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Probiotics in Aquaculture

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SUMMARY

Aquaculture is the world's fastest-growing food production sector. It contributes about 50% of world fish production. However, aquaculture is facing severe losses due to infectious diseases. The use of antimicrobial drugs, pesticides, and disinfectants in aquaculture disease prevention and growth promotion has led to the evolution of resistant strains of bacteria and antibiotic residues in cultured animals. Therefore the methods of combating diseases with antibiotics are being questioned, and alternatives are being sought. Thus, the research into probiotics for aquaculture is increasing as an eco-friendly solution for sustainable aquaculture. Probiotics have recently attracted extensive attention in aquaculture. The benefits of such supplements include improved feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, antimutagenic and anti-carcinogenic activity, and increased immune response. Probiotics are harmless bacteria that help the host animal's well-being and contribute to protecting the host animal against harmful bacterial pathogens.

INTRODUCTION

Aquaculture has a long history, originating around 475 B.C. in China and grown into a million-dollar industry. Aquaculture is the fastest developing food-producing sector with enormous potential to accomplish the growing demand for aquatic food. As per FAO SOFIA 2020 report, the global production from aquaculture was more than 45% of the total fish production. The intensification of aquaculture required cultivation at high densities, which has led to significant damage to the environment due to farm effluent discharges. These farm effluents are high in organic wastes that can deplete dissolved oxygen in the receiving water bodies leading to mass mortality of aquatic life. Further, under intensive production, aquatic organisms are subjected to high-stress conditions, increasing the incidence of diseases and mortality.

Need of Probiotic

Outbreaks of viral, bacterial, and fungal infections have caused devastating economic losses worldwide. China reported disease-associated losses of $750 million in 1993, while India reported $210 million losses from 1995 to 1996. Significant stock mortality has also been reported due to poor environmental conditions in the farms, unbalanced nutrition, generation of toxins, and genetic factors. In recent decades, the use of chemical additives and veterinary medicines, especially antibiotics, for the prevention and control of animal diseases has generated significant risks to public health by the persistence of bacterial-resistant strains.

Probiotic, Prebiotic and Synbiotic

The term "probiotic" comes from Greek *pro* and *bios*, meaning "prolife". Mr. Parker introduced it in 1974. He defined probiotics as organisms and substances that contribute to intestinal microbial balance. For many years, studies focused on microorganism characteristics from intestinal microbiota. The term "probiotic" was mainly restricted to gram-positive lactic acid bacteria, particularly representative of the genera *Bifidobacterium*, *Lactobacillus*, and *Streptococcus*. Prebiotics mainly consist of oligosaccharides promoting beneficial bacterial growth within the gastrointestinal tract of higher vertebrates. In the past decade, many substances have been investigated as prebiotics. Most studies have focused on non-digestible carbohydrates, mainly oligosaccharides. Prebiotics encompass Mannan oligosaccharides (MOS), fuctooligosaccharides (FOS), trans-galacto-oligosaccharides (TOS), xylo oligosaccharides (XOS), glucooligosaccharides (GOS), lactosucrose and many more. The idea is that the live addition in the gut would metabolise the selective substrate. Such addition would enhance probiotic survival, as well as offer the advantages of gut microbiota management techniques. A synbiotic has been defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract.

