

# Chapter 14

## Probiotics and Its Application in Aquaculture



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**Abstract** During the preceding two decades, there has been a tremendous rise in both finfish and shellfish aquaculture. Even while there have been positive impacts, some of these improvements have also led to environmental deterioration, the spread of illness, and a decreased rate of productivity. Research and probiotics have both been stimulated by the demand for improved growth performance, enhance immune system, feed efficiency, and safety in the aquatic environment. Antibiotics are very effective at treating ailments, but they are also utilized to fuel the expansion of antibiotic-resistant bacteria and resistance genes and accumulate antimicrobial residues in fish tissues. The effect could be detrimental to human health, the fish population, and the environment. A newly used approach to combat bacterial illnesses in fish involves using vaccinations. Probiotics, due to increased demand, help further push the need for ecologically friendly aquaculture. Nonetheless, we need to work on our comprehension of gut microbiology, produce adequate probiotic amounts, and ensure the safety of these supplements.

### 14.1 Introduction

Aquaculture is vital for providing an adequate supply of nutritious food for a growing population. Occasional invasions of opportunistic pathogenic microorganisms lead to substantial disease losses and environmental degradation in intensive aquaculture operations. Crowding, contaminated water, pesticides, and nutrition shortages all increase the risk of microbial infections in animals who are allowed to live in large groups (Banerjee and Ray 2017). Until preventive and therapeutic

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methods address all of these concerns, the aquaculture sector will not grow. The use of medications and chemical additives is becoming essential in modern aquaculture. Cheap chemotherapeutic medications are detrimental to the ecosystem because they are frequently used. Because antibiotics can affect the gut microbiome, they are a point of discussion for preventing illness. Because antibiotics, which have long been used in aquaculture, have helped create antibiotic resistance, their usage has further worsened the problem. Due to the overuse of antibiotics in aquaculture, an increasingly large spectrum of antibiotic-resistant human illnesses, including *Vibrio cholerae*, *Escherichia tarda*, *E. coli*, *Aeromonas sp.*, *Vibrio vulnificus*, and others, have been found (Allameh et al. 2016; Brogden et al. 2014).

For farmers, the commercial aquaculture system uses a feed containing a balanced combination of probiotics to help improve better growth, flesh quality, productivity, immune system, protein quantity, carcass quality, and intestinal health. In turn, consumers see benefits because of better growth, flesh quality, productivity, and protein quantity. This, however, is true for farmers in low-income and impoverished countries, many of whom continue to rely on naturally derived feeds to decrease production costs (typically phytoplankton and zooplankton). The approach, however, decreases growth, and mortality, all of which have a negative impact on profitability (Ige 2013). Due to the deleterious consequences on fish health, disease outbreak management is no longer recommended. A detailed chapter on probiotics discusses their use in aquaculture, application areas, numerous methods of administration, and the underlying mechanisms of action.

## 14.2 Alternatives for Medicines and Antibiotics

Bacteria that aid the human gastrointestinal tract have been recommended as environmentally safe alternatives to antibiotics. In recent years, environmental-friendly feed additives, such as microbial supplements, have increased popularity (Jahangiri and Esteban 2018). Aquatic environments harbour various microorganisms, including the ability to recycle nutrients, process organic garbage, and keep fish healthy. Probiotics are compounds that are beneficial to aquaculture. They are used to help avoid infections and, at the same time, aid animals in gaining weight, staying alive, and growing.

Additionally, there are no ill effects on the animals receiving the treatment. Increasing concern about discovering safe, non-antibiotic, and environmentally acceptable options for treating infections is another cause of the rise in infections. To combat the widespread issues with antibiotics and broad-spectrum insecticides in aquatic animals, the rapid development of probiotics in aquaculture has occurred (Lakshmi et al. 2013). There are alternatives to antibiotics, such as probiotics, that can help with infectious agent management and disease treatment. While this is certainly the case, proper usage of probiotics in aquaculture is critical for their effectiveness. At present, everyone agrees that the correct type of bacteria in the

gut is crucial for maintaining good health (Nayak 2010). Microorganisms proliferate in reaction to disease, regardless of the host species.

### 14.3 Definition

Probiotics are defined as “live bacteria that impart health benefits to the host when provided in sufficient doses”. Probiotics are microbiological origin factors that boost the growth of other organisms. Probiotic organisms can be alive or dead, or they can be part of the microbial cell composition that benefits the host by helping to boost the individual’s disease resistance, overall health, growth performance, feed usage, stress response, or overall vitality, all in part by helping to maintain the microbial balance of the host or the overall microbial balance of the surrounding environment (Merrifield et al. 2010). These non-pathogenic bacteria reside in the gastrointestinal tract, and they may contribute to health and well-being by helping maintain gut health. Since this microflora is needed to avoid sickness, it is essential to save this microflora to protect against illness, particularly GI tract infections. Probiotics are a healthy microbiome, which take time to develop and provide health benefits to the host. A healthy population of intestines in the stomach expands with the addition of probiotic strains.

