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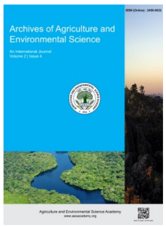

REVIEW ARTICLE

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# Probiotics in aquaculture: A pathway to safer and healthier fish farming

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**ABSTRACT**

Aquaculture benefits greatly from probiotic bacteria, which are also very helpful in preventing a number of infectious diseases. They can be use in place of antibiotics and antimicrobials. Fish that take probiotics have stronger immune systems and grow faster. They aid in the elimination of heavy metals in addition to fostering fish development. Although probiotics can be extracted from a variety of sources, the fish's own stomach is the best source for probiotics. The source of putative probiotics is the same as that of the organism ingesting them. Potential probiotics can flourish in the fish gastrointestinal tract since they are already acclimated to the conditions of the fish gut. Numerous bacteria have been used as probiotics in various experiments, primarily as a feed supplement at varying concentrations. Fish treated with probiotic bacteria have shown positive effects such as improved growth with lower production costs, improved reproductive performances, improved immunology, and disease resistance. When utilized in place of commercial antibiotics and antimicrobials, which can lead to resistance against bacterial species when overused, probiotics can be advantageous for fish farmers. In this paper, aquaculture probiotics, their types, work of mechanism and their uses have been discussed for sustainable aquaculture productivity.



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## INTRODUCTION

Probiotics are live microbial feed supplements or cultured products that improve intestinal (microbial) balance and have positive effects on the host (Irianto & Austin, 2002). By changing the intestinal microorganisms of the host animal, the live microbial feed additives known as probiotics are beneficial to the animal (Fuller, 1989). Seldom are probiotics' mechanisms of action studied. One possibility, however, is competitive exclusion, whereby the probiotics' antibacterial component prevents potential pathogens from colonizing the digestive system, or they may compete with them for nutrients and/or space (Irianto & Austin, 2002). These enzymes speed up an animal's rate of digestion. Yogurt, soy drinks, tempeh, miso, and certain juices are foods that contain probiotics. To lessen the usage of chemical preservatives in foods with a high risk of pathogen contamination, the food industry uses bacteriocins, which are produced by probiotic bacteria. Approximately 30 to 40 trillion microorganisms reside in the human gastrointestinal tract. Among the host's primary functions of the gut microbiota are vitamin production, digesting, fermentation of carbohydrates, and defense against pathogen invasion. Both Gram-negative bacteria like *Echerichia* and Gram-positive bacteria like *Lactobacillus, Ruminococcus, and Bifidobacterium* are found in a healthy human gut. Lactic acid bacteria, which make up the majority of probiotics, are isolated from animal sources. Gram-positive lactic acid bacteria are employed in veterinary and medical settings. Because they secrete lactic and acetic acid, hydrogen peroxide, and bacteriocins, lactic acid bacteria prevent the growth of different gram-positive or gram-negative bacteria. According to recent findings, dairy-fermented foods including cheese, yogurt, and other fermented milk products are the best sources of lactic acid bacteria (Ouwehand *et al*., 2002). *Lactobacillus* species make up the majority of lactic acid bacteria.

Strains of *Lactobacillus* are facultative anaerobic, non-motile, typically catalase-negative, and do not generate spores. There are several species of *Lactobacillus*, including *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus johnsonii*, and *Lactobacillus acidophilus.* These bacteria live in our genital, urinary, and digestive systems and do not cause illness.

The world's fastest-growing food production industry is aquaculture; however, the main threat to aquaculture production is the frequency of disease. The main danger to aquaculture productivity is disease. Antibiotics are one of the traditional methods of disease control, however when taken excessively and for an extended period, they negatively affect aquatic species. In the aquaculture sector, probiotics are therefore a novel tool for disease prevention and water quality enhancement. Fish illness prevalence is significantly influenced by water quality. Water quality must be maintained in order to produce fish free of disease. According to recent findings, *Bacillus* bacteria are thought of as probiotics for water treatment due to their capacity to sequester materials into $CO_2$ (Dalmin *et al.*, 2001). *Bacillus* bacteria have been shown to lower the levels of nitrite, nitrate, and ammonium in the water of ornamental fish (Lalloo *et al.*, 2007). For millions of people, aquaculture offers food and nutritional security, making it one of the fastest-growing agricultural sectors globally (Gatlin, 2002). High-quality protein, micronutrients, including phosphorus, selenium, and iron, and important fatty acids, particularly long-chain polyunsaturated fatty acids (LC-PUFA), are all better found in fish than in farm animals (Tacon & Metia, 2013). However, disease outbreaks limit aquaculture productivity, which affects people's socioeconomic standing in many nations (Gatlin, 2002). Farmers suffer significant losses because of infectious illnesses, which are a significant issue in finfish and shellfish aquaculture due to intensive farming practices (Cabello, 2006). For example, in the shrimp farming industry, illness is currently seen as the limiting factor. Various approaches, such as synthetic chemicals, antibiotics, and traditional ways, have been used to prevent disease in the aquaculture business (Panigrahi & Azad 2007). Oxolinic acid, oxytetracycline (OTC), furazolidone, amoxicillin, and perhaps sulphonamides (sulphadiazine and trimethoprim) were the most widely used antibiotics in fish farming during the 1970s and 1980s. Antibiotic resistance in bacteria has been selectively induced by the careless use of antibiotics to treat illnesses, and this trait may easily spread to other bacteria (Cabello, 2006). Large aquaculture systems cannot be effectively controlled by traditional strategies for emerging diseases. Thus, in order to preserve the health of the cultured organisms, alternative techniques must be created to keep the microbial habitat in aquaculture systems healthy. Prebiotics, probiotics, and immunological nutrients are increasingly being used to create healthy organisms (Panigrahi & Azad, 2007). Probiotics are microorganisms that are beneficial to the host's health. It has been used in aquaculture to help control disease, either in place of or in addition to antibacterial chemicals. Probiotic use has been linked to an improvement in the appetite and/or growth performance of the farmed species as well as a concurrent decrease in the levels of antimicrobial chemicals, especially antibiotics, used in aquaculture. The former is evident in that antibacterial agents will not be required if the animals are otherwise healthy (Irianto & Austin, 2002). Fish for food are becoming more and more in demand every day. Antibiotic use to promote fish development is prohibited. Healthy fish production also became better known. As a result, there is now more interest in the potential of functional diets to benefit health. Probiotics enhance fish development, immunological function, gut shape, digestive enzyme activity, illness resistance, and stress responses in addition to feed efficiency (Guerreiro *et al.*, 2017). In aquaculture and other animal production industries, functional ingredients like probiotics, prebiotics, and symbiotic are being used as alternatives to antibiotics to improve the health and wellbeing of animals (Ringø *et al.*, 2010; Dimitroglou *et al.*, 2011a; Carbone & Faggio, 2016; Dawood & Koshio, 2016). The primary goal of reviewing the literature on probiotics in aquaculture is to determine which strains of probiotics are most appropriate for a certain solution or application. Every probiotic strain will function differently, for as participating in the carbon or nitrogen cycles.

