Chapter 6

Use of Probiotics in Aquaculture

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1. Introduction

The term probiotics was first used by Lilly & Stillwell in 1965. Probiotic was defined as the microbiological origin factor that stimulates the growth of other organisms. In 1989 Roy Fuller introduced the idea that probiotics generate a beneficial effect to the host. He defined probiotics as live microorganisms which, when administered in adequate amounts, confer benefit to the host's health, improving the balance of the microbiota in the intestine.

Probiotics are defined by Food and Agriculture Organization/World Health Organization as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [1].

The purpose of its use is to install, improve or compensate for the functions of the indigenous microbiota that inhabit the digestive tract or the surface of the body.

The idea of using fermented foods for some health benefits is not new, being mentioned in the Persian version of the Old Testament (Genesis 18:8) that "Abraham attributed his longevity to the consumption of sour milk". Later, in 76 BC, a Roman historian, Pline, recommended the use of fermented milk products for the treatment of gastroenteritis cases [2].

However, a scientific approach, recognizing the beneficial role of certain microorganisms was applied only in the first decades of the 20th century, with the suggestion of using *Lactobacillus* (in 1907 Elie Metchnikoff attributed the longevity of Bulgarian populations to yoghurt consumption); *Bifidobacterium* (in 1906 Henri Tissier observed a greater presence of *Bifidobacteria* in the feces of breastfed healthy children); and *Saccharomyces boulardii* (Henri Boulard emphasized the use of a tropical fruit colonized by this yeast to treat diarrhea of local populations in the East during an episode of cholera in 1920) [3].

Several clinical studies have shown the benefits of probiotics to human health. For example, diarrhea treatment [4]; lactose intolerance [5]; irritable bowel syndrome [6]; allergies [7]; cancer [8]; among others.

The use of growth promoters allows improving the zootechnical performance of animals. Initially a large variety of substances with antibiotic function was used to improve performance of poultry, pigs and cattle, especially penicillin and tetracycline.

The use of antibiotics as additives to feeds showed great benefits to animal husbandry, expressed primarily in improved weight gain and feed conversion.

Antibiotics were used for decades, but are being banished from the zootechnical activity, mainly due to the risks posed by antibiotic-resistant bacteria, which can result in problems for animal and human health.

Accordingly, probiotics have deserved attention from researchers seeking alternatives to the use of traditional growth promoters in the field of animal nutrition.

Probiotics have also received special attention from researchers seeking animal nutrition alternatives to the use of traditional growth promoters (antibiotics). Therefore, the use of probiotics is being increasingly seen as an alternative to the use of antibiotics in animal production.

Many scientific papers show the beneficial effects of supplementation with probiotic strains in diets for poultry, pigs, cattle, fish, crustaceans, mollusks and amphibians [9-13].

Probiotics have been incorporated through diet in order to maintain the balance of the intestinal flora of animals, preventing digestive tract diseases, improving the digestibility of feed, leading to increased use of nutrients and causing better zootechnical performance of animals [14, 15].

2. Probiotic organisms

The requirements that a probiotic organism must meet are [16]:

i. Resistance to the acid stomach environment, bile and pancreatic enzymes;
ii. Accession to the cells of the intestinal mucosa;
iii. Capacity for colonization;
iv. Staying alive for a long period of time, during the transport, storage, so that they can colonize the host efficiently;
v. Production of antimicrobial substances against the pathogenic bacteria; and
vi. Absence of translocation.

The species normally used as probiotics in animal nutrition are usually non-pathogenic normal microflora, such as lactic-acid bacteria (*Bifidobacterium, Lactobacillus, Lactococcus, Streptococcus* and *Enterococcus*) and yeasts as *Saccharomyces* spp. (Table 1).

3. Mechanisms of action

The mechanisms of action of bacteria used as probiotics, although not yet fully elucidated, are described as [14, 15, 18]:

a. 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;
b. 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 environment's pH of the gastrointestinal tract, preventing the growth of various pathogens and development of certain species of *Lactobacillus*;
c. Competition for nutrients: the lack of nutrients available that may be used by pathogenic bacteria is a limiting factor for their maintenance;
d. 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.