### 14.4 History of Probiotics

Lilly and Stillwell coined the word “probiotics” in 1965. Roy Fuller proposed in 1989 that probiotics have a favourable effect on the host. He described probiotics as live bacteria that promote the host’s health by improving the gut’s balance in the intestine when given in sufficient quantities. According to FAO and the WHO, probiotics are live bacteria that are ingested orally and have been shown to have substantial health benefits (Hotel and Cordoba 2001). The term “probiotics” derives from the Greek terms “pro” and “bios”, which mean “for life”, and was coined in 1953 by German scientist Werner Kollath to describe “active substances that are required for the healthy development of life”. Unlike antibiotics, he advocated the term “all organic and inorganic food complexes” to elevate these food complexes to the level of supplements. According to him, probiotics are an “active substance necessary for the healthy growth of life”. In a different context, Lilly and Stillwell used this word in 1965 to refer to “substances released by one organism that stimulate the growth of another”. Parker (1974) defined probiotics in the context of animal feeds a few years later as “Organisms and chemicals that have a favourable influence on the host by contributing to its intestinal microbial balance”. Fuller, in 1992, defined probiotics as “a live microbial feed supplement that benefits the host animal by enhancing its gut microbial balance” (McFarland 2015). Due to their unusual functional capability, it is hypothesized that probiotics may influence the gut

microbiota of any aquatic species. Probiotics have already been revealed to conduct a different function in the host body by researchers (such as reducing disease and stress, improving immunity, modulating microbiota in the gut, assisting in nutrition, improving water quality). Furthermore, probiotics' favourable effects giving to higher feed value and animal growth and higher spawning and hatching rates in aquaculture systems. In this section, we discussed each function of probiotics and attempted to compare them with prior knowledge.

## 14.5 Salient Features of Probiotics

Probiotics with specified properties are commonly accepted as being necessary for the appropriate development of successful medications (Priyodip et al. 2017; Thakur et al. 2016). A nutritious diet with enough probiotic intake is essential for maintaining optimal gastrointestinal functioning. To be sure, non-invasive and non-carcinogenic and effective in a targeted manner must be provided; plasmid-free antibiotic and virulence resistance genes should be avoided, and it must be proven to successfully colonize and multiply within the host for an extended period (Hasan and Banerjee 2020).

### 14.5.1 *The Essential Properties of a Probiotic Encompass*

1. It should be capable of dealing with the acidic and bile salt conditions that exist in the stomach and small intestine (Pereira et al. 2003). According to WHO and FAO recommendations, probiotic organisms that are used in food must be able to survive the travel through the digestive system, including exposure to gastric acids and bile (Senok et al. 2005). They must also be able to colonize and increase in the digestive tract and maintain their safety, effectiveness, and strength throughout the product's life cycle.
2. Probiotics must have a high level of adhesion to epithelial cells (Kravtsov et al. 2008). Because adhesion is necessary for colonization, it is a critical selection factor for probiotic bacteria (Beachey 1981). The main protection mechanisms against pathogenic strains owing to competition on site of connection and food are the adhesion and colonization on mucosal surfaces (Westerdahl et al. 1991). Mucus binding proteins are found in most probiotic bacteria, which aid in the binding process.
3. It should prevent pathogen adhesion by specific rivalry (Reid et al. 2006).
4. Competition Exclusion: G. F. Gause, a Russian ecologist, established the competitive exclusion principle. This idea claims that species cannot coexist if they exploit the environment in the same way. In order to protect health, specific strains of bacteria are introduced into fish cultures to enhance the expression of various immune components and reduce pathogen burden on the intestinal mucus

layer (Banerjee and Ray 2017). Lactobacilli were found to compete with and diminish the attachment of *Yersinia ruckeri*, *Aeromonas salmonicida*, and *Carnobacterium piscicola* to rainbow trout intestinal mucus (Balcázar et al. 2006b).

5. It is feasible to stop pathogen colonization by stimulating decolonization of pathogenic microorganisms using one of several techniques. Bacteria must be able to cling to mucus and wall surfaces to establish themselves in the intestines of fish (Roeselers et al. 2011). Thus, competition for adhesion receptors with pathogens may be the first probiotic action because bacterial attachment to the tissue surface is crucial in the early stages of pathogenic infection (Chabrillón et al. 2006).

### 14.5.2 Characteristic Feature

1. Probiotics assist fish to grow, develop, and resist bacterial infection.
2. The probiotic microorganisms should have no adverse effects on the host.
3. Probiotics should not develop drug resistance; instead, they should maintain inherited features.
4. Probiotics should have the following characteristics in order to be used as an efficient feed:
  - Tolerance to acid and bile.
  - Gastric juice resistance.
  - Adherence to the surface of the digestive system.
  - Antagonism to pathogens.
  - Immune stimulation.
  - Increased intestinal motility.
  - Mucous survival.
  - Enzyme and vitamin production.
5. They should have good sensory qualities, fermentative action, freeze-drying tolerance, and viability in feed during the packaging and storage procedure (Chauhan and Singh 2018).