## HOW DO PROBIOTIC WORKS

The probiotics act in ponds water in the following manner:

- Competitive exclusion of pathogenic bacteria.
- Enhancement of digestion through production of exo-enzymes.
- By moderating and promoting direct uptake of dissolved organic materials.
- By inhibiting growth of pathogenic bacteria through pr
- duction of antibiotics.
- Controlling phytoplankton and blue-green algal bloom.
- Preventing off-flavor.
- Improve inflammation of intestines.
- Improve irritable bowel syndrome.
- Prevention of colon cancer.
- Prevent high cholesterol level.
- Improve lactose tolerance.
- Prevent gastrointestinal tract diseases.
- Prevent diarrheal diseases.
- Stabilize the gut mucosal barrier.

## CHARACTERISTICS OF PROBIOTICS

Efficient probiotics must be (a) resistant to pH and bile acids, (b) have no pathogenicity, (c) be viable, (d) be stable in storage and in field, (e) survive and potentially colonize in the gut, (f) be cultivable on a large scale, (g) be able to adhere to the epithelial lining of the gut, and (h) affect host animals beneficially. All new strains used for probiotic development should possess all the aforementioned characteristics (De *et al.*, 2009).

## TYPES OF PROBIOTICS

### Feed probiotics

Some bacterial and fungal strains can be added to food pellets, encapsulated in live feed, or given orally to animals to help them avoid illness and improve the gut's vital microbial ecology (Prasad *et al*., 2003; Nageswara & Babu, 2006). Before introducing strains to animals, their viability should be evaluated. According to Gildberg *et al.* (1997), probiotics such as lactic acid bacteria added to Atlantic cod fry feed demonstrated sufficient growth, survival, and immunological response.

### Water probiotics

Water probiotics are given directly to rearing medium to minimize organic pollutants and other toxins in water (Prasad *et al.*, 2003). These enhance the quality of the water by breaking down organic debris into smaller pieces. As organic matter breaks down, simpler compounds like glucose and amino acids are produced. These molecules are then consumed as food by beneficial bacteria, which lowers the buildup of organic pressure and gives farmed stock a comfortable environment. To reduce organic waste in aquaculture systems, probiotic bacteria like *Bacillus sp.* can convert organic matter to $CO_2$. The amount of nitrate, nitrite, and ammonia is significantly decreased by employing nitrifying bacteria.

### Probiotic bacteria

Lilley & Stillwell (1965) first used the term "probiotic" to describe substances produced by protozoa that induce other microbes. Later, it was used to describe animal food supplements that are good for the animal host (Fuller, 1989; Yilmaz *et al*., 2022; Verschuere *et al*., 2000). He even reexamined the definition of probiotic as a "living microbial feeding supplement which usefully impacts the animal host by enhancing the intestinal microbial balance. According to the aquaculture concept, a probiotic is a live food supplement for microorganisms that have positive effects on their hosts (Lara-Flores & Aguirre-Guzman, 2009). The use of probiotics in aquaculture is a relatively new idea, and procedures are required to evaluate their efficacy. The main goal of using probiotics is to maintain or foster a beneficial relationship between the pathogenic organisms that make up fish skin mucus or intestinal flora. A healthy probiotic should have the following qualities, according to Fuller (Fuller, 1989; Yilmaz *et al*., 2022; Verschuere *et al*., 2000): (i) formulation efficacy; (ii) non-toxic and non-pathogenic; (iii) use as cell viability, rather in massive quantities; (iv) sustaining and effectively participating in intestinal digestion; and (v) stabilizing and keeping stable over longer storage periods or underground conditions. Marine creatures have been found to contain both endogenous and exogenous microorganisms, which are bioactive compounds with two different origins. The Gram-positive facultative anaerobic bacteria *Vibrio* and *Pseudomonas* are the most common endogenous microorganisms found in fish species. *Aeromonads*, *Plesiomonas*, the enterobacteriaceae family, and obligatory anaerobic microbes of the classes Bacteroides, Fusobacterium, and so forth are examples of large aquatic native microbes; however, lactic acid microbes were frequently below the dominant groups in fishes (Rengpipat, 2005; Balcazar *et al*., 2007a; Balcazar *et al*., 2007b; Kesarcodi-Watson *et al*., 2007). The stability and upkeep of microbial plants within marine animals is linked to external ecological factors (Lara-Flores, 2011). Many different types of bacteria, including Gram-positive (*Bacillus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Micrococcus*, *Streptococcus*, *and Weissella*), Gram-negative (*Aeromonas*, *Alteromonas*, *Photorhodobacterium*, *Pseudomonas*, *and Vibrio*), and yeasts (*Debaryomyces*, *Phaffia*, and *Saccharomyces*) have been tested as probiotic strains (Irianto & Austin, 2002). *Bacillus subtillis*, *Bacillus circulans*, *Lactobacillus acidophilus*, *Lactococcus lactis*, *Lactobacillus rhamnosus*, *Carnobacterium maltaromaticum*, *Carnobacterium divergens*, *Carnobacterium inhibens*, *Saccharomyces cerevisiae*, and *Candida sake* are among the recently discovered probiotics that affect fish immune systems, disease resistance, and other performance indicators.