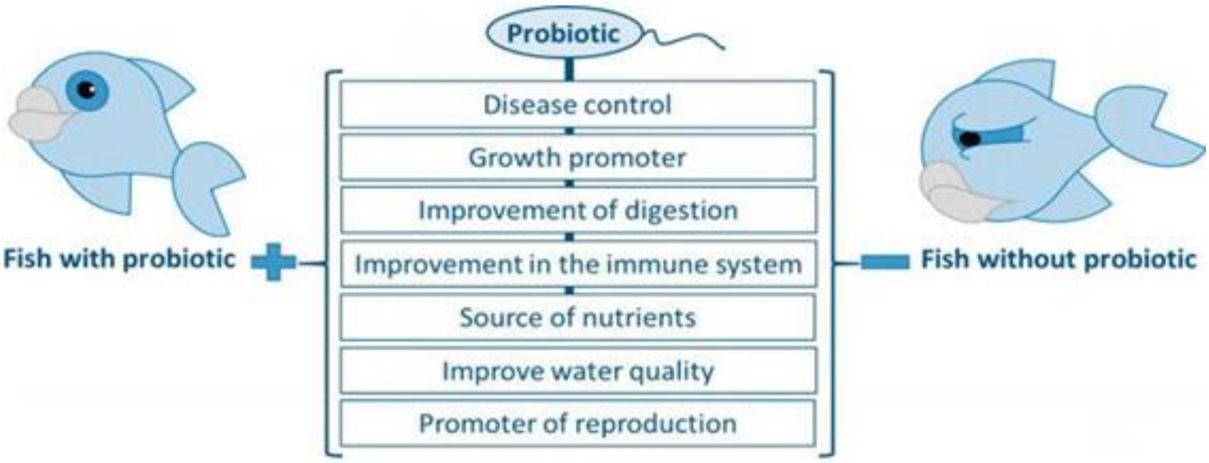

Figure 1. Beneficial effects of probiotics

(Source: Sayes et al., 2018)

The use of Probiotics in Aquaculture

The use of probiotics in fishes and other aquatic animals is increasing as an eco-friendly alternative to antibiotics. Lactic Acid Bacteria, certain photosynthetic bacteria, and yeast are used as probiotics in aquaculture. The benefits of probiotics include improved feed conversion and absorption and enzymatic contribution to digestion. They are also known to inhibit pathogenic microorganisms, antimutagenic and anti-carcinogenic activity, promoting growth and immune response. Improved water quality has been associated with probiotics and *Bacillus* sp. improved water quality, survival, and growth rates. These are in addition to the improved health status of juvenile *Penaeus monodon*. Probiotics exhibit antimicrobial effects by modifying intestinal microbiota, secreting antibacterial substances (bacteriocins and organic acids) and competing with pathogens for nutrients. Probiotics are also capable of modulating the immune system, regulating allergic response of the body, and reducing the proliferation of cancer in mammals.

Types of probiotics in aquaculture

Based on their mode of application, probiotics are classified as feed probiotics and pond probiotics.

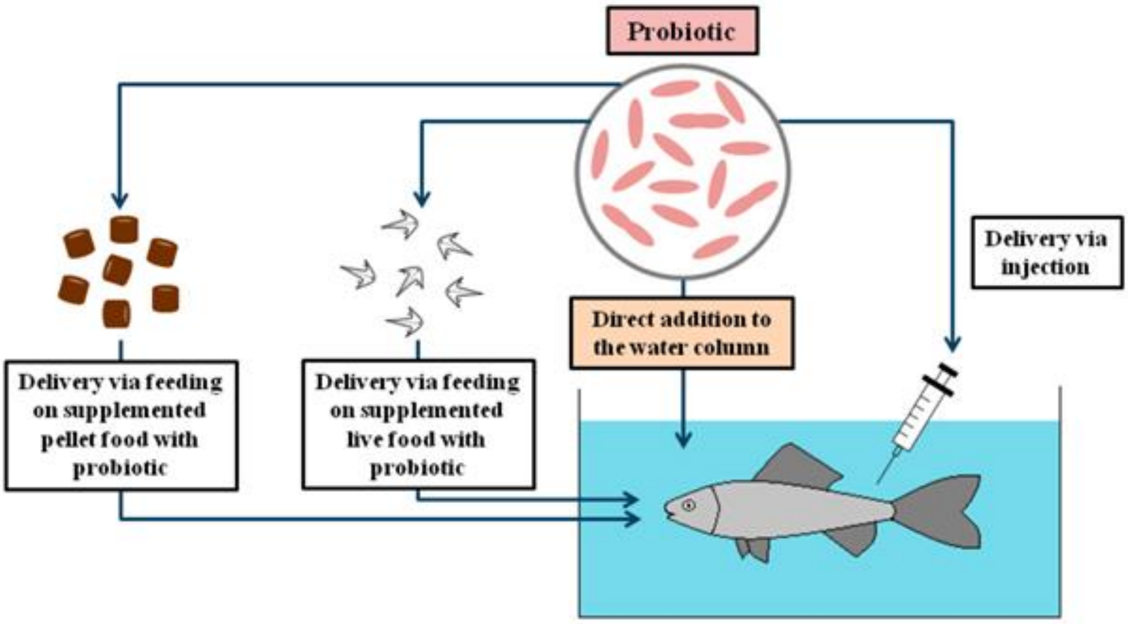

Figure 2. Method of administration of probiotics in aquatic environment

(Source: Jahangiri and Esteban., 2018)