## 14.6 Selection Criteria

Probiotics are used to develop a friendly contact between the beneficial microbiome and pathogenic strains, to preserve the mucus constituents of freshwater fish intestines or skin. In order to prevent undesired effects in fish administration, probiotics must be non-pathogenic and non-toxic. Antagonism, adhesion, and challenge, for instance, must be investigated in vitro using probiotics. Some crucial and unique characteristics contribute to the advantage of the host. In the fish farming industry,

probiotics are most often found in the fish gut and are produced from this source. *Streptococcus* is among the most common bacteria found in the lab (Giri et al. 2013). Although probiotics are a relatively recent method, they have acquired popularity due to their capability to moderate various physiological activities in aquatic species. After that, a variety of probiotics, such as *Aeromonas media*, *Enterococcus faecium*, *Bacillus subtilis*, *Carnobacterium inhibens*, *Lactobacillus helveticus*, and others, are regarded to be particularly beneficial at the time. Another microbe that was found in the GIT of fish and shellfish probiotic candidates is Gram-negative facultative symbiotic anaerobes such as *Pseudomonas*, *Vibrio*, and *Aeromonas* (Lakshmi et al. 2013; Verschuere et al. 2000). In addition to bacteria, bacteriophages, microalgae, and yeast, all forms of probiotics are forms of bacteria (Llewellyn et al. 2014). Many mono- and multi-strain probiotics are already available on the market (Van Doan et al. 2017).

## 14.7 Types of Probiotics

Aquaculture industry used two types:

1. Gut probiotics are mixed with food and orally administered to maintain and improve the beneficial microbiome of the gut. Gut probiotics are generally used in finfish culture.
2. Water probiotics live in water and consume all nutrients available, except for dangerous bacteria. This can remove harmful strains via starving (Nageswara and Babu 2006; Sahu et al. 2008). Currently, shrimp farming uses water-based probiotics. Probiotics that can be utilized for feed or as supplements for water are considered to function in fish as biological controls.

## 14.8 Forms of Probiotics

Two kinds of aquatic probiotics are available:

1. Dry forms: Dry probiotics are available in bags. They can be fed food or mixed with water and have several advantages, including safety, ease of usage, and a longer lifespan (Decamp and Moriarty 2007).
2. Liquid forms: The hatcheries mainly use live and ready-to-act liquid forms. These fluid forms are either added directly to hatchery tanks or mixed with farm feed. Liquid forms show short-lived positive results instead of dry and spore bacteria, although lower in density (Nageswara and Babu 2006). There are no reports that probiotics have adverse effects. However, it may temporarily increase biological oxygen for probiotics. Therefore, a minimum dissolved level of oxygen of 3% during probiotic administration is desirable.

## 14.9 Benefits of Probiotics

Probiotics are non-toxic to the host animal and help the host animal resist pathogenic bacteria either directly or indirectly. Due to a lot of work that has been done on the topic of probiotics, many of the theoretical advantages of their use have yet to be proved. It is critical to identify which probiotic strains are being utilized, as each strain offers specific health benefits (Senok et al. 2005).

- Despite well-established effects of probiotics, new research on the benefits of probiotics is always being conducted, and there are numerous open questions about the accuracy of some of the claims. While different probiotic strains offer distinct health advantages, it is essential to keep this in mind when purchasing supplements. By supporting and enhancing the immune system of cultured organisms, this treatment helps organisms better resist disease and stimulate the production of chemicals that hinder harmful organisms from causing disease in the host (Verschuere et al. 2000).
- It is proposed that the probiotic bacteria's activities have a direct growth-stimulating effect on fish. This may be due to either directly feeding the fish nutrients or supplementing minerals and vitamins. On the other hand, probiotics have been demonstrated in experiments to increase the ability of fish organisms to overcome host ailments or stimulate growth without harming the host.
- According to Venkateswara (2007), probiotic supplements have been shown to regulate microflora, control pathogenic ones, promote the breakdown of undesired organic substances, and improve environmental performance by reducing hazardous gases such as  $H_2O_2$ ,  $NH_3$ ,  $N_2O$ , and methane, among other things. Following probiotics, water quality improved in several trials, most notably with *Bacillus* sp. (Verschuere et al. 2000). *Bacillus* species are better at converting Gram-positive carbon to biomass or slime when compared to their Gram-negative counterparts.
- Probiotics and their products have various health benefits for both aquatic animals and terrestrial animals, but all of these benefits are provided by aquaculture, terrestrial animals, and human disease control. We also use microbial additives that help prevent intestinal infections, skin infections, and the proliferation of microbes in their environment.

## 14.10 Modes of Action of Probiotics

Probiotics can be administered in various ways, including (1) Incorporate to the pellet feed and culture water (2) enriching via live feed. Additionally, a thorough understanding of the mechanism of action and appropriate application procedures may be necessary for successful probiotic use in aquaculture. While the actual mechanism of action is unknown, it frequently exhibits host and strain-specific changes in its actions. On the other hand, probiotics generate great scientific and

economic interest on a global scale in aquaculture (Banerjee and Ray 2017). Many criteria are used to select suitable probiotic strains, including production of extra-cellular enzymes, survival in gastric juice, mucus growth, acid and bile tolerance, production of antimicrobial compounds that suppress pathogen growth in vitro, and bio-safety (haemolytic activity and antibiotics susceptibility).