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. acidophillus, L. brevis, 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*

Table 1. Microorganisms recognized as safe and used as probiotics in animals. Source: [17]

The mechanism of action of yeasts still needs substantiation by means of research. A likely mechanism of action of yeasts is related to total inhibition (*in vitro*) or partial inhibition of pathogens. Inactive yeasts contain large quantities of protein and polysaccharides in its walls, which can act positively in the immune system and in the absorption of nutrients. In addition, yeasts produce nutritious metabolites in digestive tract that boost animal performance, besides possessing minerals (Mn, Co, Zn) and vitamins (A, B_{12}, D_3) that enhance the action of beneficial microorganisms [19].

Although some mechanisms had been suggested on the action of probiotics, they are not completely clarified, but it is known that they inhibit growth of pathogenic microorganism by producing antimicrobial compounds; they compete with pathogens for adhesion sites and nutrients; and they model immune system of the host [20].

4. Selection of probiotics

Briefly, for the use of a given microorganism as probiotic, it is necessary its isolation, characterization and testing certifying its probiotic efficiency (Figure 1).

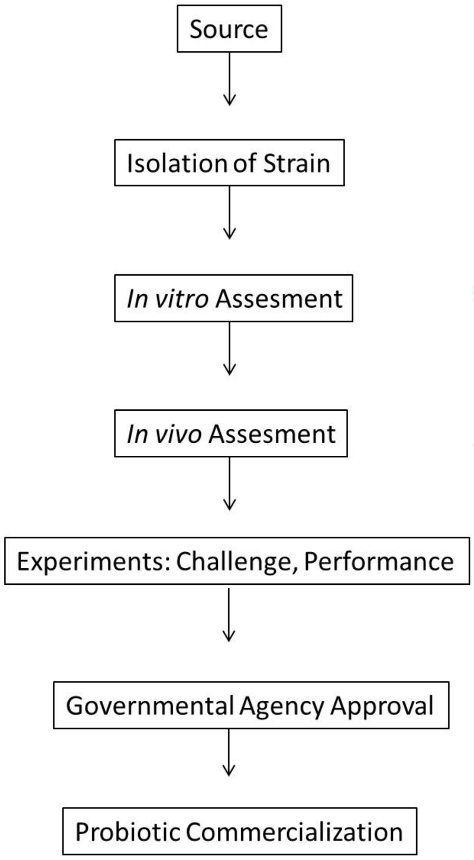

Figure 1. Diagram for selection of probiotics

First a source of microorganisms (e.g. digestive tract of healthy animals) must be selected.

After, the microorganisms with which the work is to be carried out are isolated and identified by means of selective culture.

Then a new culture with only the colonies of interest for conducting *in vitro* evaluations (inhibition of pathogens; pathogenicity to target species; resistance conditions of host; among others) is 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.

5. Use of probiotic in aquaculture

Probiotics in aquaculture may act in a manner similar to that observed for terrestrial animals.

However, the relationship of aquatic organisms with the farming environment is much more complex than the one involving terrestrial animals.

Because of this intimate relationship between animal and farming environment, the traditional definition of probiotics is insufficient for aquaculture.

In this sense, Verschuere and colleagues [21] suggest a broader definition:

"It is a microbial supplement with living microorganism with beneficial effects to the host, by modifying its microbial community associated with the host or its farming environment, ensuring better use of artificial food and its nutritional value by improving the host's response to diseases and improving the quality of the farming environment."

The microorganisms present in the aquatic environment are in direct contact with the animals, with the gills and with the food supplied, having easy access to the digestive tract of the animal.

Among the microorganisms present in the aquatic environment are potentially pathogenic microorganisms, which are opportunists, i.e., they take advantage of some animal's stress situation (high density, poor nutrition) to cause infections, worsening in zootechnical performance and even death.

For this reason, the use of probiotics for aquatic organisms aims not only the direct benefit to the animal, but also their effect on the farming environment.