Feed probiotics

Feed probiotics are those that are administered through the feed. These find their way into the gut or gastrointestinal tracts of the cultured organisms. Probiotics can be incorporated into the diets during feed preparation or natural live feed organisms reared in probiotic-enriched media.

Pond probiotics

Pond probiotics improve water quality by acting on organic matter and convert them into simpler nutrients. Beneficial bacteria break down organic matter into simpler substances like glucose and amino acids and provide a friendly environment. Probiotic bacteria such as *Bacillus* sp. convert organic matter to CO_2, minimising organic load in the aquatic system. The use of nitrifying bacteria reduces the quantity of nitrate, nitrite, and ammonia to a large extent. These lead to purification of the water, enhancing larval survival and growth in the hatchery.

Table 1. List of microorganism authorized as probiotics in feedstuffs under Council Directive 70/524/EEC.

Sl.No	Probiotic organism	Sl.No	Probiotic organism
1.	*Bacillus cereus var. toyoi*	7.	*Lactobacillus plantarum*
2.	*Bacillus licheniformis*	8.	*Lactobacillus rhamnosus*
3.	*Bacillus subtilis*	9.	*Pediococcus acidilactici*
4.	*Enterococcus faecium*	10.	*Saccharomyces cerevisiae*
5.	*Lactobacillus casei*	11.	*Streptococcus infantarius*
6.	*Lactobacillus farciminis*		

Benefits of Probiotics in aquaculture:

Growth Promoter:

Probiotics have been used in aquaculture to increase the growth of cultivated species. In reality it is not known whether these products increase the appetite or improve digestibility. Some people are inclined to think that it could be both factors; furthermore, it would be better to determine whether probiotics taste good for the aquatic animal. Probiotic microorganisms can colonize the gastrointestinal tract when administered over a long period due to a higher multiplication rate than expulsion rate (Balcázar et al., 2006). Hence, as probiotics are constantly added to fish, they adhere to the intestinal mucosa, developing and exercising their multiple benefits.

Inhibition of Pathogens

Antibiotics were used for a long time in aquaculture to prevent diseases in the crop. However, this caused various problems such as the presence of antibiotic residues in animal tissues, the generation of bacterial resistance mechanisms, and an imbalance in the gastrointestinal microbiota of aquatic species, which affected their health. The European Union has regulated the use of antibiotics in organisms for human consumption. Today, consumers demand natural products free of additives such as antibiotics. Moreover, there is a tendency to prevent diseases rather than treating them. Thus, probiotics are a viable alternative for the inhibition of pathogens and disease control in aquaculture species. Probiotic microorganisms can release chemical substances with bactericidal or bacteriostatic effects on pathogenic bacteria in the host's intestine, thus constituting a barrier against the proliferation of opportunistic pathogens. In general, the antibacterial effect is due to one or more of the following factors: production of antibiotics, bacteriocins, siderophores, enzymes (lysozymes, proteases), and hydrogen peroxide, as well as alteration of the intestinal pH due to the generation of organic acids. In shrimp, use of *Vibrio alginolyticus* strains as probiotics to increase survival and growth of white shrimp (*Penaeus vannamei*).

Improvement in Nutrient Digestion

A study has suggested that probiotics have a beneficial effect on the digestive processes of aquatic animals because probiotic strains synthesize extracellular enzymes such as proteases, amylases, and lipases that provide growth factors such as vitamins, fatty acids, and aminoacids. Therefore, nutrients absorption is more efficient when the feed is supplemented with probiotics. Various microorganisms have a beneficial effect on the digestive system of aquatic organisms (Michael *et al*. 2015). *Bacteroides* and *Clostridium* sp. have been observed to supply nutrients like fatty acids and vitamins to the host in aquaculture. In white shrimp *Litopenaeus vannamei* and *Fenneropenaeus indicus culture,* various Bacillus strains have been used as probiotics to increase the apparent digestibility of dry matter, crude protein, and phosphorus. Aquaculture practices demand intensive production in shorter times, causing stress in crop species. The use of probiotics in aquaculture reduces stress and increases the yield.

