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# Emerging Probiotic Approaches and Concepts for Sustainable Aquaculture

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## Abstract

Aquaculture plays a vital role in the food-producing sector to compensate for the shortage of protein. Bacterial and viral diseases are the most important obstacles to expanding and developing sustainable aquaculture. Prolonged usage of antibiotics and chemotherapeutic drugs against microbial infection leads to selective pressure to the emergence of multi-drug resistant pathogens. Therefore for growing concerns, ecofriendly harmless alternative treatments require sustainable development in aquaculture to support the country's economy. The use of probiotics is an efficient alternative solution that allows restoring the balanced microflora in the gastrointestinal tract. Probiotics have many benefits, including promoting development, improving digestion, stimulating the immune response, enhancing reproduction, controlling infections, providing nutrients, and improving water quality. The purpose of this review is to focus on various probiotic approaches used in aquaculture and the role of marine probiotics in this context.

**Key words:** Probiotics; Synbiotics; Paraprobiotics; Marine microorganisms.

### Introduction

Aquaculture is one of the rapidly growing sectors of the global food production industry. In comparison to capture fisheries and terrestrial farmed meat, aquaculture helps to meet global protein food demand (Chauhan and Singh, 2019). The infectious disease takes the lead of production-limiting threats, causing multibillion-dollar losses each year (Assefa and Abunna, 2018). Among infectious diseases, bacterial infections are the most prevalent, resulting in decreased fish production. Bacterial disease outbreaks are usually caused by the genera *Aeromonas*, *Vibrio*, *Edwardsiella*, *Pseudomonas*, and *Streptococcus*, with the epithelial mucosal surfaces of the intestinal tract of fish serving as the main routes of infection (Kavitha *et al*., 2018).

Antibiotics and chemotherapeutic agents have traditionally been using to manage bacterial problems in fish hatcheries. But their continued and often unregulated use has resulted in resistant bacterial strains (Puvanendran *et al*., 2021). Furthermore, antibiotics in the flesh can be transferred from fish to humans through the food chain, causing harm to human health and destroying beneficial microflora in the gut, resulting in an unbalanced gastrointestinal ecosystem that directly influences nutrition, physiology, and immune response (Banerjee and Ray, 2017). Vaccination is an alternative prevention mechanism for infectious diseases; nevertheless, since juvenile fish are not immunocompetent, their effectiveness is reduced or ineffective. In addition, there are only a few authorized vaccines available in aquaculture due to the complicated process of commercializing the product (Perez-Sanchez *et al*., 2018).

In humans, domesticated terrestrial animals, and fish, the gut microbiota affects a variety of biological processes (Giatsis *et al*., 2016). Especially fish and other aquatic animals have a trusting relationship with their surroundings and, as a result, with the microorganisms that live there. Fish may have a mutualistic or pathogenic relationship with microorganisms in their environment and associated symbiotic gut microbiota,

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which play a role in nutritional provider, metabolic regulator, and immune defender (Egerton *et al*., 2018). The use of probiotics is a successful approach for restoring the fish intestinal microbiota and physiological condition (Zuenko *et al*., 2017). Moreover, they effectively protect the host from invading pathogenic microorganisms through various mechanisms such as epithelial cell adhesion, immune system modulation, and secretion of antimicrobial substances (Somashekaraiah *et al*., 2019). Other beneficial effects of probiotic strains include the production of extracellular enzymes, growth augmentation, water quality maintenance, and improve reproduction (Banerjee and Ray, 2017). Therefore, probiotic cultures development is significant as an alternative and complementary approach to antibiotics, chemotherapy, and vaccination to prevent fish diseases (Munoz-Atienza *et al*., 2014).

The marine fish gut microbiota has been examined for a variety of reasons, but little is known about the efficacy of probiotics isolated from wild marine fish and their positive effects on the host. To that end, the main aim of this review is to provide valuable information on the use of probiotics in aquaculture and various probiotic approaches used for long-term aquaculture sustainability.