## CONCEPT FOR USAGE OF PROBIOTICS

There are many different kinds of aquaculture systems, including tanks, pens, cages, RAS, ponds, and liner ponds. Each of them will have a distinct environment. Probiotic use will also differ depending on the system. Here, the primary focus of our research is on how well probiotics work to maintain ponds. We have liner ponds and earthier ponds here as well. The water and soil will not come into contact in bordered ponds. Typically, the majority of the waste generated in the pond throughout the culture is dump into the soil, which also serves as a substratum. Those waste materials will naturally be broken down and disintegrated by the bacteria that are present in the soil. Feed waste, feces, dead plankton, and other wastes are typically produce in ponds, both earthen and liner. The nitrogen and carbon cycles are the biological processes that are important in maintaining the ecosystem of the pond. A key component of effective pond management is the carbon to nitrogen ratio, or C: N ratio.

The involvement of bacteria in the nitrogen cycle is evident when we look at it first, and the significance of carbon as a food source for bacteria is evident when we look at the carbon cycle. Increases in ammonia, nitrates, and nitrites in pond water are a sign of waste buildup from an ineffective nitrogen cycle. The C: N ratio will not be balanced in this case. The amount of nitrogen will exceed the normal range of 6:1 to 12:1 (Boyd and Gross, 1998). Microbes use organic matter as a source of energy in their respiratory systems, and as organic matter breaks down, $CO_2$ is dissolved in the soil. While $N_2$ is only retain with residues from microbial activity, this reduces the amount of organic material in the decomposing wastes. As a result of the substance's breakdown, the carbon to nitrogen ratio decreases. The amount of $N_2$ in decomposition microbes is higher (10 percent in bacteria and 5 percent in fungi). Biological wastes often dissolve more quickly with $N_2$ than they do with considerably less nitrogen because microorganisms require a lot of $N_2$ to produce younger cells. In general, particles have a higher carbon intensity (30–45%), although bacteria and viruses make up 50%. While bacteria respire slowly in fresh organic material, a reasonably constant carbon to nitrogen ratio is attained in stabilized organic material because carbon is eliminated by microbial metabolism (Boyd & Gross, 1998).

## PROBIOTICS IN FISH FARMING

Probiotics used in aquaculture are not the same as those utilized in that terrestrial environment. Animals in the water interact with their surroundings considerably more frequently. Aquatic animals breathe and eat, which exposes them to several infections. Therefore, as aquatic organisms share a complicated relationship with their external environment, the definition of probiotics for aquatic application needs to be adjusted. "A whole or components of a micro-organism that is beneficial to the health of the host" is how Irantio & Austin described probiotics for aquatic usage (Irianto & Austin, 2002). By improving their feed conversion efficiency and protein efficiency, probiotics help fish grow. Probiotics produce cell products and micronutrients such as vital fatty acids, vitamins, minerals, and even enzymes, which accelerate the growth rate of marine larvae. Probiotics offer advantages such as immunological stimulation, growth enhancement, and illness resistance against pathogens when introduced to Rohu fingerlings' diet. Additionally, using probiotics strengthens the body's natural defenses, inhibits harmful pathogens, and increases the growth of microbiota. Probiotics can prevent the common pathogen infection pathway and have a propensity to adhere to the mucus in the stomach. Inhibitory chemicals that are detrimental to the growth of infections are also produced, which inhibits their growth. According to Gohila (2013), probiotics can sometimes aid in the completion of the larval cycle without the need for antibiotics. Probiotics are increasingly being used in aquaculture and fish feed to improve the health advantages for fish. Probiotics that are resistant to feed processing must be produced. A lot of study has been done on probiotics and their beneficial benefits. Even though probiotic clinical research has begun, this subject is still regarded as being in its "infancy." Probiotics can therefore be the subject of a wide spectrum of research. Furthermore, a variety of probiotic species, including Lactobacillus, Bacillus, and Pseudomonas, can be investigated (Ige, 2013). Most people do not know how probiotics work. Additionally, there is a chance that probiotics could spread antibody resistance to the public through freshwater diets. This risk can be eliminated or reduced by extensive investigation. Aquaculture disease control has entered a new era thanks to probiotics. Probiotics offer a wide range of research opportunities that will aid in assessing the potential of probiotics in the future as well as their safe application, in addition to helping to understand how they work. One way to satisfy the rising need for freshwater food is through aquaculture. Additionally, probiotic usage has grown recently (Zhou *et al*., 2009). Probiotic resistance to antibodies and the possibility that consuming aquaculture products could spread this resistance to other gastrointestinal tract pathogens and ultimately to humans are equally important research topics in addition to assessing the probiotics' qualities (Mancuso, 2014).