1. Probiotics may influence the host's immune system, especially the innate and acquired immune systems. The ability of this mechanism to accomplish this is essential not only for the inhibition and treatment of infectious illness but also for resolving intestinal inflammation.
2. Additionally, probiotics can influence commensals and potentially harmful organisms. This is an idea of high importance in the field of infection prevention and treatment and restoring microbial homeostasis in animals' digestive tracts.
3. Toxin inactivation and detoxification of host and food components may occur in the gut due to the action of probiotics on microbial products, host products, and dietary factors.
4. Probiotic substances function as an antagonist to the quorum-sensing system.
5. The role of enzymes in digesting.
6. Iron competition: For harmful bacteria to survive in the host, iron acquisition is crucial. Because of this, a large number of genes that play a role in iron uptake are associated with pathogenicity. Furthermore, because siderophores have a great affinity for ferric ions, they inhibit the growth of dangerous bacteria by limiting iron availability.
7. Some recent studies have indicated that probiotics can significantly impact various key physiological systems in aquatic animals. Some studies have found that it helps raise the insulin-like growth factor (IGF-I) levels in European seabass (Carnevali et al. 2014).
8. As furthermore, research shows that probiotics lower cortisol levels and support antioxidative enzymes to help the body better cope with stress (Zolotukhin et al. 2018).
9. While the use of probiotics to enhance disease resistance is widely documented, research on the effects and mechanism of probiotics on aquatic animal reproductive function is sparse (Abasali and Mohamad 2011). Using diverse strains such as *Lactobacillus subtilis*, *L. acidophilus*, *L. casei* and *B. subtilis*, very few studies have attempted to demonstrate the effect of probiotic supplementation on reproductive performance in aquaculture. It is well established that probiotics affect reproduction in various ways, including fertilization, gonadosomatic index, fecundity, and fry production by females (Ghosh et al. 2007).
10. While probiotic bacteria studies reveal that many bacterial strains have antiviral properties in aquaculture, the exact mechanism by which they inhibit viruses has not yet been determined. In the scientific community, the effectiveness of probiotic agents such as *Pseudomonas* sp. and *Vibrios* sp. against the infectious haematopoietic necrosis virus is generally acknowledged (Sahu et al. 2008).
11. Probiotic bacteria play an essential function in limiting algal development, notably red tide plankton (Qi et al. 2009). While unicellular algae are being



developed in the culture pond, bacteria antagonistic to algae will be undesirable in green water larval-raising techniques in hatcheries, where bacterial antagonists to algae will be useful.

12. Probiotic bacterial populations can produce numerous chemical compounds bactericidal or bacteriostatic to both Gram-negative and Gram-positive bacteria. These inhibitory molecules are of various origins, including proteinaceous substances (lysozyme and other types of proteases), chemicals (hydrogen peroxides), and iron-chelating compounds such as siderophores (Giri et al. 2013). These inhibitory chemicals are vital in pathogen suppression and growth, reducing pathogen load.
13. Physical competition for attachment sites on the host's gut mucosal layer is one putative strategy for inhibiting pathogen colonization. It is well understood that the capacity to cling to mucus and wall surfaces is required for bacteria to establish themselves in fish intestines (Roeselers et al. 2011).
14. Probiotics have a favourable role as immunostimulants in protecting cultured aquatic species by lowering the impact of illnesses and pathogen entry. As a result, its usage as an immunostimulant is an efficient method to improving aquaculture success. Many authors have validated the use of probiotics in carp species to improve immune response, disease resistance and minimize abnormalities (Chi et al. 2014; Dawood and Koshio 2016; Wu et al. 2015). Unfortunately, the precise impact of probiotic supplementation in the expression of immunological parameters is still unknown.

### ***14.10.1 Trials of Probiotics in Fish Culture***

Probiotics in aquaculture may act similarly to those found in terrestrial animals. The interaction between aquatic animals and the culture situation is significantly more complicated than that of terrestrial animals. Probiotics are microbial supplements containing living microorganisms that benefit the host by altering the host's or its culture environment. The microorganisms found in water bodies are harmful bacteria that are opportunists, which means they reap the benefits of an animal's stress situation, which causes a decline in growth and survival (Verschuere et al. 2000). Consequently, probiotics in aquaculture aims not only at animal's significant benefit but also at the effect on the farming environment.

