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## Application of probiotics in aquaculture

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### Abstract

Aquaculture is one of the world's fastest-growing sectors, with Asia accounting for about 90% of worldwide output. However, disease outbreaks in many countries in the Asia-Pacific region are restricted to the production of aquaculture, thereby affecting both the economic growth of the country and the socio-economic status of local citizens. Disease management in the aquaculture industry has been accomplished by using conventional techniques, synthetic chemicals and antibiotics in various ways. However, the use of such costly chemotherapeutants for disease control has been widely criticized for their negative effects, such as residue accumulation, development of drug resistance, immunosuppression and decreased consumer demand for antibiotic-treated aqua products, and conventional methods are ineffective in large aquaculture systems to control new diseases. As a result, alternate strategies for maintaining a stable microbial environment in aquaculture systems must be developed in order to sustain the health of farmed species. One of these methods is the use of probiotics, which is gaining popularity in the management of possible pathogens. This analysis summarizes the requirements for the selection, significance and future prospects of potential probiotics in the aquaculture industry.

**Keywords:** probiotics, aquaculture, economic

### Introduction

Aquaculture has become a significant economic operation in the field of a number of countries. In large-scale manufacturing premises, where aquatic animals are subject to harsh environments, disease-related issues and environmental degradation also occur, leading to severe economic problems. During this time, the prevention and control of diseases led to the use of veterinary medicinal products has increased significantly in recent decades. However, given the extensive evidence of antimicrobial evolution between pathogenic bacteria, resistance, the usefulness of antimicrobial agents has been questioned as a preventive measure (Balcazar JL (2003) [4]. Globally, during an antibiotic period of just around 60 years length, antibiotic tones were dispersed throughout the biosphere. Out of the 18,000 tons of antibiotics developed annually for medical and agricultural purposes in the United States, 12,600 tons are used for non-therapeutic treatment of livestock for growth promotion purposes (SCAN 2003) [27]. 1600 t of antibiotics, representing approximately 30% of the overall use of antibiotics in farm animals, are similarly used for growth promotion purposes in the European Union and Switzerland (SCAN 2003) [27]. These quantities of antibiotics exert a very strong selection pressure on the resistance of bacteria adapted to this situation, primarily through the horizontal and promiscuous flow of genes of resistance (SCAN 2003) [27]. Mechanisms of resistance can evolve in one of two ways: chromosomal mutation or plasmid acquisition. Chromosomal mutations can not be transmitted to other bacteria, but tolerance can be easily transferred by plasmids (Lewin CS 1992) [14]. Several bacterial pathogens can develop resistance mediated by plasmids. In marine *Vibrio* species, plasmids carrying genes for resistance to antibiotics have been identified and could be laterally exchanged. Genetic exchange mechanisms could all be probable at high population densities of bacteria found in aquaculture ponds, transfer via plasmids, transduction via viruses, and even direct transformation from DNA absorbed into particles in the water or on sediment surfaces (Moriarty D 1997) [17]. Furthermore other evidence of resistance transmission between aquaculture ecosystems and humans has been shown in *Salmonella typhimurium* DT104, with a novel florofenicol resistance gene floR, which confers resistance to chloramphenicol and is almost identical by molecular sequence to the florofenicol resistance gene first identified in *Photobacterium damsela*. There is a growing interest in the regulation or removal of antimicrobial usage within the industry at present. Therefore to maintain a healthy microbial environment in aquaculture systems, alternative

methods need to be created. The use of probiotic bacteria to control possible pathogens is one such technique that is gaining significance within the industry.

### Definition of probiotic

The term probiotic means "for life," derived from the Greek words "pro" and "bios" (Gismondo *et al.*, 1999) [4]. Lilley and Stillwell (1965) [15] originally used the concept of probiotics to mean a substance (s) that promotes the growth of other microorganisms (Chukeatirote, 2002) [5]. The description was changed by Parker in 1974 to "organisms and substances that contribute to intestinal balance."

" Fuller (1992) [8] revised the definitions to include a live microbial feed supplement that benefits the host animal by enhancing the microbial balance in its intestine. The meaning of live cells as the live cells has been put forward by this definition "A potential probiotic's essential component and its clears the confusion created by the use of the term "substance". According to Gram *et al* (1999) [10], a probiotic is any live microbial supplement that improves the microbial balance of the host animal. In this instance, there is no connection to food. In addition, Salminen *et al* (1999) [25] suggested that a probiotic is regarded as any microbial (but not necessarily living) preparation or component of the microbial cells that have a beneficial impact on the host's health. Verschuere *et al.* (2000) [32] proposed the concept "a live microbial adjunct that has a beneficial effect on the host by modifying the host" by ensuring improved use of the feed or improving its nutritional value by improving the host response to disease, or by improving the quality of its environmental climate, the related or ambient microbial population. A portion of the probiotic requirement to be a live organism culture, as defined by Irianto and Austin (2002) [11], this definition is a long way to describe a probiotic, so a probiotic is an entire or component of a microorganism that is beneficial to the host's health." The use of microorganisms or their products (element of microbial cells or cell-free supernatant factors) in these definitions reflects in tanks and ponds in which animals live as biological control or their ability to change the bacterial composition of the intestine, water and sediment of aquatic animals or to use feed as a health supplement and/or as a feed supplement and/or biological oversight.