Although the aquaculture sector is currently the one with the fastest rate of growth in the food industry worldwide, bacterial infections are a serious threat to this sector. Antimicrobial medications and antibiotics can be used to address this issue, but they carry the danger of introducing genes that are resistant to antibiotics into the human environment. The usage of probiotics can help with this issue. "Live micro-organisms that when administered in adequate amounts confer a health benefit on the host" is how a joint FAO/WHO panel characterized probiotics (WHO, 2001). Different concentrations of the isolated probiotic strain were added to the fishes' food. In comparison to control groups, it was found that the fish had improved growth, specific growth rate, and feed conversion efficiency. These findings unequivocally suggested that *Bacillus subtilis* can be employed as a growth-promoting agent in common carp cultivation. Gohila (2013) conducted a study in which probiotic-containing food was given to rohu fingerlings. They demonstrated a notable improvement in protein efficiency, feed conversion efficiency, and feed conversion ratio. These findings unequivocally demonstrated the value of probiotics in aquaculture. Bacillus cereus was isolated, identified, and described by Bhatnagar & Lamba (2014) from the stomach of *Cirhinus mrigala* (Bhatnagar & Lamba, 2015). The bacteria was cultivated and added to *Cirhinus mrigala*'s diet. In comparison to control groups, the results indicated a low feed conversion ratio, high protein digestibility, a high growth rate of fish, and an increase in the percentage rise in body weight. Providing nutrients and digestive enzymes also improved fish digestion (Bhatnagar & Lamba, 2015). By suppressing pathogenic *Vibrio spp*., probiotics were found to reduce their numbers and enhance the beneficial microbial load in fish. Ammonium, nitrite, nitrate, and phosphate ion concentrations were also reduced. *Bacillus circulans* was isolated from the Rohu fingerlings' gut by Ghosh *et al.* (2003), and the isolated strain was cultivated and fed to the fingerlings. It was found that experimental groups outperformed control groups in terms of growth, feed conversion ratio, and protein efficiency. However, it was shown that as the amount of Bacillus subtilis increased, the lipid digestibility decreased. The probiotic qualities of bacteria isolated from the gut of freshwater fishes (*Labeo rohita* and *Catla catla*) were assessed by Sahoo *et al.* (2015). Two probiotic bacterial strains were identified from *Catla catla* and three from *Labeo rohita*. *Lactobacillus gasseri* and *Lactobacillus animalis* were the probiotic strains from *Catla catla*, while *Enterococcus avium*, *Enterococcus pseudoavium*, and *Enterococcus raffinosus* were the probiotic strains from *Labeo rohita*, according to biochemical testing and PCR detection. The probiotic bacteria from *Catla catla* have inhibitory properties against pathogens such as *Aeromonas hydrophila* and are resistant to bile and acid. Vancomycin resistance was present in several strains. High cell surface characteristics as hydrophobicity, auto- and co-aggregation were also displayed by them. According to this study, the best probiotics for aquaculture are *Lactobacillus gaseri* and *Lactobacillus animalis*. Probiotics can also be made from gram-negative bacteria. The impact of the probiotic *Pseudomonas pseudoalcaligenes* on the growth performance of Rohu fingerlings was examined in a study conducted by Chaudary & Qazi (2007). Compared to the control groups, the experimental groups exhibited superior growth. Compared to the control groups, the experimental groups' body weight protein efficiency ratio increased (Chaudhary and Javed, 2007). This unequivocally supports the application of probiotics in aquaculture. *Bacillus infantis*, which was isolated from *Labeo rohita*'s intestines, was evaluated for probiotic qualities by Dharamraj & Rajendren (2014). One of the seven isolated bacterial strains showed a stronger inhibitory effect on harmful germs. Significant hydrophobicity, antibiotic resistance, and acid and bile tolerance were all displayed by this strain (Dharamraj & Rajendren, 2014). These findings led to the conclusion that the isolated probiotic had exceptional probiotic qualities and was perfect for use in cattle production. *Bacillus sp.* was isolated and identified from the gut of common carp (*Cyprinus carpio*) by Al-Faragi & Alsaphar (2012). Additionally, the effectiveness of *Bacillus* species against *Aeromonas hydrophila*, a common fish disease, was assessed (Al-Faragi & Alsaphar, 2012). After 24 hours, *Bacillus sp.* was shown to suppress the pathogen, and 48 hours later, the highest concentration of antibacterial compounds was created. According to Ghosh *et al.* (2003), probiotics were isolated from the stomach of 28 main Indian carps, including *Labeo rohita*, *Catla catla*, and *Cirhinus mrigala*. Of these strains, four showed the strongest antibacterial activity against *Pseudomonas flouresens*, *Aeromonas hydrophilla*, and *Edwardsiella tarde*. The strain was identified by biochemical testing as belonging to the potentially probiotic species *Bacillus subtilis*. The competition for adhesion sites has been proposed as the mechanism of action. In one investigation, it was found that probiotic bacteria prevent infections from sticking to the gut mucous (Vine *et al.*, 2004). Fish immune systems have been observed to be strengthened by probiotics. Probiotic *Pseudomonas aeruginosa* VSG-2 supplementation has been shown to significantly boost serum lysozyme and alternative complement pathway (ACP) activity, as well as phagocytosis and several macrophages. Additionally, treatment groups had greater serum IgM levels than control groups. Additionally, the fish's survival rates against the *Aeromonas hydrophila* disease were noticeably greater.

According to Prasad *et al.* (2003), lactic acid bacteria, a common probiotic strain, can be used to manage bacterial infections. Additionally, *Bacillus sp.*, another well-known probiotic, is employed to eliminate metabolic waste in aquatic systems. Numerous strains of *Pseudomonas*, *Vibrio*, and *Aeromomas* exhibit antiviral action against the infectious hematopoietic necrosis virus (Kamei, 1998). Individual or combined supplementation of *Lactobacillus rhamnosus* and *Lactobacillus sporogenes* can improve common carp health and disease resistance. These probiotic organisms can be utilized alone or in combination (Allameh, 2014; Chi, 2014; Faramazi, 2011; Harikrishnan, 2010). Because probiotics are environmentally benign, they do not contaminate water. In order to make it increasingly appropriate for aquaculture systems. They protect consumer health safety in addition to promoting animal health (Prasad, 2003). Table 1 lists the uses of probiotics and the aquatic species they target.

