
Role of Probiotics in Aquaculture

Shashi Patil¹ and Sonia Johri²

¹Department of Life Science and Technology, Boston College for Professional Studies Gwalior, M.P., India

²School of Life Sciences, ITM University, Gwalior, M.P., India

*drpatil@gmail.com

Abstract

Fish a protein, vitamin rich and fat deficient diet is considered as a vital brain food. Aquaculture has emerged to provide nutritional and food security to people. The three Indian major carps, namely, catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) are the major contributor of Indian aquaculture production. The use of probiotics in aquaculture is becoming increasingly popular, microbial cells. Probiotics should be defined as live microbes which, when administered in adequate amounts, confer a health benefit on the host. Thus Probiotics are defined as live cells or substrates that provide benefits by stimulating growth and improving immune response. The review focuses on the use of Probiotics as prophylactic rather than therapeutic. Commercial production of Probiotics requires a consideration of safety issues.

Keywords: Probiotics, Aquaculture, Fishes, Immune modulation, *Spirulina*, Seafood.

Introduction

Fish is a worldwide distributed food commodity and plays a pivotal role in human diet. Since ancient times fish was recognized as being a 'brain food', a reference to its importance in the development of a healthy brain. In developing countries like India, where problems of nutritional deficiencies persist fish serves as rich source of easily digestible protein, nutrients, vitamins and minerals and relatively low caloric content. Most fishes due to their low fat content are considered excellent in calorie restriction and reduction of plasma lipids in human food. Fish protein is easily digestible because of very low stroma protein and has an excellent spectrum of essential amino acids. Fish protein has very high biological value. Fish is an excellent source of lysine that is limited in vegetable foods. It may be classed as either whitefish, oily or shellfish. Whitefish, such as haddock and seer, contain very little fat (usually less than 1%) whereas oily fish, such as sardines, contain between 10-25%. The latter, as a result of its high fat content, contain a range of fat-soluble vitamins (A, D, E and K) and essential fatty acids, all of which are vital for the healthy functioning of the body.

Fish, especially liver oils is rich in vitamins A and D. Vitamin E is present in significant amounts in many sea foods, for example salmon and some shellfish. Vitamin E acts as an antioxidant protecting polyunsaturated fats and Low Density Lipoprotein (LDL) cholesterol from oxidation by free radicals and may also have anti-inflammatory effects. B vitamins are essential for the metabolism of food, particularly carbohydrate. Most fish are a source of some B vitamin particularly thiamine, riboflavin and pyridoxine.

Fishes are source of vitamin B6 and B12, which help in producing red blood cells, antibodies and maintain the central nervous system. Clams, herrings, crabs, mackerel, mussel and oysters contain high quantities of B12 and B6 vitamins. Fish generally provide little or no vitamin C. Besides vitamins; fishes also contain minerals like iodine and calcium that are vital to our health. Fish/shell fish and many other aquatic animals are used as powerful research models. They are unique due to their biology, genetics, breeding and reproductive cycles. Research areas include developmental biology, pharmacology,

toxicology, molecular biology, transgenic fish, gene banking and human disease research. In vitro experiments with eggs from frogs and fish allow for non-invasive research otherwise unavailable using traditional mammalian models such as mice and rats. Aquatic animals are suitable for research in biotechnology due to availability of large numbers of gametes (germ cells), use of external fertilization, and ease of in vitro rearing of embryos. Fish are useful in cell biological studies also, particularly pigment cell biology (Patil and Jain, 1989, 1991, 1993; Jain and Patil, 1990, 1992; Sharma *et al.*, 1996; Fujii, 2000; Patil, 2004).

Aquaculture

The rapid expansion of population throughout the world and changed eating habits have created a devastating problem of hunger and malnutrition. In developing countries poor are deprived of sufficient food. With ever increasing demand for animal protein, aquaculture has emerged to provide nutritional and food security to people. It has become the most viable and promising food producing sector. To a large extent this enterprise provides employment to people in the growth sector itself and also to supporting system. In situations where major fishery resources have depleted due to increased pressure, environment degradation or loss of wild fisheries catches, aquaculture development has accelerated. It has become an attractive component of rural livelihood as well. In aquaculture practices exposure of aquatic animals to disease outbreak, stress, and deterioration of environmental conditions may cause economic losses.