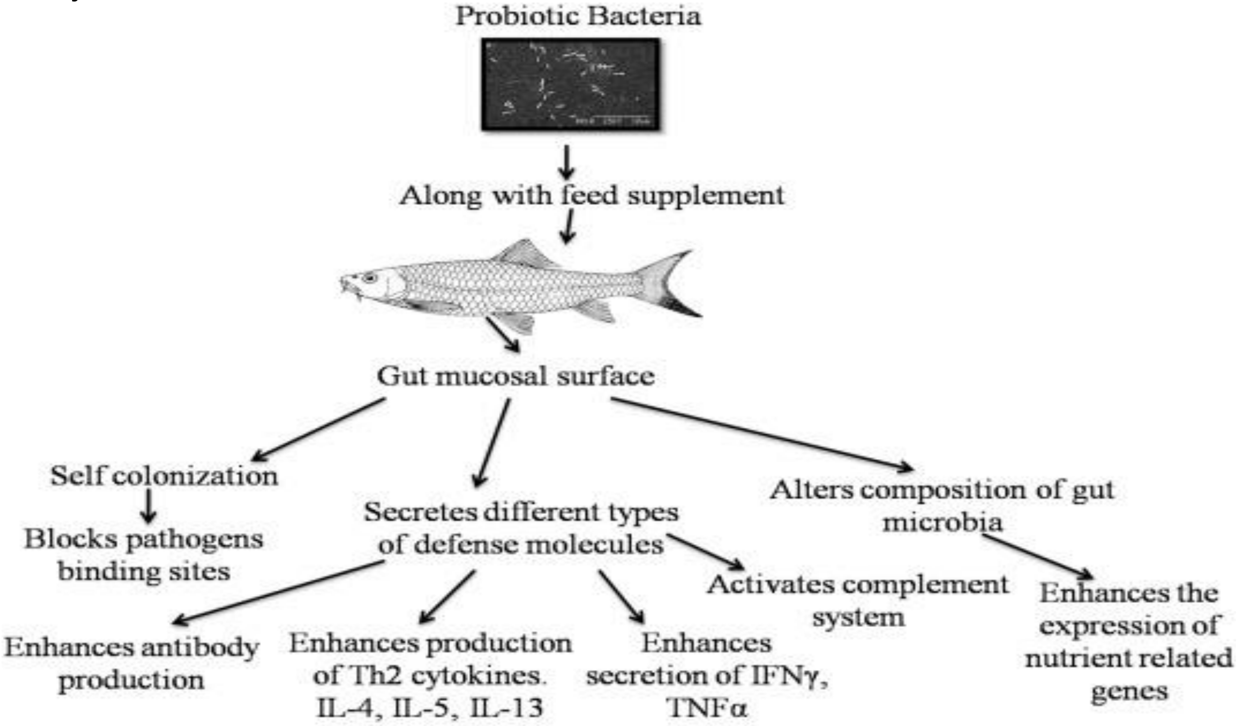

Figure 3. Mechanism of probiotic in immune modulation in fish after colonization

(Source: Banerjee and Ray, 2017)

Improvement of Water Quality

In several studies, water quality was recorded during probiotic strains, especially of the gram-positive *Bacillus*. Probably since this bacterial group is more efficient than gram-negative in transforming organic matter to CO_2, by maintaining high levels of probiotics in production ponds, fish farmers can minimize the accumulation of dissolved and particulate organic carbon during culture. Besides, this can balance the production of phytoplankton. However, this hypothesis could not be confirmed in the cultivation of shrimps or channel catfish, using one or more Bacillus species, *Nitrobacter*, *Pseudomonas*, *Enterobacter*, *Cellulomonas*, and *Rhodopseudomonas*. Thus published evidence for improving water quality is limited, except for nitrification.