## FISH RESPONSES TO DIETARY SUPPLEMENTATION WITH VARIOUS PROBIOTICS

Numerous positive effects of probiotics, including enhanced food availability and disease resistance, have been documented in terrestrial animals. Table 2 lists the Summary of fish responses to dietary supplementation with various probiotics.

## ROLE OF PROBIOTICS IN CARP AQUACULTURE

Researchers have demonstrated the use of probiotics have positive impact in carp species. Probiotic applications in carps refer to Table 3.

**Table 1.** Uses of Probiotic in aquaculture system.

| Uses of Probiotic | Probiotic Species | Target aquatic species | Reference |
|---|---|---|---|
| Water quality | *Lactobacillus acidophilus* | *Clarias gariepinus* | Dohail *et al.* (2009) |
| Control of diseases | *Enterococcus faecium* SF 68<br>*Pseudomon fluorescens*<br>*Lactococcus lactis*<br>*Pseudomonas* sp. | *Anguilla Anguilla*<br>*Oncorhynchus mykiss*<br>*Epinephelus coioides*<br>*Oncorhynchus mykiss* | Chang & Liu (2002), Gram *et al.* (1999), Spanggaard *et al.* (2001) |
| Growth promoter | *Lactobacillus lactis* AR21<br>*Bacillus* sp.<br>*Streptococcus thermophiles*<br>*Bacillus coagulans* | *Brachionus plicatilis*<br>Catfish *Scophthalmus maximus*<br>*Cyprinus carpio koi* | Harzeveli *et al.* (1998)<br>Queiroz, (1998), Gatesoupe *et al.* (1999), Lin *et al.* (2012) |
| Digestion | *Lactobacillusa cidophilus*<br>*Lactobacillus helveticus* | *Clarias gariepinus*<br>*Scophthalmus maximus* | Dohail (2009)<br>Gatesoupe (1999) |
| Improvement of immune response | *Clostridium butyricum L.*<br>*Casei L.*<br>*Acidophilus* | *Rainbow trout Poecilopsis gracilis*<br>*Paralichthys olivaceus* | Sakai, (1995)<br>Hernandez, ( 2001)<br>Taoka, (2006) |

**Table 2.** Summary of fish responses to dietary supplementation with various probiotics.

| Probiotic | Species | Measured response | Reference |
|---|---|---|---|
| Live bacteriophage | Ayu | Resistance to *Pseudomonas plecoglossicida* | Park *et al.* (2000) |
| *Aeromonas media* strain A199 | Eel | Resistance to*Saprolegnia parasitica* | Lategan *et al.* (2004) |
| *Bacillus subtilis* and *B. licheniformis* | Rainbow trout | Resistance to *Yersinia ruckeri* | Raida *et al.* (2003) |
| *Bacillus circulans* | Rui | Immune enhancerand Control *hydrophila* | A.Bandyophyay & DasM hapatra (2009) |
| *B. pumilus* | Tilapia | Immunity enhancerand Better survival | Aly *et al.* (2008) |
| *Bacilllus subtillis* and *Lactobacillus delbriieckii* | Gilthead seabream | Cellular innateimmune response | Salinas *et al.* (2005) |
| *Carnobacterium divergens* | Atlantic cod | Survival , Resistance to *Vibrio anguillarum* | Gildberg *et al.* (1997), Gildberg & Mikkelson (1998) |
| *Enterococcus faecium* | European eel | Resistance to *Edwardsiella tarda* | Chang and Liu (2002) |
| *L. acidophilus* | African catfish | Better growth performance, hematological parameters and immunological profile | Al-Dohail *et al.* (2009) |
| *Saccharomyces cerevisiae* | Nile tilapia | Weight gain and feed efficiency | Lara-Flores *et al.* (2002) |

**Table 3.** Studies using probiotics in carp aquaculture.

| Species | Probiotic | Results | References |
|---|---|---|---|
| Catla | *B. circulans PB7* | Weight gain, feed conversion ratio, protein efficiency ratio increase | Bandyopadhyay & Mohapatra (2009) |
| Common carp | *Streptococcus faecium*, *L. acidophilus* and *S. cerevisiae* | Weight gain, specific growth rate, protein efficiency ratio increase | Faramarzi *et al.* (2011) |
| Rui | *L. plantarum VSG3* | Specific growth rate, feed conversion ratioincrease | Giri *et al.* (2013) |
| Gibel carp | *S. cerevisiae* | Final weight, weight gain, specific growth rate, feed conversion ratio | He *et al.* (2011) |
| Koi carp | *L. acidophilus and/or S. cerevisiae* | Weight gain, specific growth rate, feed conversion ratio increase | Dhanaraj *et al.* (2010) |
| Grass carp | *B. subtilis Ch9* | Specific growth rate,feed conversion ratio increase | Wu *et al.* (2012) |

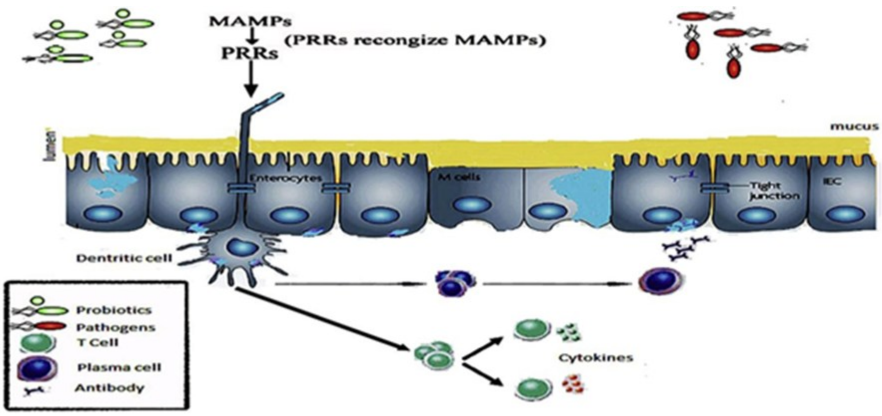


**Figure 1.** *Probiotics showing the activity of host immuno-modulation. Abbreviations: MAMPs / Microbe associated molecular patters, PRRs / Pathogen pattern recognition receptors (Source: Akhter et al., 2015).*