### ***14.10.2 Role in Immune System***

Probiotic bacteria are beneficial bacteria, which can prevent and regulate the host immune system as well as infections (Yousefi et al. 2018). Furthermore, a study on the GALT (gut-associated lymphoid tissue) of seabream found that oral administration of a probiotic combo (*Lactobacillus plantarum* and *Lactobacillus fructivorans*)

increased the production of granulocytes and antibody (Picchietti et al. 2007). Fish fed with *Flavobacterium sasangense*, *Aeromonas veronii*, and *Vibrio lentus* reported the rises in the expression of TNF, lysozyme-C, and IL-1b (Dawood et al. 2016). Probiotics administration increased leucocyte numbers (Korkea-Aho et al. 2012), lymphocytes (Brunt et al. 2007), monocytes, and erythrocytes (Aly et al. 2008; Sun et al. 2010), and serum bacterial agglutination titres, albumin levels (Abbass et al. 2010), and serum peroxidase (Heo et al. 2013) have also been shown to increase. Immunological parameters such as tumour necrosis factor, serum lysozyme activity, and expression of the cytokine genes (interleukin-1, heat shock protein 70) and respiratory bursts were considerably improved in fish fed with *Rummeliibacillus stabekisii* (Tan et al. 2019). Innate immune responses are the initial line of defence against infections, and they involve many cells and mechanisms that secure the host organism from infectious diseases. *Clostridium butyricum* treatment improved the phagocytic activity of rainbow trout leucocytes (Sakai et al. 1995).

Probiotics have been reported to affect non-specific immune system factors such as neutrophils, macrophages, monocytes, and natural cell killers (Hoseinifar et al. 2020). Improved fish immunity is associated with a rise in granulocytes and lymphocytes, which is linked to cell-mediated mucosal defence system (Lazado and Caipang 2014). After the administration of probiotics such as, *Lactobacillus belbrüeckii*, *Bacillus subtilis*, and *Debaryomyces hansenii*, with *Sparus aurata* has been shown to increase innate immune responses (Salinas et al. 2005). When treated with the probiotic strain *Bacillus subtilis* and *Pseudomonas*, *Oreochromis niloticus* demonstrated notable improvements in growth performance and immunological response (Wang et al. 2010). Gatesoupe (1994) discovered that turbot larvae fed with rotifera supplemented with lactic acid bacteria developed resistance to *Vibrio* spp. infection. The feeding trial of *Lactobacillus fructivorans* and *L. plantarum* with sea bream larvae (*Sparus aurata*) showed increased in colonization of the intestine as well as growth and survival (Carnevali et al. 2014). Probiotic *Lactobacillus rhamnosus* was found to accelerate the respiratory burst in rainbow trout (Nikoskelainen et al. 2003).

### 14.10.3 Immunostimulants

Probiotics play a vital role as immunostimulants in the protection of aquatic animals by reducing disease and pathogen entry. As a result, its usage as an immunostimulant is a very practical method to improving aquaculture success. Numerous studies have validated the usage of probiotics in carp species to increase disease resistance, immune response and reduce abnormalities. The studies showed on probiotics indicated favourable effects on host gut defences, which is critical in disease prevention and treatment of digestive tract inflammation and producing inhibitory substances (Akhter et al. 2015; Azimirad et al. 2016; Dawood and Koshio 2016). Probiotics or their derivatives for host health benefits have been demonstrated to be effective in aquaculture disease management. These comprise microbial adjuncts

that inhibit harmful bacteria from multiplying in the intestine, on the surface of the skin, as well as within the culture atmosphere of the species (Verschuere et al. 2000). Probiotics have an effect on culture organisms by improving their immune systems, increasing their susceptibility to pathogens, or generating inhibitory substances that can stop harmful organisms from infecting the host.

#### **14.10.4 Colonization Capacity**

Competitive exclusion and immunomodulation are the two basic modes of action in aquatic species. Probiotics have the potential to be an effective substitute to the preventive usage of antibiotics and additives. Oxygen, chemicals, and nutrition can be competitive with or even better than water and feed utilities in encouraging the health and growth of livestock. The intestinal tract's mucosal epithelium is inhabited by probiotics (Korkea-Aho et al. 2012), and exhibit adherence (Mahdhi et al. 2012) and proliferation in intestinal mucus (Sorroza et al. 2012). Probiotics such as *Bifidobacterium infantis*, *Bacillus coagulans*, and *Bacillus mesentericus* effectively colonized in the gut of freshwater ornamental fish *Puntius conchonius* (Divya et al. 2012). While *Pseudoalteromonas* RA7–14, *Ruegeria* RA4–1, *Microbacterium* ID3–10, and *Vibrio* RD5–30 formed in the intestines of Atlantic cod larvae, their colonization was just a temporary occurrence (Skjermo et al. 2015). Probiotics (*Bifidobacterium infantis*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bacillus coagulans*, and *Bacillus mesentericus*) effectively colonized in the gut of fish, *Brachydanio rerio* larvae (Akbar et al. 2014b).