### Criteria for selection of probiotics in aquaculture

The main objective of the initial use of probiotics is to preserve or restore a favorable relationship between friendly and pathogenic microorganisms that make up the intestinal flora or skin mucus of fish. A good probiotic is supposed to have a few unique characteristics. And the following steps should be taken in order to produce probiotics for commercialization.

- A healthy source of microorganisms must be selected from the digestive tract of healthy aquatic animals.
- The microorganisms in which to carry out the work are isolated by means of selective culture and established.
- A modern society of interest only in the colonies for conducting *in vitro* assessments, e.g. pathogen inhibition; pathogenicity to target species; host conditions of resistance; among others are conducted.

In the absence of any limits on the use of the target species, *in vivo* supplementation experiments, on a small and wide scale, it is carried out to verify whether the host has real advantages.

Finally, the probiotic that produced a considerably satisfactory outcome commercially and used may be manufactured.

### Characteristics of good probiotics

The following were identified by Fuller (1989) [7] as features of healthy probiotic bacteria:

- i. A strain capable of exerting a beneficial effect on the host animal, such as increased growth or resistance to disease, should be considered.
- ii. It should be non-toxic and non-pathogenic.
- iii. They should be present, ideally in large numbers, as viable cells.
- iv. It should be capable of survival and metabolization, e.g. tolerance to low pH and organic acid, in the gut environment.
- v. Under storage and field conditions, it should be stable and capable of remaining viable for periods.

### Probiotics significance in aquaculture

There are some potential advantages linked to the administration of probiotics that are already proposed as:

#### Improvement of water quality

Pollution of nitrogenous compounds such as ammonia, nitrite, nitrate has become a severe problem in fish culture systems/ponds. In general, the vulnerability of cultivated aquatic organisms to high concentrations of these compounds is species-specific, but these compounds can be extremely harmful at high concentrations and cause mass mortality in all cases. The capacity of *Lactobacillus* spp. was stated by Ma *et al* (2009) [16]. JK-8 and JK-11 extract all nitrogen and pathogens from infected shrimp farms at the same time. In several other studies, the addition of probiotics, particularly *Bacillus* spp., has improved the quality of water (Verschuere *et al.*, 2000 and Kolndadacha *et al.*, 2009) [32]. Gram-positive *Bacillus* spp. is the explanation for this. According to Stanier *et al.* (1963) [29], organic matter is typically more effective than gram-negative bacteria in converting organic matter back to CO<sub>2</sub>, which will transform a higher percentage of organic carbon to bacterial biomass or slime.

#### As growth promoters

It has been experimentally shown that probiotics can actually improve fish development. It was a probiotic bacterium because of the ability of species to out-grow the pathogens in favor of the host or to enhance the growth of the host and still no side effect on the host. In an attempt to use probiotic bacteria as a growth promoter for tilapia (*Oreochromis niloticus*), Yassir *et al.*, 2002 [34] identified that the highest growth output with *Micrococcus luteus* was documented as a probiotic and the best feed conversion ratio with the same organism was observed. So *M. luteus*, as a growth promoter in fish aquaculture, can be considered. As growth promoters, lactic acid bacteria have had an effect on growth rates in juvenile carps, but not in sea bass (Noh 1994) [18].

#### For disease prevention

Probiotics or their host health products have been shown to be useful in aquaculture, terrestrial animals and human diseases. These include microbial adjuncts that prevent the production of pathogens, proliferation of cultivated organisms in the intestinal tract, superficial surfaces and in the cultural environment (Verschuere *et al.*, 2000) [32]. The effect of these

beneficial organisms is accomplished by improving the culture organism's immune system, enhancing their resistance to disease, or creating inhibitory substances that prevent the host disease from being developed by pathogenic organisms.

### Enhancement of the immune response

Among the various beneficial effects of probiotics, one of the most widely claimed benefits of probiotics is immune system regulation. The ability of *Lactobacillus fermentum* LbFF4 isolated from Nigerian fermented food ('fufu') and *L. Plantarum* Fish larvae shrimps and other invertebrates have immune systems that are less well evolved than the adult stage and are primarily dependent on non-specific immune responses for their resistance to infection (Verschuere *et al.*, 2000 and Ogunshe 2009) [32, 19] evaluated LbOGI from the drink 'Ogi' to induce immunity against some selected fish bacterial pathogens in *Clarias gariepinus*.