## PROBIOTICS IN IMMUNE SYSTEM

Probiotics are good bacteria that can control the human immune system in addition to blocking infections (Figure 1). Probiotic-induced immunomodulation is regarded as a collaborative effort including the host, commensals, and the invasive microbe. Pathogen pattern recognition receptors (PRRs) allow the host to determine whether an organism is harmful or not. The microbial associated molecular patterns (MAMPs), which are found in both pathogenic and non-pathogenic microorganisms, are used to discover these recognition receptors. Microbial nucleic acids, flagellin, peptidoglycan, and lipopolysaccharides (LPS) are a few examples of MAMPs. MAMPs attach to PRRs to initiate an intracellular signaling cascade that promotes the production of particular cytokines, sends messages to neighboring cells, or has antiviral, pro-, or anti-inflammatory workout effects. The mucosal commensal microbiota's homeostasis is regulated by the same recognition system. Additionally, probiotics have the ability to alter the commensal microbiota's diversity and richness (Nayak, 2010).

**Table 4.** Non-specific immune response of *Cyprinus carpio* fry fed basal diet and diet supplemented with *Bacillus coagulans*, *B. licheniformes* & *Paenibacillus polymexa* as probiotics for 80 day. B0 (control): fish fed with basal diet. B1, B2 and B3: fish fed with basal diet supplemented with *Bacillus coagulans*, *B. licheniformis* and *Paenibacillus polymyxa* at $10^9$CFU/g, respectively.

| Immune response | B0 control) | B1 | B2 | B3 |
|---|---|---|---|---|
| Lysozyme (U/ml) | 31.16 | 42.3 | 42.53 | 44.39 |
| Respiratory burst (OD 540nm ) | 0.41 | 0.49 | 0.94 | 0.98 |
| Myeloperoxidase (OD 450 nm) | 0.11 | 0.13 | 0.15 | 0.19 |

(Source: Gupta *et al.*, 2014)

A study on the immune response and disease resistance of *Cyprinus carpio* fry is carried out by Gupta *et al.* (2014). By using the agar well diffusion experiment, the activity against fish pathogens was investigated using laboratory-maintained *B. coagulans*, *B. licheniformis*, and *P. polymyxa*. They test healthy fish fry for this bacterium to determine its safety. Over the course of 80 days, they fed fish a control basal diet (B0) and experimental diets containing *B. coagulans* (B1), *B. licheniformis* (B2), and *P. polymyxa* (B3) at a rate of 109 CFU/g diet. At 80 days after feeding, several studies on immunological parameters and disease resistance were carried out. The antagonism investigation revealed an inhibitory zone against *Vibrio harveyi* and *Aeromonas hydrophila*. There were no recorded deaths or illnesses as a result of the challenge. Therefore, it is possible to conclude that the probiotic bacterial strains did not affect fish fry. Fish fry fed the B3 diet had significantly increased levels of several non-specific innate immunological measures, such as respiratory burst assay, myeloperoxidase content, and lysozyme activity, at $10^9$ CFU/g (Table 4).

An essential part of the immune system is lysozyme. In vertebrates, it served as a protective agent against invasive microorganisms (Ellis, 1990). By cleaving glycosidic bonds in the peptidoglycan layers, lysozyme breaks down bacterial cell walls (Alexander, 1992). After 80 days of feeding, fish in the current study that received diets supplemented with various probiotics had noticeably greater lysozyme activity than the control group. During phagocytosis, phagocytes create respiratory bursts to kill invading bacteria. These bursts have been frequently employed to assess the host's ability to defend against infections (Dalmo, 1997). This study demonstrated that the respiratory bursts of fish fed various probiotic supplements were noticeably higher than those of the control group. An essential enzyme called myeloperoxidase uses oxidative radicals to create hypochlorous acid, which kills infections. It is mostly secreted by neutrophil azurophilic granules during oxidative respiratory burst (Das, 2013; Dalmo, 1997). Following eighty days of feeding with diets enriched with *Bacillus coagulans*, *B. licheniformis*, and *P. polymyxa*, the myeloperoxidase level of the serum was significantly increased in the current study.

The challenge test revealed that adding *B. coagulans*, *B. licheniformis*, and *P. polymyxa* to the food greatly increased the fish fry's resistance to bacterial challenge. According to these findings, *Paenibacillus polymyxa* is a possible probiotic species that can be utilized in aquaculture to enhance the immune system, growth performance, digestion, and illness resistance of common carp fry (*Cyprinus carpio*).

## DIETARY PROBIOTICS INFLUENCE GROWTH PERFORMANCE

The impact of feeding snakehead (*Channa striata*) fingerlings specific probiotics (*Saccharomyces cerevisiae*, *Lactobacillus acidophilus*) and β-glucan, galacto-oligosaccharide (GOS), and mannan-oligosaccharide (MOS) stimulants was assessed in a study by Munir *et al.* (2016). Fish fed a supplement of *Lactobacillus acidophilus* performed the best. When compared to fish on probiotic-supplemented diets, the growth trends were lower in all other groups. The study's findings demonstrated that *L. acidophilus* supplementation is optimal for growth (Figure 2). A study on the impact of probiotics on shrimp (*Penaeus vannamei*) growth performance was carried out by Wang *et al.* (2008). Three concentrations of photosynthetic bacteria and *Bacillus sp.* were added as probiotics to shrimp basal diets: T1, 2 $gkg^{-1}$ (1 $gkg^{-1}$ lyophilized photosynthetic bacteria cells (PSB) and 1 $gkg^{-1}$ lyophilized Bacillus sp. (BS); T2, 10 $gkg^{-1}$ (5 $gkg^{-1}$ PSB and 5 $gkg^{-1}$ BS); and T3, 20 g $kg^{-1}$ (10 g $kg^{-1}$ PSB and 10 g $kg^{-1}$ BS). After 28 days, the shrimp given the probiotic-supplemented diets outperformed the shrimp on the basal diet in terms of growth.