#### **14.10.5 Antagonistic Activity**

Few bacteria yield a variety of antibiotic and antagonistic components that can be used as probiotics. Probiotics are utilized as a substitute to antibiotics and pesticides for prophylaxis (Decamp et al. 2008). To compete for nutrients and habitats, they developed anti-bacterial compounds (Moriarty 1998). Probiotics produced enough organic acid and a decrease in pH to inhibit many harmful bacteria. *Bacillus licheniformis* and *Bacillus pumilus* both have antibiotic activity and could survive low pH and high bile levels (Ramesh et al. 2015). Bacteriocidal proteins, organic acids, diacetyl, and hydro peroxide were all generated by *Lactobacillus* spp. (Farzanfar 2006; Verschuere et al. 2000). These compounds activated animal immune systems, making them more resistant to pathogens or suppressed bacterial pathogens in aquaculture systems. In white leg prawns, *Bacillus licheniformis* displayed in vitro inhibitory properties contrary to *Vibrio alginolyticus* (Ferreira et al. 2015).

### ***14.10.6 To Improve Disease Resistance***

Probiotics have been demonstrated to be disease-resistant and are good pathogen-prevention measures. Probiotics are vital in the development of anti-bacterial compounds that prevent pathogenic bacteria from entering organisms and develop resistance to infectious diseases. Probiotics improved the gut flora's ability to fight infections. Because probiotics are a beneficial contributor to disease management policies in aquaculture (Balcázar et al. 2006a). Probiotics administration has been shown to increase disease resistance in fish by increasing both innate and adaptive immune responses. Several studies have shown that probiotics can protect aquatic animals from pathogenic infection. The probiotic *Pediococcus acidilactici* fed to *Litopenaeus stylirostris* demonstrated vibriosis resistance (Castex et al. 2008). Similar studies have been reported by *Vibrio* spp. vs. *Brevibacillus brevis* (Mahdhi et al. 2012), *Flavobacterium psychrophilum* vs. *Pseudomonas* M174 and M162 (Ambas et al. 2013), *Bacillus* spp. vs. *Strep. iniae* (Cha et al. 2013). *Pseudomonas aeruginosa* supplementation increased innate immunity and illness resistance in rats (Giri et al. 2012). *Streptococcus phocae* is a fish disease; however it increased the growth of *Penaeus monodon* post larvae and protection against *Vibrio harveyi* (Austin and Austin 2012). *Aeromonas hydrophila* and *A. sobria* have been shown to be fish pathogens (Austin and Austin 2012), and they have been shown to diminish infections of *Aeromonas salmonicida* (Irianto and Austin 2002). Studies have reported in *Bacillus pumilus* in Nile tilapia (Aly et al. 2008), *Oncorhynchus mykiss* (Brunt et al. 2007) and *Bacillus* S11 in white scampi (Rengpipat et al. 2000). Rohu was protected against *A. hydrophila* infection by *Bacillus licheniformis* and *Bacillus pumilus* (Ramesh et al. 2015). Dietary probiotic *Lactococcus lactis* on Japanese flounder (Heo et al. 2013), *B. pumilus* improved Nile tilapia health and disease resistance (Aly et al. 2008). The occurrence of white spot syndrome virus was reduced in white leg prawns fed a mixture of *Staphylococcus hemolyticus* and *Pediococcus pentosaceus* (Leyva-Madrigal et al. 2011).

### ***14.10.7 Improve Water Quality***

Probiotics have been proved to help enhance water quality in different ways. Probiotics have enhanced water quality by reducing pathogenic water bacteria (Dalmin et al. 2001), enhanced organic matter decomposition, reduced ammonia concentration, hydrogen sulphide, nitrogen and phosphorus, nitrite (Boyd and Massaut 1999; Cha et al. 2013). Probiotics reduced phosphate pollution in the sediments (Wang and He 2009), organic matter accumulation, mitigated nitrogen (Verschuere et al. 2000; Wang et al. 2005), probiotics reduced metabolic wastes in cardinal tetras during transportation (*Paracheirodon aequalis*) (Gomes et al. 2009).

### 14.10.8 Improvement in Nutrient Utilization and Digestion

Studies have demonstrated that administering probiotics to a diet will benefit the health and disease resistance of fish. Recent research has also shown that dietary probiotics can improve nutritional digestibility in aquatic animals by boosting the activity of digestive enzymes. *Bacillus* species have been widely used in aquaculture due to their ability to sporulate and beneficial impacts on feed utilization, immunological response, and digestive enzyme activity. Furthermore, some *Bacillus* species supplementation via water bath or live feed has been found to modify the gut microbial composition in fish. However, Lactic acid bacteria are the most often utilized probiotic formulations for this purpose. It enhanced nutrient digestibility and enhanced the production of digestive enzymes in the host (Ghosh et al. 2017). Few bacteria, for example, *Rhodobacter sphaeroides* and *Bacillus* sp., participate successfully in improving digestive enzymes and cellulase enzymes considerably in white shrimp (*Litopenaeus vannamei*) and bivalves (Sahu et al. 2008; Wang et al. 2008). Bacterial species *Staphylococcus* sp., *Brevibacterium* sp., *Pseudomonas* sp., *Microbacterium* sp., and *Agrobacterium* sp. have all been identified as essential to a charr's metabolic and nutritional well-being in the Arctic (*Salvelinus alpinus*) (Ringø et al. 2014).