Cultured species in India

The three Indian major carps, namely, catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) contribute as much as 87 percent of the total Indian aquaculture production. The three exotic carps namely, silver carp (*Hypophthalmichthys molitrix*); grass carp (*Ctenopharyngodon idellus*) and common carp (*Cyprinus carpio*) introduced for polyculture now form a second important group. The commercial farming of some of the cultivable medium and minor carp species which show high regional demand, including, *Labeo calbasu*, *L. fimbriatus*, *L. gonius*, *L. bata*, *L. ariza*, *Cirrhinus mrigala*, *Puntius sarana*, *Hypselobarbus pulchellus*, *H. kolus* and *Amblypharyngodon mola* as well as several others, has been almost non-existent (Ayyappan and Jena, 2003). Among other are walking catfish, 'magur' (*Clarias batrachus*), Stinging catfish, 'Singhi' (*Heteropneustes fossilis*). In recent years, attempts have been made to develop the culture of non-air breathing catfishes like *Pangasius pangasius*, *Wallago attu*, *Sperata seenghala*, *S. aor* and *Ompok pabda*. The other finfish species of importance include climbing perch (*Anabas testudineus*), murrels (*Channa striata* and *C. marulius*) and tilapia (*Oreochromis mossambicus* and *Oreochromis niloticus*). Among the freshwater prawns, the giant river prawn (*Macrobrachium rosenbergii*), is the most important species followed by the monsoon river prawn, *M. malcolmsonii*. The brackish water aquaculture sector is mainly supported by shrimp production as well as giant tiger prawn (*Penaeus monodon*), which are responsible for the bulk of production followed by the Indian white prawn, *P. indicus*. Although India possesses several other potential species of finfish and shellfish, production of these is still very low key. In seawater the major farmed species are the green mussel (*Perna viridis*), brown mussel (*Perna indica*), Indian backwater oyster (*Crassostrea madrasensis*), Japanese pearl oyster (*Pinctada fucata*) and seaweed species like (*Gracilaria edulis*).

To solve the high demand for fish, aquaculture production remains the best option to bridge the wide gap between fish demand and domestic production. Without clear recognition of its dependence on natural ecosystems, the aquaculture industry is unlikely to develop to its full potential or continue to supplement ocean fisheries. Aquaculture industry can be developed by farming of species lower on the food web, by feed and resource management, by integrated fish farming and by proper aquaculture practices. India is a large country with great potential for aquaculture. The use of probiotics in aquaculture is becoming increasingly popular, having recently been defined as 'microbial cells that are administered in such a way as to enter the gastrointestinal tract and to be kept alive, with the aim of improving health (Gatesoupe, 1999). The probiotics application came first as it is environmentally safe and cost effective (Moriarty,

1997). The application and development of the probiotics in Indian aquaculture is very limited compared to other countries. Asia produces roughly 90 percent of global aquaculture output, and China alone contributes more than two thirds of the total. The one-tenth of global output is contributed by Europe, North America, and Japan together. These regions consume the bulk of farmed seafood that is traded internationally. Various species of carp dominate the tonnage of farmed fish produced worldwide, and carp production for local or regional use by relatively low income households has increased dramatically in Asia (mainly China). In contrast, increased volumes of salmon, shrimp, and other high-value species are marketed mainly in industrialized countries. Farmed output and markets for other lower value species such as tilapia and milkfish have increased in both developing and industrialized countries. Most farmed mollusks are still consumed locally and regionally in China and in other developing countries. However, production of certain species for global markets has increased in several developed countries. These species include the Pacific cupped oyster, blue mussel, New Zealand mussel, and *Yesso scallop*.

Probiotics and Aquaculture

In 2001, a Joint Food and Agriculture Organization/World Health Organization Working Group on drafting “Guidelines for the evaluation of Probiotics in food” recommended that probiotics should be defined as “live micro organisms which, when administered in adequate amounts, confer a health benefit on the host”(FAO/WHO, 2001).