Bergh and colleagues [22] observed that, when starting its first feeding, the intestinal flora of the Atlantic halibut (*Hippoglossus hippoglossus*) changed from a prevalence of *Flavobacterium* spp. to *Aeromonas* spp./*Vibrio* spp. showing the influence of the external environment and food on the microbial community of this fish.

Vibrio spp., *Plesiomonas shigelloides*, and *Aeromonas* spp. are the main causative agents of diseases in aquaculture, and may even cause food infections in humans.

The interaction between the environment and the host in an aquatic environment is complex. The microorganisms present in the water influence the microbiota of the host's intestine and vice versa.

Makridis and colleagues [23] demonstrated that the provision of two strains of bacteria via food directly into the farming water of the incubators of turbot larvae (*Scophthalmus maximus*) promoted the maintenance of the bacteria in the environment, as well as the colonization of the digestive tract of the larvae.

Changes in salinity, temperature and dissolved oxygen variations, change the conditions that are favorable to different organisms, with consequent changes in dominant species, which could lead to the loss of effectiveness of the product.

Accordingly, the addition of a given probiotic in the farming water of aquatic organisms must be constant, because the conditions of environment suffer periodic changes.

Thus, the variety of microorganisms present must therefore be considered in the choice of probiotic to be used in aquaculture.

Intensive farming systems utilize high stocking densities, among other stressors (e.g. management), which often end up resulting in low growth and feed efficiency rates, besides of weakness in the immune system, making these animals susceptible to the presence of opportunistic pathogens present in the environment.

In this sense, the effect of probiotics on the immune system has led to a large number of researches with beneficial results on the health of aquatic organisms, although it has not yet been clarified how they act.

In addition, probiotics can also be used to promote the growth of aquatic organisms, whether by direct aid in the absorption of nutrients, or by their supply.

Probiotics most used in aquaculture are those belonging to the genus *Bacillus* spp. (*B. subtilis*, *B. licheniformis* and *B. circulans*), *Bifidobacterium* spp. (*B. bifidum*, *B. lactis*, *and B. thermophilum*), lactic-acid bacteria (*Lactobacillus* spp. e *Carnobacterium* spp.) and yeast *Saccharomyces cerevisiae* [24,25].

The benefits observed in the supplementation of probiotics in aquaculture include [21, 26-28]:

1. Improvement of the nutritional value of food;
2. Enzymatic contribution to digestion;
3. Inhibition of pathogens;
4. Growth promoting factors;
5. Improvement in immune response; and
6. Farming water quality.

Among the most recent studies that point to the effect of the use of probiotics for various aquatic organisms stand those for fish [21], shrimps [26], mollusks [30] and frogs [29].

5.1. Results of probiotics in fish farming

5.1.1. Immune system

Gatesoupe [31] observed that turbot larvae (*Scophthalmus maximus*) fed rotifera enriched with lactic-acid bacteria increased resistance against infection by *Vibrio* spp.

The joint administration of *Lactobacillus fructivorans* and *Lactobacillus plantarum* through dry or live feed promoted the colonization of the intestine of sea bream larvae (*Sparus aurata*) and the decrease in mortality of animals during larviculture and nursery [32].

Gram and colleagues [33] showed that the use of *Pseudomonas fluorescens* AH2 as probiotics decreased the mortality of juveniles of rainbow trout (*Oncorhynchus mykiss*) exposed to *Vibrio anguillarum*.

Kumar and colleagues [34] observed higher survival rate of carp *Labeo rohita* fed *Bacillus subtilis*, submitted to intraperitoneal injection with *Aeromonas hydrophila*.

Oral administration of *Clostridium butyricum* increased phagocytic activity of leucocytes of rainbow trout [35].

Nikoskelainen and colleagues [36] observed that the administration of *Lactobacillus rhamnosus* at 105 UFC g^{-1}, stimulated the respiratory burst in rainbow trout.