The probiotics like *Lactobacillus*, *Brevibacillus brevis*, *Vibrio harveyi*, and *Vagococcus fluvialis* are all known to help aid digestion and gastrointestinal health (Lazado et al. 2011; Mahdhi et al. 2012), adhere to the mucus, and also improve feed digestibility by increasing the activity of digestive enzymes (Zokaefar et al. 2012). They also create body components, such as fatty acids, vitamins, antibiotics, bacteriocins, siderophores, and lysozyme, which are beneficial to host health (Vine et al. 2006). Probiotic supplemented food, including *Streptomyces* sp. and *Bacillus subtilis*, increased growth and survival rates in *Xiphophorus maculatus*, *Poecilia reticulata*, and *Xiphophorus helleri* (Dharmaraj and Dhevendaran 2010). Khat tab et al. (2005) reported *Micrococcus luteus* as probiotics in Tilapia, which exhibited improved feed conversion ratio and growth performance. Nile tilapia fed with *Rummeliibacillus stabekisii* for eight weeks resulted in greater digestive enzyme secretion, faster growth, and more significant weight gain (Tan et al. 2019). Feeding a probiotic strain of *Lactobacillus* sp. to Nile tilapia (*Oreochromis niloticus*) improved crude lipid, total protein, and body weight (Hamdan et al. 2016). It is generally accepted that probiotics help animals grow and develop strong immune systems and better digestion by increasing the number of beneficial microorganisms in the intestinal tract, resulting in improved feed quality, growth-promoting substances, and digestive enzymes. Probiotics alter the population of microbes in the gastrointestinal tract in several ways, one of which is to generate various types of fatty acids, minerals, vitamins, and important amino acids (Newaj-Fyzul et al. 2014). *L. vannamei* growth and feed efficiency are increased by probiotic *B. subtilis* E20. *Bacillus* contains probiotic strains that release extracellular enzymes that break down numerous nutrients into smaller molecules such as proteins, lipids, and carbohydrates (Ochoa-Solano and Olmos-Soto 2006). Van Hai and Fotedar (2009), for

example, demonstrated that dietary probiotics (*Pseudomonas Synxantha* and *P. aeruginosa*) increased the growth performance of western king prawns. Rohyati (2015) recently discovered that feeding probiotics (Vibrio Alg3.1RfR-Abn1.2RfR-enriched protein) increased the growth rate of *Haliotis asinina*; probiotics may be a supplemental food source due to their availability of essential nutrients such as biotin, fatty acids, and vitamins (Verschuere et al. 2000).

### 14.10.9 Growth and Survival

Probiotics are live microbial cells that, when given in appropriate quantities, helps boost the growth and health of fish (Ringø and Gatesoupe 1998). Another important characteristic of probiotics is that it takes less time for gut loading of live feeds such as *Artemia* (Akbar et al. 2014a). Several research investigations reveal that probiotic supplementation has been shown to increase the rate of development, the strength of the immune system, and the resistance to sickness in fish. Kumar et al. (2006) discovered that *Bacillus subtilis* fed *Labeo rohita* had a greater survival rate when subjected to an intraperitoneal injection of *Aeromonas hydrophila*. Growth stimulation *Bacillus* spp. has been previously reported in *Paralichthys olivaceus* (Gatesoupe et al. 1989), *Tilapia*, *Scophthalmus maximus* (Gatesoupe 1991), and Nile tilapia (Aly et al. 2008). Probiotics have increased the growth rates of aquatic animals, the feed utility, and survival rates by modulating digestive enzyme activities (Ringø et al. 2010). Bergh et al. (1992) discovered that when the Atlantic halibut (*Hippoglossus hippoglossus*) started feeding for the first time, the intestinal flora changed from a predominance of *Flavobacterium* spp. to *Aeromonas* spp. demonstrating the impact of the external environment and food on the microbial community of this fish. Probiotics enhanced the growth of *Penaeus monodon* (Maeda and Liao 1992) and *Macrobrachium rosenbergii*, a giant freshwater prawn (Mujeeb Rahiman et al. 2010). The growth of western king prawns was boosted by *Pseudomonas aeruginosa* and *Pseudomonas synxantha* (Van Hai et al. 2010). Probiotic *Lactobacillus plantarum* helps the fish gut by creating antibiotic compounds that prevent dangerous intestinal bacteria from multiplying and suppressing the growth of competing bacteria (Kesarcodi-Watson et al. 2008). As a result, supplementation with *L. plantarum* resulted in immunological modulation, enhanced growth parameters, and elevated disease resistance in a wide range of fish species (Dawood et al. 2015; Son et al. 2009). Probiotics help in digestion by enhancing the production of extracellular enzymes like proteases, carbohydrases, and lipases, as well as providing growth factors. Flocculated algae (flocculated with probiotics *Bacillus subtilis* (MTCC 441) and *Lactobacillus acidophilus* (10,307 MTCC)) enriched *Artemia* has a remarkable effect on digestive enzyme production, growth, and gut microflora of freshwater fish *Catla catla* (Kandathil Radhakrishnan et al. 2020). *Vibrio midae* SY9 improved the digestive enzyme activity (protease), digestion and absorption of proteins, and growth performance of *Haliotis midae* (Huddy and Coyne 2015). With an increase in digestive enzymes, photosynthetic bacteria and *Bacillus* spp.

