Probiotics: An Essential Tool in Intensive Aquaculture

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Abstract—*The growth of aquaculture as an industry has accelerated over the years; this has resulted in food production and positive economic impact. But the emergence of disease outbreak has been the constraint. The need for increased disease resistance, growth of aquatic organisms and feed efficiency has brought about the use of probiotics as nonantibiotic agent in aquaculture productions. There are documented evidences that probiotics can improve the water quality, growth promoters, disease resistance and enhancement of immune response. The increase of productivity in aquaculture has been accompanied by ecological impacts including emergence of a large variety of pathogens and bacterial resistance. These impacts are in part due to the indiscriminate use of chemotherapeutic agents as a result of management practices in production cycles. The field of probiotics as well as the selection steps to acquire probiotic strain for the management of disease in aquaculture is discussed. Currently, there are commercial probiotic products prepared from various bacterial species such as Bacillus sp., Lactobacillus sp., Enterococcus sp., Carnobacterium sp., and the yeast Saccharomyces cerevisiae among others, and their use is regulated by careful management recommendations. The present review shows the current knowledge of the use of probiotics in aquaculture, its antecedents, and safety measures to be carried out and discusses the prospects for study in this field.*

Keywords*: Probiotics, Aquaculture, antibiotic, chemotherapeuticagents.*

1. INTRODUCTION

Aquaculture is the farming of aquatic organisms by intervention in the rearing process to enhance production and private ownership of the stock being cultivated. Compared to fishing, this activity allows a selective increase in the production of species used for human consumption, industry or sport fishing. Due to overfishing of wild populations, aquaculture has become an economic activity of great importance around the world. Aquaculture's contribution to world food production, raw materials for industrial and pharmaceutical use, and aquatic organisms for stocking or ornamental trade has increased dramatically in recent decades. In 2014, overall fish production was 167.3 million tonnes (value of US$ 160.4 billion) grown by 5.9 percent from 158.0 million tonnes in 2012. Global aquaculture production was boosted by 10.9 percent to 73.8 million tonnes in 2014 as compared to 66.6 million tonnes in 2012 while compensated for a 2.4 percent expansion in wild fish output to 93.5 million tonnes in 2014 which was 91.3 million tonnes in 2012. The world production of farmed fishes other than finfishes (crustaceans, molluscs and other aquatic animals) was 23.4 million tonnes grown by 6.1 percent from 22.1 million tonnes in 2012. The contribution of aquaculture to the world total fish production reached 44.1 percent in 2014, up from 42.1 percent in 2012. Aquaculture now accounts for almost half of total fish supply for human consumption [1].

Additionally, aquaculture has appropriated of water bodies used for recreational purpose, and sometimes makes a water's waste because this natural resource is not reused in extensive aquaculture systems [2, 3]. Moreover, under these conditions of intensive production, aquatic species are subjected to high-stress conditions, increasing the incidence of diseases and causing a decrease in productivity [4]. Outbreaks of viral, bacterial, and fungal infections have caused devastating economic losses worldwide, that is, China reported disease-associated losses of $750 million in 1993, while India reported $210 million losses from 1995 to 1996. Added to this, significant stock mortality has been reported due to poor environmental conditions on farms, unbalanced nutrition, generation of toxins, and genetic factors [5].

Probiotic is a relatively new term which is used to name microorganisms that are associated with the beneficial effects for the host. Kozasa made the first empirical application of probiotics in aquaculture [6], considering the benefits exerted by the use of probiotics on humans and poultry. He used spores of *Bacillus toyoi* as feed additive to increase the growth rate of yellow tail, *Seriola quinqueradiata*. In 1991, Porubcan [7] documented the use of *Bacillus* spp, to test its ability to increase productivity of *Penaeus monodon* farming and to improve water quality by decreasing the concentrations of ammonia and nitrite. In order to avoid or reduce the use of certain antimicrobials, biological control was tested, described as the use of natural enemies to reduce the damage caused by harmful organisms. Strictly speaking, a probiotic should not be classified as a biological control agent because it is not necessarily a natural enemy of the pathogen [8]. However, certain probiotics have the ability to inhibit the growth of pathogenic bacteria. Moriarty determined the ability of *Bacillus* spp. to decrease the proportion of *Vibrio* spp [9].