Types of Probiotics

Probiotics are mainly of two types a) gut probiotics which can be blended with feed and administered orally to enhance the useful microbial flora of the gut and, b) water probiotics which can proliferate in water medium and exclude the pathogenic bacteria by consuming all available nutrients. Thus, the pathogenic bacteria are eliminated through starvation (Nageswara and Babu, 2006).

Probiotics are important natural ingredients in aquaculture with many beneficial effects like improved water quality, improved activity of gastrointestinal micro biota, and enhanced immune status of host, growth performance and feed utilization. The use of probiotics is now prevalent in the aquaculture industry as a means of controlling disease, improving water quality and enhancing the immune system of cultured species (Wang, 2007; Wang *et al.*, 2008; Ma *et al.*, 2009). Nowadays, a number of probiotic products are commercially available in aquaculture. Probiotics selection criteria have been proposed for cultured aquatic species (Fuller, 1989; Verschuere *et al.*, 2000; Vine *et al.*, 2006; Watson-Kesarcodi *et al.*, 2008; Gómez and Balcázar, 2008; Kolndadacha *et al.*, 2009).

The 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. Merrifield *et al.*, 2010 proposed that the potential probiotics must have following features:

Non pathogenic, to the host species, resistant to bile salts, able to adhere to and /or grow well within intestinal mucosa, display advantageous growth characteristic, exhibit antagonistic properties toward one or more key pathogens, remain viable under normal storage conditions and robust enough to survive industrial processes.

Balcázar (2002) reported that the microbial manipulation can reduce or eliminate the incidence of opportunist pathogens. By ingestion of other microorganisms the micro biota in the gastrointestinal tract of aquatic animals can be modified. Some researchers have suggested that microorganisms have a beneficial effect in the digestive processes of aquatic animals (Yanbo and Zirong, 2006; Balcázar *et al.*, 2007). 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 (Sakata, 1990).

In bivalves some bacteria produce extracellular enzymes, such as proteases, lipases in digestion process; as well they provide necessary growth factors (Prieur *et al.*, 1990). It has been reported that use of Bacillus

sp. improved water quality, survival and growth rates and increased the health status of juvenile *Penaeus monodon* and reduced the pathogenic *Vibrios* (Dalmin *et al.*, 2001). In fish culture systems/ponds contamination by nitrogenous compounds such as ammonia, nitrite and nitrate has been a serious concern. In many studies the addition of probiotics especially *Bacillus spp.* (Verschuere *et al.*, 2000; Kolndadacha *et al.*, 2009), *Nitrosomonas* and *Nitrobacter* (Sunitha and Padmavathi, 2013) water quality has been improved.

Competitive exclusion of potential pathogenic bacteria effectively reduces or eliminates the need for antibiotic prophylaxis in intensive larviculture systems (Garriques and Arevalo, 1995). Recently a marine bacterial strain, *Pseudomonas* I2, was isolated from estuarine environmental samples that produced inhibitory compounds against shrimp pathogenic *Vibrios*. This antibacterial compound was shown to be of low molecular weight, heat stable, soluble in chloroform, and resistant to proteolytic enzymes (Chythanya *et al.*, 2002). Several studies have been carried out using probiotics to understand immunity responses in different teleost fish (Nayak, 2010).

The non-specific immune system can be stimulated by probiotics. It has been demonstrated that oral administration of *Clostridium butyricum* bacteria to rainbow trout enhanced the resistance of fish to vibriosis, by increasing the phagocytic activity of leucocytes (Sakai *et al.*, 1995). Rengpipat *et al.*, (2000) mentioned that the use of *Bacillus sp.* (strain S11) that the administration of a mixture of bacterial strains (*Bacillus* and *Vibrios sp.*) positively influenced the growth and survival of juveniles of white shrimp and presented a protective effect against the pathogens *Vibrio harveyi* and white spot syndrome virus. This protection was due to a stimulation of the immune system, by increasing phagocytosis and antibacterial activity. Some bacteria used as candidate probiotics have antiviral effects.