Effect on Reproduction of Aquatic Species

Breeding aquaculture species require high nutritional requirements, as reproductive capacity depends on appropriate concentrations of lipids, proteins, fatty acids, vitamins C and E, and carotenoids. Inter-relationship of these components influences reproduction in various processes such as fertility, fertilization, birth, and larval development. At present, for most cultured fish species, there are commercially available broodstock diets that are just larger-sized diets. In practice, many fish hatcheries improve the nutrition of their broodstock by feeding them solely on fresh fish byproducts or in combination with commercial diets. The most common new organisms used to feed broodstock fishes include squid, cuttlefish, mussels, krill, and small crustaceans. The use of these unprocessed fish products often does not provide adequate levels of nutrients needed by broodstock fishes, increasing the risk of pathogens transmission to the parents and offspring, including parasites, bacteria, and viruses. Therefore, probiotics added to food or water were used to prevent infections and explore their effect on reproduction.

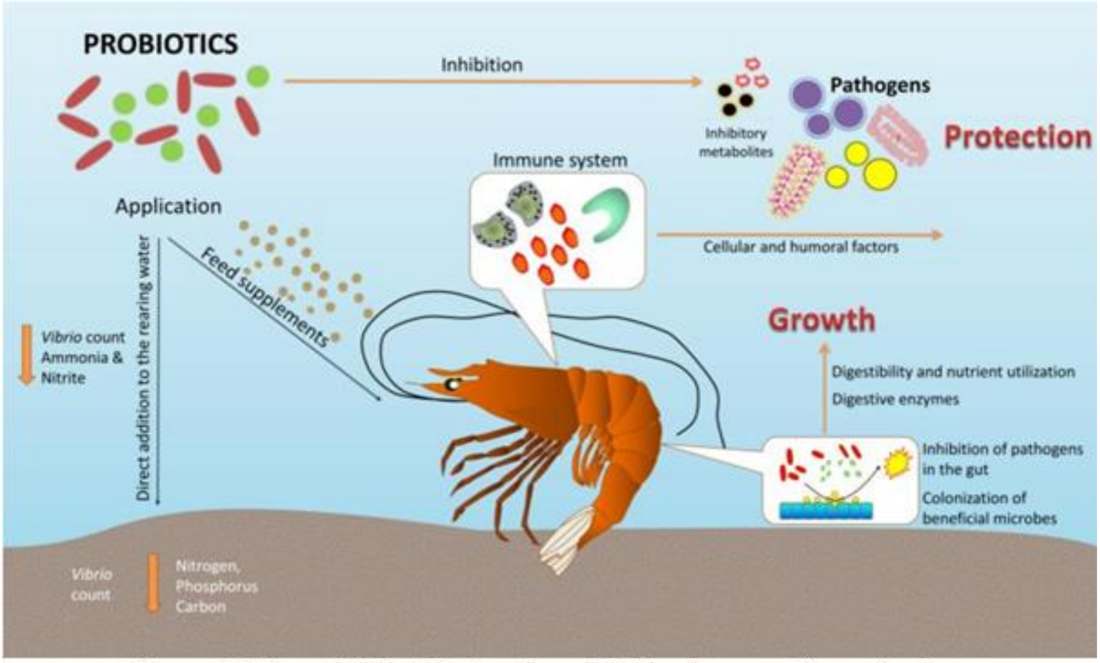

Figure 4. Beneficial effects of probiotics in aquatic animals growth and Health
(Source: Lazado et al., 2015)

Table 2. Probiotics considered as biological control agents in aquaculture of finfishes, crustaceans and mollusc:

Probiotic strain	Source	Used on	Method of application
Finfish			
Lactobacillus sp. and Carnobacterium sp.	Rotifers (Brachionus plicatilis)	Turbot larvae	Enrichment of rotifers
Carnobacterium divergens	Intestines of Atlantic salmon	Atlantic cod fry	Addition to diet
Bacillus megaterium, B. subtilis, B. polymyxa, B. licheniformis	Commercial product (Biostart)	Channel catfish	Addition to pond water
Vibrio pelagius	Turbot larvae	Turbot	Addition to culture water
G-probiotic	Commercial product	*Oreochromis niloticus*	Addition to diet
Pseudomonas fluorescens	Iced freshwater fish (Lates niloticus)	Rainbow trout *(Oncorhynchus mykiss)*	Addition to culture water
Carnobacterium sp.	Intestines of Atlantic salmon	Atlantic salmon	Addition to diet
Lactobacillus rhamnosus ATCC 53103	Culture collection	Rainbow trout	Addition to diet
Aeromonas hydrophila, Vibrio fluvialis, Carnobacterium sp., Micrococcus luteus	Digestive tract of rainbow trout	Rainbow trout	Addition to diet
Enterococcus faecium SF68	Commercial product (Cernivet)	*Anguilla anguilla*	Addition to diet

L. rhamnosus JCM 1136	Culture collection	Rainbow trout	Addition to diet
Roseobacter sp. strain 27-4	Turbot larvae, Tetraselmis copepod-fed larvae	Turbot larvae	Addition to culture water
Bacillus circulans	Intestines of *Labeo rohita*	*L. rohita*	Addition to diet
Shellfishes			
Crustaceans			
Bacillus sp. S11	*Penaeus monodon*	*P. monodon*	Addition to diet
Bacillus sp.	Commercial product (DMS)	*P. monodon*	Addition to culture water
Lactobacillus spp.	Digestive tract of chicken	*P. monodon*	Addition to diet
Saccharomyces cerevisiae, S. exiguus, Phaffia rhodozyma	Commercial product	*Penaeus vannamei*	Addition to diet
Vibrio hepatarius, Vibrio sp., Bacillus sp.	*P. vannamei*	*P. vannamei*	Addition to diet
Vibrio P62, Vibrio P63, Bacillus P64	*P. vannamei*	*P. vannamei*	Addition to culture water
Pseudomonas sp., Vibrio fluvialis	*P. monodon*	*P. monodon*	Addition to culture water
Molluscs			
Aeromonas media strain A199	-	*Crassostrea gigas*	Addition to culture water
Roseobacter sp. BS107	Scallop larval cultures	*Pecten maximus*	Addition to culture water
Alteromona haloplanktis	Microalgal cultures	*Argopecten purpuratus*	Addition to culture water

(**Source:** Balcázar et al., 2006)

Safety Considerations of Probiotic

Traditionally, probiotics used in the food industry have been deemed safe; in fact, no human risks have been determined, which is the best proof of safety. Theoretically, probiotics may be responsible for four types of side effects in susceptible individuals: systemic infections, harmful metabolic activities, excessive immune stimulation, and gene transfer. However, no hard evidence has been found. In practice, there are few reports of bacteremia in humans, where isolation of probiotic bacteria from infections seems to be the result of an opportunistic infection caused by skin lesions, cancer, chronic illness, or a drug-induced abnormality. These conditions lead to a decreased intestinal barrier that promotes the passage of the bacteria through the mucosal epithelium. Subsequently, these microorganisms are transported to the mesenteric lymph nodes and other organs, leading to bacteremia that may progress to septicemia. All reported cases of bacteremia occurred in patients with chronic illness or a weakened immune system. Since there was no international consensus to ensure the efficiency and safety of probiotics, FAO and WHO recognized the need to create guidelines for a systematic approach, which is essential for the evaluation of probiotics in food to substantiate their health claims. A working group with experts in the field was formed to recommend criteria and methodology for evaluating probiotics based on scientific evidence. As a result, a ‘Guide for the evaluation of probiotics in food’, was presented for providing guidelines on assessing the health and nutrition properties of probiotics. The working group stated that no pathogenic or virulent properties were found in *lactobacilli*, *bifidobacteria*, or *lactococci*. However, they acknowledged that some strains of lactobacilli have been associated with rare cases of bacteremia under certain conditions. However, its incidence does not increase with raising the use of lactobacillus in probiotics. It was also mentioned that enterococci may possess virulence characteristics; therefore, it is not recommended as a probiotic for human consumption. Although the guide is not focused on aquaculture products, it creates a precedent for conducting studies to evaluate the safety of probiotics in this area.

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