## Probiotic approaches in aquaculture sector

### Single strain vs. Microbial consortium

Aquaculture can benefit from the use of probiotics in single or combinations of strains. The majority of research on probiotics in aquaculture used a single probiotic strain. According to some research, using combined probiotics have more potential for marine animals than using single species. For instance, a probiotic mixture containing *Bacillus subtilis* E20, *Lactobacillus pentosus* BD6, *L. fermentum* LW2, and *Saccharomyces cerevisiae* P13 had introduced to the diets of Asian sea bass and white shrimp, the probiotic mixture had significantly improved the development, immunity, and disease resistance than those sole probiotics in the diet (Lin *et al*., 2017; Wang *et al*., 2019). In another study, compared the activity of mixed *Bacillus* sp. FI99 and FI162 in European sea bass juveniles found that the carnivorous fish ability to metabolize plant-based diet was higher than the single strain (Serra *et al*., 2019). Also, recent research reported that microbial consortium formulated with three strains, *Vibrio diabolicus* (Ili strain), *Vibrio heptarius* (P62 strain), and *Bacillus cereus sensu stricto* (P64 strain) significantly, protected the *Penaeus vannamei* shrimp larvae from *V. parahaemolyticus* (Ramirez *et al.,* 2022).

### Native source vs. Commercial /terrestrial sources

Aquaculture research has previously concentrated on well-known probiotic strains derived from terrestrial hosts such as mammals, birds, dairy products, fermented food products, and commercially available products by ignoring fundamental variations in the physiology of cultured organisms. The use of host-associated microorganisms as probiotics has recently received more attention. Because probiotics from native sources perform more efficiently within the original host than those from other terrestrial or warm-blooded animals, they can comfortably adapt to other farmed fish species (Wanka *et al*., 2018). Various studies supported that wild marine fish can be used as a better source for isolating probiotics because of the resistance ability to the harsh environment in the intestinal region (Nguyen *et al*., 2017). For instance, Serra *et al*. (2019); Puvanendran *et al*. (2021); Amenyogbe *et al.* (2022) have reported the host-associated probiotics from wild marine fish with significant results.

### Synbiotics

Synbiotics are probiotics combined with a variety of prebiotics, which acts as dietary supplements can improve health by assisting in the modulation of the host intestinal microflora (Hassaan *et al*., 2014). It has been characterized as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the intestinal tract, and thus

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improves host health" (Ai *et al*., 2011). Prebiotics used in aquaculture includes galactooligosaccharide (Safari and Paolucci, 2017), mannan-oligosaccharide (Ohtani *et al*., 2020), fructooligosaccharide (Ai *et al*., 2011), chitin (Askarian *et al*., 2012), β-glucan (Munir *et al*., 2016), and xylooligosaccharide (Hoseinifar *et al*., 2014). In contrast to feeding probiotic and prebiotic separately, feeding synbiotic *Lactococcus lactis* HNL12 and algal meal (*Schizochytrium limacinum*) resulted in better PWG (percent weight gain), SGR (specific growth rate), and protection against *Vibrio harveyi* in humpback grouper (Sun *et al*., 2019). Similarly, a mixture of probiotic and prebiotic have shown to produce the highest WG (Weight Gain) and SGR in olive flounder (Hasan *et al*., 2018), WG, and immune-related gene expression in Asian sea bass (Ashouri *et al*., 2020), WG, SGR and PER (Protein efficiency rate) in Pacific white shrimp (Chen *et al*., 2020), and enhanced growth, disease resistance against *Vibrio* pathogens in white shrimp (Prabawati *et al*., 2022).

**Paraprobiotics**

During recent years, new terminologies added to the probiotic concepts such as Paraprobiotics and Postbiotics. Probiotics are historically characterized as live microorganisms that impart positive health effects to the host. Despite some research suggesting that dead/killed microorganisms (Paraprobiotics) or microbial substances/cellular extracts (Postbiotics) are advantageous to the host health (Zendeboodi *et al*., 2020). Some critical concerns in probiotic practical application and performance issues, such as the viability of the probiotic strains in products, different colonization patterns, persistence in the intestinal region, and the risk of acquiring the virulent gene from pathogenic bacteria via horizontal gene transfer, have triggered the use of non-viable probiotic to benefit the host in a similar way of viable probiotics (Choudhury and Kamilya, 2019). Various reports on the use of paraprobiotics in the form of dead or cellular extracts are available. When *Litopenaeus vannamei* (white shrimp) was fed both live and a cell-free extract of *Clostridium butyricum* CBG01 against *Vibrio parahaemolyticus,* the metabolite supplements boosted the shrimp's growth efficiency and disease tolerance compared to the live cells alone (Li *et al*., 2019). Supplementing the gut microbiota of the *Penaeus vannamei* (whiteleg shrimp) with *L. plantarum* cell-free extracts modulated the gut microflora by increasing the possible beneficial bacteria while substantially decreasing the potentially pathogenic bacteria (Zheng *et al*., 2020).