### Source of nutrients and enzymatic contribution to digestion

Some studies have suggested that probiotic microorganisms have a beneficial effect on marine animals' digestive processes. Bacteroides and Clostridium sp. have been recorded in fish. They have contributed to the nutrition of its host, especially by providing fatty acids and vitamins (Sakata T 1990) [24]. Some microorganisms, such as Agrobacterium sp., Pseudomonas sp., Microbacterium sp., Staphylococcus sp., and Brevibacterium sp. Arctic charr (*Salvelinus alpinus* L.) may contribute to nutritional processes (Ringo *et al.*, 1995). In addition, by producing extracellular enzymes such as proteases, lipases and providing required growth factors, some bacteria can participate in the digestion processes of bivalves (Prieur *et al.*, 1990) [21]. Similar findings have been recorded for the adult penaeid shrimp microbial flora (*Penaeus chinensis*), where there is a complement of digestive enzymes and synthesis compounds that are assimilated by the animal (Wang *et al.*, 2000) [33]. Microbiota may serve as a supplementary food source, and vitamins or essential amino acids may be a source of microbial activity in the digestive tract (Dall and Moriarty, 1983) [6].

### Production of inhibitory compounds

A number of chemical compounds that are inhibitory to both gram-positive and gram-negative bacteria are released by probiotic bacteria. These include bacteriocins, lysozymes, siderophores, proteases, peroxides of hydrogen, etc. Lactic acid bacteria (LAB) are known to generate compounds inhibitory to other microbes, such as bacteriocins (Saurabh *et al.*, 2005) [26].

### Competition for adhesion sites

Probiotic species compete with the pathogens in the gut epithelial surface for adhesion sites and food and eventually avoid their colonization (Vanbelle *et al.*, 1990) [30]. Fish pathogens such as *Vibrio anguillarum* and *Aeromonas hydrophila* have been shown to bind to and expand on or in the intestinal or external mucous membrane *in vitro* (Krovacek *et al.*, 1987) [13].

### Competition for nutrients

Nutrients otherwise ingested by pathogenic bacteria are used by probiotics. The composition of the intestinal tract microbiota or the environment of cultured aquatic organisms may be influenced by nutrient competition (Ringo and

Gatesoupe 1998) [22]. Therefore it is not easy to effectively apply the concept of competition to natural settings, and this remains a major challenge for microbial ecologists.

### Probiotics in aquaculture management

These species may be administered through the feeding, infusion or immersion of probiotic bacteria for aquaculture management (Irianto and Austin, 2002) [11].

### Application in feed

With the feed and binder (egg or cod liver oil), probiotics are added and most commercial preparations contain either *Lactobacillus* sp or *Saccharomyces cerevisiae* (Abidi R.,2003) [1]. Probiotic species used in food must be able to withstand passages through the gut according to FAO and WHO guidelines, i.e. they must have the ability to avoid gastric juices and bile exposure (Senok AC *et al.*, 2005) [28]. They must also be able to proliferate and colonize the digestive tract and for the duration of the shelf life of the product, they must be healthy, efficient and retain their efficacy and potency (Senok AC *et al.*, 2005) [28].

### Direct application of probiotics to pond water

There are some strains of bacteria in the water probiotics, such as *Bacillus acidophilus*, *B. subtilis*, *Nitrobacter* sp, *Aerobacter* and *Sacharomyces cerevisiae*, *Lecheniformis*. Probiotic application through the water of tanks and ponds can also have an impact on fish health by improving many water qualities, as the bacterial composition of water and sediments is changed (Ashraf, A., 2000 and Venkateswara AR.,2007) [2, 31].

### Application of probiotics through injection

Applying probiotics by injection is an alternative. The idea of freeze-drying the probiotic like a vaccine was proposed by Austin *et al.* in 1995 [3] and applied either by bathing or injection. The experimental administration of probiotic, *Micrococcus luteus* by injection through intra peritoneal route to *Oreochromis niloticus*, which had only 25 percent mortality compared to 90 percent with *Pseudomonas* using the same route, was demonstrated by Yassir *et al.*, 2002 [34]. The use of probiotics promotes Rainbow trout immunity by stimulating the activity of phagocytes, complementing mediated bacterial killing and production of immunoglobulin, according to Yassir *et al.*, 2002 [34] and Noh SH *et al.*, 1994) [18].

### Conclusion

The efficacy of probiotics was associated with strain multiplications and/or their existence after application in the environment, and this attribute was associated with host strain colonization and some beneficial health effects. These are not in accordance with all products of probiotics and help to achieve conflicting results about their impact on aquatic species. Probiotic evolution is correlated with a greater understanding of the use of these types of products, properties, and the particular strain-host in intestinal ecology. A particular point of environmental science consideration is the direct use of a probiotic on water (from fresh to seawater from farms and laboratories). These products (probiotics) are usually foreign or exogenous strains and pose a potential risk of infection by microorganisms, in particular with the use of genetically modified strains, complex adhesions or colonization niches, the development of antibiotics and

synergistic action. Before wide application to aquaculture, it is critical to understand the use and environmental effects of those new generations of probiotics. Nonetheless a range of probiotic products have been extensively tested, demonstrating their effectiveness and potential use in aquaculture. The aquaculture group has become more commonly available with beneficial bacterial preparations that are species-specific probiotics. As disease prevention, these preparations display particular beneficial effects and provide a natural factor to obtain a stable gut environment and immune system. To increase the development of aquatic organisms, the establishment of a strong disease prevention program, including probiotic and good management practices, can be beneficial.

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