactis* (Sun *et al*., 2019); Disease resistance of rainbow trout against *Lactococcus garvieae* was enhanced by *Enterococcus faecalis* present in commercial probiotics (Baños et al., 2019); Numerous significant fish infections, such as *Aeromonas hydrophila*, *Aeromonas sobria*, *Aeromonas veroni*, *Eelwardsiella tarda*, *Plesiomonas shigelloide*, and *Lactococcus garviae* are effectively combatted by *Pediococcus pentosaceus* (Raheem *et al*., 2021).

Immune response

The pathogens can be fought by producing inhibitory substances. Probiotics can prevent pathogen infection by boosting the host immune system and cellular and non-specific immunity in the body (Raheem *et al*., 2021). According to Munirasu *et al.* (2017) and Avella *et al*. (2010), the effects of probiotics towards fish is to increase survivorship, increase growth and development and reduce stress in fish. Based on Avella *et al*. (2010), for the probiotic to be most effective is when the fish is in its early stage of development. This can increase the survivorship by reducing the chances of getting infected by disease of the fish in future. Probiotics can also help alleviate fish stress and foster growth. Munirasu *et al*. (2017) proved that when the fish were fed with probiotics as supplements, the fish's feed value, enzymatic contribution to digestion, inhibition of pathogenic microbes, antimutagenic and anticarcinogenic activity, growth-promoting factors, and elevated biochemical activities all improved. Most lactic acid bacteria, including *Leuconostoc mesenteroides*, *Lactococcus lactis*, and *Lactobacillus sakei* increased the fraction of phagocytically active head kidney cells and activated the complement receptor expression in rainbow trout, improving both cellular and hormonal immune capabilities (Balcázar *et al*., 2007). In nile tilapia, *Lactobacillus plantarum* and even *Bacillus velezensis* increased innate immune markers such as lysozyme and peroxidase activity in skin mucus, serum lysozyme and peroxidase, alternative complement, phagocytosis, and respiratory burst activities (Doan *et al*., 2018). Complement activity was greatly improved by *Lactobacillus plantarum* at 10^8 and 10^9 CFU/g supplementation. Lysozyme and respiratory burst activity were also significantly increased in black eared catfish, *Pangasius larnaudii* (Silarudee *et al*., 2019). The application of *Pediococcus pentosaceus* to common carp causes a significant increase in the number of erythrocytes, phagocytes, hematocrit, total serum antibody level, alternative complement protein, protease and lysozyme activities, and antibacterial activity (Ahmadifar *et al*., 2019).

CONCLUSION

Probiotics used as fish feed supplement comes in two forms; bacterial or nonbacterial microorganisms that represent the alternative additive for sustainable aquaculture. The most essential step in increasing the efficacy of probiotics is to choose the right strain from the favourable characteristics. Only the most effective probiotic at the right dose has the most beneficial effect on a certain species. There are pros and cons to using these probiotics upon the fish feed. One of the benefits is that fish growth has improved a lot. Meanwhile, long-term human exposure to bacteria from aquaculture still poses potential risk. Besides, the application of probiotics in either way is still costly to most small-scale farmers. Overall, the probiotic application shows positive advancement as it influences growth, reduces stress in fish, and enhances immunisation in fish by increasing their immune system.

ACKNOWLEDGMENT

The cooperation of the supporting laboratory staffs is appreciated. The first author appreciated the financial support from TWAS-UNESCO Associateship Scheme (Ref. no: 3240319965) and Putra Grant, Universiti Putra Malaysia (Grant no. 9692700).

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