Other studies showed an increase in immune response with the use of probiotics for different species, such *Carnobacterium maltaromaticum* B26 and *Carnobacterium divergens* B33 for rainbow trout [38], *Lactobacillus belbrüeckii*, *Bacillus subtilis* and *Debaryomyces hansenii* for gilthead seabream [39-41], *B. subtilis* and *Pseudomonas aeruginosa* for *Labeo rohita* [42,43], *Lactococcus lactis* for Nile tilapia (*Oreochromis niloticus*) [44] and *B. simplex* DR-834 to carp (*Cyprinus carpio*) [45].

5.1.2. Performance

Tovar and colleagues [37] incorporated the yeast *Debaryomyces hansenii* to the feed of sea bass larvae and observed improvement in the maturation of the digestive tract of this species. According to the authors this satisfactory effect was due to the high secretion rate of spermine and spermidine by yeasts.

Increase of weight gain and survival was observed for turbot larvae fed rotifera enriched with acid-lactic bacteria [31].

Queiroz and Boyd [46] observed enhancement of the zootechnical performance and survival of channel catfish (*Ictalurus punctatus*) when a mixture of *Bacillus* spp. was added to the farming water.

Using yeast *Saccharomyces cerevisiae* as probiotic for Israeli carp, Noh and colleagues [47] observed an increase in the food efficiency of this species.

Lara-Flores and colleagues [48] concluded that the use of *Saccharomyces cerevisiae* as probiotic for fry of Nile tilapia resulted in better growth and food efficiency, suggesting that this yeast promotes adequate growth in tilapia farming. In this study it was observed that fish fed control diet showed reduced survival and digestibility of feed with increased storage density, considered a stressful factor for growing fish. This result highlighted the efficiency of the use of this probiotic in stressful situations.

Other positive results of the probiotic on the performance of fish are found for *Labeo rohita* fingerlings [49], Nile tilapia [50] and common carp [51].

5.2. Results of the use of probiotics in shrimp farming

5.2.1. Immune system

In relation to farmed shrimp, bacterial diseases are considered as the largest cause of mortality in larvae.

The administration of a mixture of bacteria (*Bacillus* spp. and *Vibrio* spp.) positively influenced on survival and had protective effect against *Vibrio harveyi* and the white spot syndrome virus (WSSV) [15]. This result was due to stimulation of the immune system, by increasing phagocytosis and antibacterial activity.

The administration of a commercial probiotic for the larvae of *Marsupenaeus japonicus* resulted in increased survival (97%) being significantly higher than the control treatment [52].

Thus, the use of *Bacillus coagulans* SC8168 as probiotic for postlarvae of *Litopenaeus vannamei* resulted in higher survival of animals [53].

In a study with tiger shrimp (*Penaeus monodon*), the inoculation of *Bacillus* S11, a saprophyte strain, resulted in higher survival of postlarvae challenged by a luminescent pathogenic bacterial culture [54].

Bacillus subtilis and *Lactobacillus plantarum* for *Litopenaeus vannamei* [55-58], *Pediococcus acidilactici* to *Litopenaeus stylirostris* [59] and *Bacillus* NL110 and *Vibrio* NE17 for *Macrobrachium rosenberguii* [60] also proved effective in improving the immune system of these animals.

5.2.2. Performance

Lin and colleagues [61] used *Bacillus* spp. in the diet of *Litopenaeus vannamei* enhancing digestibility rates of the feed.

Ziaei-Nejad and colleagues [26] added the probiotic *Bacillus* spp. in the farming of *Fenneropenaeus indicus* larvae and observed survival increase, and also an increase in the activities of lipase, protease and amylase enzymes in the digestive tract of shrimps.

Several studies have shown that the bacteria of the genus *Bacillus* spp. secrete exoenzymes (proteases, lipases and carbohydrases) that can help improve digestion and nutrient absorption increase, resulting in better use of food and animal growth [62].

5.3. Results from the use of probiotics in the farming of others aquatic organisms

5.3.1. Mollusks

The culture of oysters and scallops has been introduced in many countries, however, mass mortalities of larvae have frequently occurred and to prevent these mortalities, most farmers use antibiotics [63]. Thus, the use of probiotic bacteria has been fueled, especially during the hatchery [64].