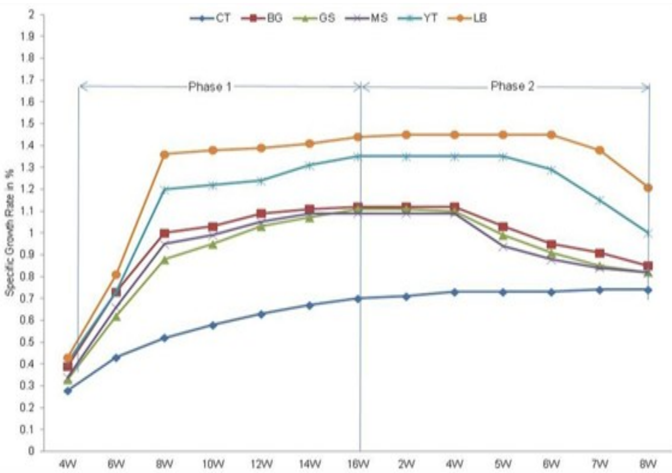


**Figure 2.** *Comparison of specific growth rate of Channa striata fingerlings feeding with different diet. CT=control without any supplementation; BG=feed with β-glucan; GS=feed with glacto- oligosaccharides; MS=feed with mannan-oligosaccharides; YT = feed with live yeast; LB= feed with Lactobacillus acidophilus (Source: Munir et al., 2016).*

## DEVELOPMENT AND DIGESTIVE METHOD PROMOTER

Fish will use less Abs and chemical compounds in their diet if probiotics are added (Fuller, 1989; Yilmaz *et al.*, 2022). As a result, it is becoming usual to add vitamins to fish farming diets. Probiotic use results in lower feed costs, indicating that aquaculture plays a significant impact in decision-making. It's interesting to note that earlier research has shown that probiotic strains may help marine animals eat more because they supplement their gut bacteria, improve feed quality and growth, prevent gastrointestinal disorders, and have pro-nutrition qualities in the complex feed (Balcazar *et al.*, 2007a; Balcazar *et al.*, 2007b; Suzer *et al.*, 2008). Probiotics have been shown to improve fish growth and feed, especially by increasing nutrient digestibility (Faramarzi *et al.*, 2011a; Faramarzi *et al.*, 2011b). However, a lot of probiotics repopulate the host and have a big effect on the gastrointestinal tract by multiplying and creating microorganisms, which helps to improve the intestines' microbial composition and, in turn, the feed's digestibility and absorption (Mohapatra *et al.*, 2012; El-Haroun *et al.*, 2006). After undergoing metamorphosis in the belly, the bacteria settle in the digestive tract and produce a wide range of proteolytic enzymes by using a significant amount of carbohydrates (El-Haroun *et al.*, 2006). However, to avoid deactivation or destruction of beneficial probiotic organisms in the culture, it is essential to examine the feeding process (Mohapatra *et al.*, 2012). The digestive system's microbiome can serve as a biological growth and alternative supply of protein. It can also be a source of nutrients, amino acids, and micronutrients (Balcazar *et al.*, 2007a; Balcazar *et al.*, 2007b). In actuality, a variety of compounds and substances are being shown to have probiotic-like stimulating effects on specific growth rates, food palatability, consumption productivity gains, and the sustainability of marine species. In fact, the digestive organs significantly alter the gastrointestinal enzymatic activity and are highly responsive to fortified diets (Mohapatra *et al.*, 2012). However, using larger amounts of probiotics might not always result in better growth efficiency (Son *et al.*, 2009). Different probiotics boost different aspects of fishing species' nutritional absorption and development (Mohapatra *et al.*, 2012). Accordingly, the effectiveness of probiotic strains on fish culture depended on the hydrobiont species, temperature, enzyme levels, inherited tolerance, and water quality (Cruz *et al.*, 2012). Furthermore, the effectiveness of probiotic administration is frequently influenced by the hosting life phase. This was evident in the creation of beneficial microbes in bivalve larvae, as the microbes' transition period became too short and the microbial colonies seemed difficult to establish (Kesarcodi-Watson *et al.*, 2008; Avendano & Riquelme, 1999; Jorquera *et al.*, 2001; Jaseera *et al.*, 2021). Additionally, prebiotics were categorized as indigestible dietary supplements that improved host microbial colonies by boosting digestive microorganisms (Gatesoupe, 1999; Gatesoupe, 2005; Hassaan *et al.*, 2021; Tuyet Hoa *et al.*, 2021). This helped to accelerate health. However, a number of authors noted that prebiotic intake had no beneficial effects on feed digestion or aquaculture (Cruz *et al.*, 2012). As the amount of substances in the abdomen increases, there is a chance that infections could break down the intestinal components (Gatesoupe, 1999; Gatesoupe, 2005; Tuyet Hoa *et al.*, 2021). Therefore, before using prebiotics in farming, fish hatcheries, or shrimp, more research on their effectiveness in aquaculture is needed.