**Quorum quenching probiotics**

Quorum sensing (QS) is a cell-to-cell communication process that regulates the establishment of virulent factors such as extracellular polysaccharides, lytic enzymes, siderophores, and other factors to sustain the cell population (Defoirdt, 2018). Quorum quenching (QQ) is opposite to QS. The significant thing is that QQ bacteria can degrade the molecules of QS as well as utilize them as their source of food. Disruption of the pathogenic bacterial QS system is an effective and popular strategy to target as anti-virulence therapy for a healthy aquatic life (Defoirdt *et al*., 2004). Among the QS molecule, acyl-homoserine lactones (AHL) are the primary signal molecule produced by Gram-negative bacteria (Huang *et al*., 2016). Therefore, AHLs are a potential target to control the virulence of pathogens (Chen *et al*., 2020). As stated by Ghanei-Motlagh *et al*. (2021), the Asian sea bass acquired resistance against AHL secreting *V. harveyi* via incorporation of *Bacillus cereus* QQ2 and *B. thuringiensis* QQ1 bacteria, isolated from *Lates calcarifer* (Asian Sea bass) (Ghanei-Motlagh *et al*., 2020). Furthermore, compared to widely used probiotics with potent antimicrobial activity in aquaculture, QQ probiotics produce less selective pressure against pathogens (Ghanei-Motlagh *et al*., 2021).

**Encapsulation**

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Encapsulation is the process of enhancing live feed with probiotics such that the probiotic bacteria can survive and reproduce by using the live feed as a vector. As a consequence, probiotics can efficiently transmit to the host through the live feed. *Artemia* and rotifer are well-known examples of live carriers (Chauhan and Singh, 2019). A microencapsulated diet is a mix of nutrients and agents encased in a digestible capsule used in aquaculture (Willer and Aldridge, 2019). It is the method of encapsulating probiotics in alginate-based nanofibers to increase their survivability (Ceylan *et al*., 2018; Amir *et al*., 2019). Moreover, it protects probiotic bacteria from severe external circumstances like high temperatures during feed preparation and the acidic nature of the gastrointestinal tract. Alginate is the most often used encapsulating material for probiotic organisms for its ease of handling, non-toxicity, inexpensiveness, and acceptance as a food ingredient (Jantarathin *et al*., 2017). According to Cordero *et al*. (2015), the microbiota and the immunological response of gilthead sea bream modulated by encapsulation of *Shewanella putrefaciens* Pdp11 in calcium alginate. The use of microencapsulated synbiotic dietary supplementation feed was significantly improved growth performance and immune system effects against *V. harveyi* infection in White shrimp (Zubaidah *et al*., 2015).

**Conclusion and future perspectives**

Antibiotic-resistant microorganisms are becoming a major public health concern around the world. This threat develops and spreads in the aquaculture industry due to the unrestricted use of antibiotics to treat infectious diseases. The microbiome-based research is primarily focusing on disease management and improving the host growth performance. Moreover, the marine ecosystem has a wide range of bioactive compounds with a variety of health-promoting properties. The marine environment is an excellent source for exploring novel probiotics with a broad spectrum of antimicrobial activity and unique functional features. This review paper concentrates on introducing the marine probiotics that play a crucial role in achieving sustainable aquaculture production. The use of probiotics is an effective strategy for combating the spread of antibiotic resistance. There has been a plethora of research on the use of probiotics, prebiotics, synbiotics, paraprobiotics, postbiotics, and quorum quenching probiotics in aquaculture, and they are all considered environmental friendly strategies for preventing infectious disease. Although adequate information on the mode of action and how the immune system of fish reacts to specific probiotics are needed, this information could aid in the management of the microbial population to prevent disease and treatment. Accelerating recent advances in technology, such as metagenomics and proteomics, will provide extensive knowledge on the probiotic mechanism also the possibility of discovering new probiotics.

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