Riquelme and colleagues [65] identified a bacteria (*Alteromonas haloplanktis*) capable of reducing the mortality of Chilean scallop larvae (*Argopecten purpuratus*) when exposed to 10^3 colony forming units per milliliter (UFC ml^{-1}) of *Vibrio anguillarum*.

Cultures of *Alteromonas media* control *Vibrio tubiashii* infections in larvae of Pacific oysters (*Crassostrea gigas*) [66].

Other bacteria with probiotic potential for mollusks such as Pacific oysters (*Alteromonas* spp.) [67, 68], Scallop larvae (*Roseobacter* spp., *Vibrio* spp., *Pseudomonas* spp., *Arthrobacter* spp.) [69-71], promoted growth, survival and immune response of animals.

5.3.2. Frogs

For Bull Frog (*Lithobates catesbeianus*) with an average weight of 3.13 g, the addition of probiotic *Bacillus subtilis* in different doses (2.5, 5.0 and 10 g kg^{-1} feed) resulted in improved weight gain, feed conversion and apparent survival, when compared to control treatment (without added probiotic); however, the immunostimulant effect was demonstrated through the increased phagocytic capacity of animals [72].

Likewise, Dias and colleagues [29] observed the beneficial effect of two commercial probiotics on the immune system of *L. catesbeianus*.

5.4. Probiotics and quality of water in aquaculture

Another aspect of the use of probiotics in aquaculture is the improvement of the quality of the water in the farming nurseries. Increases in organic load, levels of phosphorous and nitrogen compounds are growing concerns in aquaculture.

Boyd [73] noted the beneficial effect of probiotics on organic matter decomposition and reduction of the levels of phosphate and nitrogen compounds.

Aerobic denitrifying bacteria are considered good candidates to reduce nitrate or nitrite to N_2 in aquaculture waters.

To this end some bacteria were isolated in shrimp farming tanks. *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Cellulosimicrobium*, *Halomonas*, *Microbacterium*, *Paracoccus*, *Pseudomonas*, *Sphingobacterium* and *Stenotrophomas* are some of the denitrifying bacteria already identified [28].

Reduction in levels of phosphorous and nitrogen compounds in the farming water of shrimp *Litopenaeus vannamei* was also observed when commercial probiotics were added to the water [27].

Similarly, for the shrimp *Penaeus monodon*, an improvement in the quality of farming water was observed with the addition of *Bacillus* spp. as probiotic [74].

Gram-positive bacteria are better converting organic matter into CO_2 than gram-negative bacteria. Thus, during a production cycle, higher levels of these bacteria can reduce the accumulation of particulate organic carbon. Thus, maintaining higher levels of these gram-positive bacteria in production pond, farmers can minimize the buildup of dissolved and particulate organic carbon during the culture cycle while promoting more stable phytoplankton blooms through the increased production of CO_2 [21].

6. Conclusion

The results reported so far with the use of probiotics for aquatic organisms are promising. However, many works have not achieved satisfactory results.

Sometimes in experiments in which aquatic organisms are challenged by some pathogenic agent, the probiotic organism does not exhibit inhibiting action against the pathogen, resulting in mortality.

Similarly, the conditions to which the animals are subjected during farming may directly influence the effectiveness of probiotics. Thus, when not subjected to stressful situations, the results often do not show a significant effect of probiotics on the performance of animals.

In general, the effects of adding probiotics tend to be most striking in unsuitable operating conditions or in conditions of stress, when the microflora is unbalanced, primarily in young animals.

Among these factors, the most commonly featured are: temperature above or below the thermal comfort zone; presence of pathogens; poor sanitary conditions; stressful management; change in nutrition; transport; high storage density; after treatment with antibiotics; sudden change of environment.

Also, the results obtained in experiments with probiotics may be affected by factors such as: type of probiotic microorganism; method and quantity administered; condition of the host; condition of intestinal microbiota; age of the animal.

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