boosted the growth of white leg prawns (Wang 2007). Specific digestive enzyme (lipase, protease, and amylase) activities were elevated in probiotic-fed *Fenneropenaeus indicus* (Ziaei-Nejad et al. 2006). Furthermore, probiotics resulted in the production of vital nutrients such as fatty acids, biotin, and vitamin B12 (Vine et al. 2006). Bacteria are a key food component in natural environments by holothurian deposit-feeding, and probiotics can act as a supplementary food or meal digestion aid (Verschuere et al. 2000). Yassir et al. (2002) attempted to use probiotic bacteria as a growth promoter on *Oreochromis niloticus* and discovered that *Micrococcus luteus* resulted in the best growth parameters and the feed conversion ratio on Tilapia. As a result, probiotics can be used as growth promoters in aquaculture while also providing other profits. The growth and survival of *Haliotis rufescens* (Silva-Aciaries et al. 2011) and *H. midae* was improved by *Vibrio* C21-UMA and *Vibrio midae*, respectively (Macey and Coyne 2006). *B. subtilis* or *Lactobacillus acidophilus* increased the survival rate of Nile tilapia (Aly et al. 2008), and *L. acidophilus* improved the survival rate of Nile tilapia (Villamil et al. 2014). *Pseudomonas aeruginosa* YC58 increased the survival of pearl oyster juveniles (Aguilar-Macías et al. 2010). Sahandi et al. (2012) found that direct inoculation of *Bacillus* probiotic into fish culture tanks has good impacts on the growth of *Oreochromis mossambicus*, *Ctenopharyngodon idella*, and *Cyprinus carpio*, and Tilapia had considerably increased growth performance when the commercial probiotic BZT® BIOAQUA was introduced to tanks, and probiotic treated fish inclined to perform well survival in all treatments (Mohamed et al. 2013). Devaraja et al. (2013) demonstrated that *Bacillus* sp. increased the growth of *Penaeus monodon* in a culture system with no water exchange. *Bacillus* is a frequent probiotic used in aquaculture. Due to the fact that *Bacillus* spp. are non-toxic and good to shrimp, this microbe is a popular one in aquaculture. For example, the introduction of *Bacillus* to the meal helped the tiger shrimp's survival, growth, and immunity (Rengpipat et al. 2000). Some beneficial bacterial strains (*Micrococcus*, *Bacillus*, *Lactobacillus*, *Saccharomyces*, *Debaryomyces*, *Lactococcus*, *Enterococcus*, and *Photobacterium*) have been added shrimp diets or water to enhance their growth (Tseng et al. 2009; Wang et al. 2019). *Bacillus subtilis* E20 was used as a probiotic for *L. vannamei*, and the larval development was improved by either orally administering it to water or by adding it to water (Liu et al. 2010). Numerous studies have demonstrated that specific probiotics administration may be a substitute for preserving aquatic life. Farmers, however, cannot predict when sickness will strike to offer probiotic feed ahead of infection. Consider that in aquaculture, good in vitro results don't always translate to good in vivo effects. Also, the lifespan of probiotic benefits is unknown. Nobody knows what happens to living probiotics in aquatic environments. It is suspected that short-term cyclic probiotic feeding may avoid stimulating the immune system while sustaining some level of protection or immunostimulant. As a result, more research is required to explore this application technique. However, more research is needed to find out how often, in what manner, for how long, and with what kinds of symbiotic to be used. Probiotic bacteria can improve feed efficiency by producing digestive enzymes, which is especially useful in aquaculture because there is a shortage of fish meal. Probiotics are critical when animal protein



alternatives such as plant protein sources are used as a supplement. It is critical to explore the metabolic abilities of probiotics when trying to deconstruct antinutrients, particularly in plant-based proteins. Dosage-dependent research is scarce and inconsistent. Additional investigation is also required before providing any level of certainty. As more in-depth research is done on every area of probiotics, more fruitful results are anticipated about the application of probiotics in aquaculture. In order to ensure the integrity of probiotics, they will have to be grown and developed in industry settings that follow strict quality control requirements. This would enable the worldwide distribution of organic aquaculture products, which are essential for human nutrition and wellness. A harvest that uses natural and sustainable methods can help to keep food fresh and safe for humans.

## 14.11 Conclusion

More recently, probiotics have enhanced fish health, water quality, disease prevention, and immune response support. Probiotics could be used in aquaculture, but there is much work ahead of us. Developing screening criteria for potential probiotics are critical, as understanding the mechanisms of action is essential to discovering new probiotics. To obtain a complete understanding of the microbiome and probiotics, further in-depth research on host-microbe interactions in vivo and the development of new tools for microbial surveillance is required. The effectiveness of probiotics is dependent on knowledge of species and strain competition.

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