## WATER QUALITY IMPROVEMENT

Nitrogen chemicals such as $NH_3$, $NO_{2-}$, and $NO_{3-}$ are generally non-specific, but they have an impact on aquaculture species and are likely to cause a greater death rate. This makes cultivated marine organisms vulnerable to these substances. A greater amount of natural carbon is transformed into microbial activity when gram +ve *Bacillus* species are used instead of gram -ve microorganisms to convert organic molecules back to carbon dioxide because the former are typically less expensive (Fuller, 1989). By altering the composition and production of water-borne disease populations associated with collected organisms, endospore-producing bacteria such as *Bacillus* sp. have been shown to be effective in improving the quality of water (Bandyopadhyay & Mohapatra, 2009). Enhancing water quality, reducing hazardous *Vibrio* species in the environment, increasing shelf life, and improving the health of juvenile *Penaeus monodon* are all associated with *Bacillus* species (Al-Faragi & Alsaphar, 2012; Dalmin *et al.*, 2001). Furthermore, probiotics derived from plants, such as yucca extract, tannic and citric acid, and others, as well as other intestinal organisms that belong to the genus *Nitrobacteria* and *Pseudomonas*, are utilized in culture systems and have been shown to significantly improve water quality (Bhatnagar & Lamba, 2015). Increased decomposition of natural substances, decreased levels of $N_2$ and P, improved algal blooms, increased water oxygen accessibility, decreased cyanobacteria blooms, controlled levels of $NH_3$, $NO_2$, and $H_2$ sulfide, decreased disease incidence, increased survival, and improved production were all requirements for using prototype probiotics in aquaculture ponds (Bhatnagar & Lamba, 2015). Hura *et al.* (2018) evaluated the impact of commercial probiotic *Bacillus megaterium* on water quality measures through an experimental investigation. The study's findings demonstrated that *Bacillus megaterium*, a commercial probiotic, is showing encouraging benefits on water parameters. Ammonia, BOD, COD, and dissolved oxygen were the parameters that demonstrated the biggest impact. Additionally, the results demonstrated that the treated water had fewer total dissolved solids than the control. This may be because probiotics increase digestion, assimilation, and help aquatic cultured organisms use feed more effectively.

## PROSPECTS AND CHALLENGES FOR THE FUTURE

Probiotics are increasingly being used in aquaculture. As mentioned below, using probiotics has a number of advantages, including improved water quality, greater production, feed performance, and a stronger immune response. To fully understand the probiotics' mechanisms, more research is required.

Probiotics are more effective when we start using them in the early stages of the community. Marine life communicates directly with its environment, therefore adding probiotics to the water could be helpful. A healthy transitional gastrointestinal tract may develop later on if probiotics are exposed in food during the bacterial stage, for example (Hassaan *et al.*, 2021; Tuyet Hoa *et al.*, 2021). The bacterial community in the gut system could be maintained at a level that can communicate sufficient flexibility by routinely administering probiotics to animals grown in captivity. Theoretically, the bacterial species were isolated from the intestinal system and then given to this host species; this is what many commercial probiotics have recently been found to use. Both animals and the environment can be positively or negatively impacted by probiotics. It is important to identify the microbiota's species and hosts because this usually specifies the characteristics of interactions. As a result, probiotic origin and variety often play a big part in maximizing probiotic usage and preventing needless expenses. In addition, the probiotics' dominating communities may become infectious due to alterations in the habitat environment, which could endanger the host's injured or distressed organisms (Hassaan *et al.*, 2021; Tuyet Hoa *et al.*, 2021). The efficacy of the various probiotic strains tested in aquaculture was demonstrated. Intestinal microbe inoculum, or organism-specific probiotics, are increasingly available in the aquaculture industry. With the addition of probiotics, these formulations were refined to serve a more effective function. Furthermore, a thorough analysis of the bioactive components' consistency is required. It is expected that the use of cutting-edge research methods, such as molecular strategies for probiotic product analysis and in vivo testing, would significantly improve the consistency and functional qualities of probiotics.

## Conclusion

Probiotics are now a crucial component of aquaculture procedures to enhance growth performance. Fish weight gain, growth rates, and feed conversion are all significantly impacted by probiotics. It improves the growth performance of shrimp, common carp, grass carp, rui, and catla. However, probiotics also have a number of positive impacts, primarily on fish's ability to withstand sickness and their availability of nutrients. Probiotics aid in disease prevention measures for fish and shellfish. Some of the inhibitory compounds currently employed in aquaculture may be replaced with their use. Probiotics boost myeloperoxidase activity, respiratory bursts, and lysozyme, all of which improve immunological response. Numerous food animals have shown that gut bacteria have a significant impact on the host organism's nutrition and overall health. Shrimp exhibit increased activity of the enzymes lipase, amylase, and protease. Higher GSI, fecundity, and fry survival were all indicators of increased reproductive success when probiotics were added to feed. It has a beneficial impact on pond water quality. BOD, dissolved oxygen, ammonia, and chemical oxygen demand were all significantly impacted. Probiotics have a lot of promise to improve fish production's sustainability and efficiency. The mechanism underlying these benefits is yet unknown, despite the fact that probiotics present a prospective substitute for pesticides and antibiotics in aquatic animals and can help protect cultured species from illness. The source, dosage, and length of probiotic administration are some of the variables that can affect the immune-modulatory function of probiotics. As a result, using the right administration techniques contributes to creating an environment where probiotics may function effectively. Furthermore, the secret to using probiotics in aquatic systems may lie in knowing their mechanisms of action and applying them correctly.

## DECLARATIONS

### Author contribution statement

Conceptualization: MHM and RI; Formal analysis and investigation: MHM and RI; Resources: MHM.; Data curation: MHM and RI; Writing—original draft preparation: MHM and RI; Writing—review and editing: MHM and RI; Visualization: MHM and RI; Supervision: MHM and RI; Project administration: MHM and RI; All authors have read and agreed to the published version of the manuscript.

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