It has been reported that strains of *Pseudomonas sp.*, *Vibrios sp.*, *Aeromonas sp.*, and groups of Coryneforms isolated from salmonid. Hatcheries, showed antiviral activity against infectious hematopoietic necrosis virus (IHNV) with more than 50% plaque reduction (Kamei *et al.*, 1988).

Ramakrishnan *et al.*, 2008, studied effect of probiotics and *Spirulina* on survival and growth of juvenile common carp (*Cyprinus carpio*). They used two probiotics the bacteria *Lactobacillus acidophilus* and the yeast *Saccharomyces cerevisiae* and *Spirulina*, a freshwater microalgae. *Spirulina* is a good source of protein and energy (Harel *et al.*, 2002). They reported improved growth performance and total heterotrophic load. *Spirulina* is a valuable feed supplement for *Cyprinus carpio* (Nandeeshha *et al.*, 1993). Similar results were reported for *Spirulina* based diet for Indian carp, *Labeo rohita* (Ghosh *et al.*, 2003), Nile tilapia (Lara-Flores *et al.*, 2003) and Indian white shrimp, *Fenneropenaeus indicus* (Ziaei-Nejad *et al.*, 2006), Common carp, *Catla catla* (Krishnavenik *et al.*, 2013). Similar to earlier reports (Manohar, 2005), the dietary incorporation of *S. cerevisiae* increased the gut flora of *C. carpio*. Probiotics promoted colonization of bacteria in fish gut and stimulated fish growth (Mukhopadhyay and Paul, 1996; Gatesoupe, 2007). Mohapatra *et al.*, 2012 used different microbial probiotics in the diet of Rohu, *Labeo rohita* fingerlings. The diet was supplemented with two species of bacteria (*Lactococcus lactis* and *Bacillus subtilis*) and one species of yeast (*Saccharomyces cerevisiae*) in equal proportion as probiotic. This combination showed better growth performance. It resulted in better food conversion, higher protein digestion, utilization and gain, reduction in total heterotrophic bacteria (THB) count in the intestine and better adhesion of probiotic micro flora in gut. Velmurugan and Rajagopal (2009) used probiotics in mass scale production of highly economical marine ornamental fish *Neopomacentrus nemurus*. At present keeping ornamental fishes has become a popular hobby so they are in high demand as pets. The farmed marine ornamental fishes are threatened by pathogenic microorganisms like *Vibriosis harveyi*, *V. splendidus* and *V. parahaemolyticus*. The use of probiotics is becoming popular as alternative to antibiotics. They used commercially available water probiotic; PROACT a unique combination of aerobic, microaerophilic and anaerobic microorganisms to keep water in environmentally balanced condition. Aerobic bacterium in this helped in degradation of organic matter by mineralization. The unutilized feed was degraded by bacterium *Celulomonas*. The microorganisms in probiotic had

bactericidal effect on *Vibrio* sp. to bring down their population. In their study probiotics maintained good water quality and helped in growth, survival and disease resistance of fish. In India commercial shrimp culture started in mid eighties and now due to its taste and nutritive value they are in great demand. The diseases of shrimp interfered with shrimp culture Soundarapandian et al., 2010 studied the effect of probiotics on the growth and survival of *Penaeus monodon* (Fabricius). They applied probiotics, viz., super biotic, Super Ps, Zymetin and Mutagen in culture ponds. These probiotics maintained good water quality and helped in growth, survival and resistance to diseases. Recently, the use of *Alteromonas haloplanktis* (strain 77) and *Vibrio* sp. (strain 11) has been effective at controlling infections by *V. anguillarum* in scallop (*Argopecten purpuratus*) larvae (Riquelme et al., 2000).

Limitation of probiotics use

Probiotics can be used as preventive tools rather than for treatment of diseases. They should be applied immediately to sterilized water medium to avoid any microbial contamination. During application of probiotics the use of any other chemical or drug for treatment of other diseases can destroy the probiotics.

Present status in India

Indian aquaculture industry is growing fast but compared to other countries the application of probiotics is very less. To develop putative probiotics it is required to carry screening of novel probiotic strains from local aquaculture rearing systems to suit the specific requirement in India during commercial production of probiotics safety issues should also be considered.

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