2. DEFINITION OF PROBIOTIC

According to Browdy [10], one of the most significant technologies that evolved in response to disease control problems is the use of probiotics. The term probiotic means life; it was derived from two Greek words ‘pro’ and ‘bios’ [11]. Probiotics are live microbes that can be used to improve the host intestinal microbial balance and growth performance. Development of probiotics in aquaculture management will reduce the use of antimicrobial drugs which were prophylactive alone and whose over dependence in recent times poses potential hazards to man who consume them [12]. Gatesoupe in 1999, defined them as “microbial cells administered in a certain way, which reaches the gastrointestinal tract and remain alive with the aim of improving health” [13]. In the same year, studies were carried out on the inhibition of pathogens using probiotics, this expanded the definition to “live microbial supplement which benefits the host by improving its microbial balance” [14].

3. SELECTION OF PROBIOTICS

The initial major purpose of using probiotics is to maintain or reestablish a favourable relationship between friendly and pathogenic microorganism that constitute the flora of intestinal or skin mucus of fish. A successful probiotic is expected to have a few specific properties in order to certify, a beneficial effect. And in order to produce probiotics for commercialization, the following steps are to be put into consideration as in Figure 1: A healthy source of microorganisms from a digestive tract of healthy aquatic animals must be selected. The microorganisms with which the work is to be carried out are isolated and identified by means of selective culture. A new culture with only the colonies of interest for conducting in vitro evaluations such as inhibition of pathogens; pathogenicity to target species; resistance conditions of host; among others are performed. In case of the absence of restrictions on the use of the target species, experiments with in vivo supplementation, and small and large scale, are carried out to check if there are real benefits to the host. Finally, the probiotic that presented significantly satisfactory result can be produced commercially and utilized [15].

4. CHARACTERISTICS OF GOOD PROBIOTICS

Fuller [16] listed the following as features of good probiotic bacteria:

1. It should be a strain, which is capable of exerting a beneficial effect on the host animal e.g. increased growth or resistance to disease.
2. It should be non-pathogenic and non-toxic.
3. It should be present as viable cells preferable in large numbers.
4. It should be capable of surviving and metabolizing in the gut environment e.g. resistance to low pH and organic acid.
5. It should be stable and capable of remaining viable for periods under storage and field conditions.

5. MECHANISMS OF ACTION

The mechanisms of action of bacteria used as probiotics, although not yet fully elucidated, are described as [17, 18]:

1. Competition for binding sites: also known as "competitive exclusion", where probiotics bacteria bind with the binding sites in the intestinal mucosa, forming a physical barrier, preventing the connection by pathogenic bacteria.
2. Production of antibacterial substances: probiotic bacteria synthesize compounds like hydrogen peroxide and bacteriocins, which have antibacterial action, mainly in relation to pathogenic bacteria. They also produce organic acids that lower the environmen's pH of the gastrointestinal tract, preventing the growth of various pathogens and development of certain species of *Lactobacillus*.
3. Competition for nutrients: the lack of nutrients available that may be used by pathogenic bacteria is a limiting factor for their maintenance.
4. Stimulation of immune system: some probiotics bacteria are directly linked to the stimulation of the immune response, by increasing the production of antibodies, activation of macrophages, T-cell proliferation and production of interferon.

Table 1: Microorganisms recognized as safe and used as probiotics in animals. Source: [19]

Aspergillus	*A. niger, A. orizae*
Bacillus	*B. coagulans, B. lentus, B. licheniformis, B. subtilis*
Bifidobacterium	*B. animalis, B. bifidum, B. longun, B. thermophylum*
Lactobacillus	*L. acidophilus, L. brevi, L. bulgaricus, L. casei, L. cellobiosis, L. fermentarum, L. curvatus, L. lactis, L. plantarum, L. reuterii, L. delbruekii,*

Pediococcus	*P. acidilacticii, P. cerevisae, P. pentosaceus, P. damnosus*
Saccharomyces	*S. cerevisiae, S. boulardii*
Streptococcus	*S. cremoris, S. faecium, S. lactis, S. intermedius, S. thermophyllus, S. diacetylatis*

6. CONSTRAINTS TO PROBIOTICS IN AQUACULTURE

1. Inability of strains to be produced in commercial quantities and consequent demonstration on a large scale.
2. Difficulty in proving performance at the farm level.
3. Inability of companies to conduct extensive research on how to make product specifically for aquaculture purposes.

7. PROBIOTICS SIGNIFICANCE IN AQUACULTURE

There are some possible benefits linked to the administering of probiotics which have already been suggested as:

1. Improvement in water qualities

Nitrogenous compounds contamination such as ammonia, nitrite and nitrate in fish culture systems/ponds has been a serious concern. The susceptibility of cultured aquatic species to high concentration of these compounds is generally species-specific, but in high concentrations, these compounds may be extremely harmful and cause mass mortality in all cases. Ma et al. [20] reported the ability of *Lactobacillus spp.* JK-8 and JK-11 simultaneously removes nitrogen and pathogens from contaminated shrimp farms. In several other studies, water quality has been improved by the addition of probiotics especially Bacillus spp. [21]. The reason is that gram – positive Bacillus spp. according to Stanier et al. [22] are generally more efficient in converting organic matter back to CO2 than gram – negative bacteria, which would convert a greater percentage of organic carbon to bacterial biomass or slime.

2. As growth promoters

It has been demonstrated experimentally that probiotics indeed may enhance the growth of fish. The ability of organisms to out-grow the pathogens in favour of host or to improve the growth of the host and yet no side effect on the host made it a probiotic bacteria.Yassir et al. [23] in attempt to use probiotic bacteria as growth promoter on tilapia (*Oreochromis niloticus*) identified that the highest growth performance was recorded with *Micrococcus luteus* a probiotic and the best feed conversion ratio was observed with the same organism. So *M. luteus* may be considered as a growth promoters in fish aquaculture. Lactic acid bacteria also had an effect as growth promoters on the growth rate in juvenile carp though not in Sea bass [19].

3. For disease prevention

Probiotics or their products for health benefits to the host have been found useful in aquaculture, terrestrial animals and in human disease control. These include microbial adjunct that prevent pathogens from proliferating in the intestinal tract, on the superficial surfaces and in culture environment of the culture species [24]. The effect of these beneficial organisms is achieved through optimizing the immune system of culture organism, increasing their resistance to disease, or producing inhibitory-substance that prevent the pathogenic organisms from establishing disease in the host.

4. Source of nutrients and enzymatic contribution to digestion

Some researchers have suggested that microorganisms have a beneficial effect in the digestive processes of aquatic animals. In fish, it has been reported that *Bacteroides* and *Clostridium sp*. have contributed to the host's nutrition, especially by supplying fatty acids and vitamins [25]. Some microorganisms such as *Agrobacterium sp., Pseudomonas sp., Brevi-bacterium sp., Microbacterium sp., and Staphylococcus sp*. may contribute to nutritional processes in *Salvelinus alpinus L* [26].

5. Enhancement of the immune response

Among the numerous beneficial effects of probiotics, modulation of immune system is one of the most commonly purported benefits of probiotics. Fish larvae shrimps and other invertebrates have immune systems that are less well developed than adult stage and are dependent primarily on non-specific immune responses for their resistance to infection [24] evaluated the ability of *Lactobacillus fermentum* LbFF4 isolated from Nigerian fermented food ('fufu') and *L. plantarum* LbOGI from a beverage 'Ogi' to induce immunity in *Clarias gariepinus* (Burchell) against some selected fish bacterial pathogens.

Table 2: Probiotics considered as biological control agents in aquaculture of fishes. Source: [18]

Probiotic strain	Source	Used on	Method of application
Streptococcus lactis and Lactobacillus bulgaricus	-	Turbot larvae (Scophthalmus maximus)	Enrichment of live food
Lactobacillus sp. and Carnobacterium sp.	Rotifers (Brachionus plicatilis)	Turbot larvae	nrichment of rotifers
Vibrio alginolyticus	Commercial shrimp hatchery	Atlantic salmon (Salmo salar L.)	Bathing in bacterial suspension
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

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.,	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

Table 3 Probiotics considered as biological control agents in aquaculture of crustaceans, molluscs, and live food. Source: [18]

Probiotic strain	**Source**	**Used on**	**Method of application**
Crustaceans			
Bacillus sp. S11	Penaeus monodon	P. monodon	Addition to diet
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 A	Microalgal cultures	rgopecten purpuratus	Addition to culture water
Live food			
Flavobacterium sp.	Chaetoceros gracilis culture	C. gracilis, I. galvana, P. lutheri	Addition to culture water
Lactococcus lactis AR21	Rotifer culture	Rotifers	Addition to culture water
V. alginolyticus C7b	Seawater	Chaetoceros muelleri	Addition to culture water
Pediococcus acidilactici	Commercial product	Artemia	Addition to culture water
Lactobacillus casei, L. brevis, L. helveticus, Lactococcus lactic spp.	Culture collection	Artemia nauplii	Addition to culture water

8. CONCLUSION

Increased use of antibiotics has led to the high proportion of antibiotic-resistant bacteria which provide threat to fish and man through consumption of the infected fish. Inefficiencies in antibiotic treatment of fish illnesses lead to significant economic losses. But the use of probiotics in aquaculture has shown to have beneficial impact on fish health and thereby economic performance of fish farming. At the same time, the use of probiotics has also important environmental benefits. By reducing the risk of diseases, the necessity of medication and thereby the risk of residues left in the environment is reduced. Therefore the use of probiotics in fish feed should also be seen as an important step in aquaculture sustainability.

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