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Study on the use of Probiotic Products in Indian Shrimp Aquaculture

Mahidhar Bole¹, Shaik Baji Shaheed¹, A. Udaya Shankar² and Gadadhar Dash³

¹ Research Scholar, K. L. U. Business School, K. L. University, Greenfields, Vaddeswaram, Guntur District, Andhra Pradesh, India

² Associate Professor, K. L. U. Business School, K. L. University, Greenfields, Vaddeswaram, Guntur District, Andhra Pradesh, India

³ Professor, Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Abstract: The study was carried out to understand the current status of the use of probiotics in Indian shrimp aquaculture is presented. The growth of aquaculture as an industry has accelerated over the past decades; this has resulted in environmental damages and low productivity of various crops. The need for increased disease resistance, growth of aquatic organisms, and feed efficiency has brought about the use of probiotic products in aquaculture practices. Thus, the research into the use of probiotic products for aquaculture is increasing with the demand for environment friendly sustainable aquaculture. Probiotics are defined as live cells or a substrate that provides benefits through stimulation of growth, improved digestion, and improved immune response. Probiotics can also improve water quality. Other approaches to disease prevention (crop holiday, pond preparation, regulating stocking density, effluent treatment systems) are considered, and overview of commercial probiotic strains available in India are summarized. It is concluded that although probiotics plays a vital role in growth, survival and disease resistance of shrimp by maintaining good water quality parameter throughout the culture period, even though more studies are require on use of probiotics under culture conditions, in particular the consideration of species, source, quality and application methods to improve the performance of shrimp culture.

Keywords: Shrimp Aquaculture, Probiotics, Infectious diseases, Bacillus, Commercial probiotic product

INTRODUCTION

The term 'probiotics' was derived from the Greek word, meaning "for life" (Reid *et al.*, 2003). An expert panel commissioned by Food and Agriculture Organization (FAO) and World Health Organization (WHO) defined probiotic as "live micro-organisms," which, when administered in adequate amounts confers a health benefits on the host (FAO/WHO, 2006). Recently, there is an increasing scientific and commercial interest in the use of beneficial microorganisms for the prevention and treatment of diseases (Figure 1).

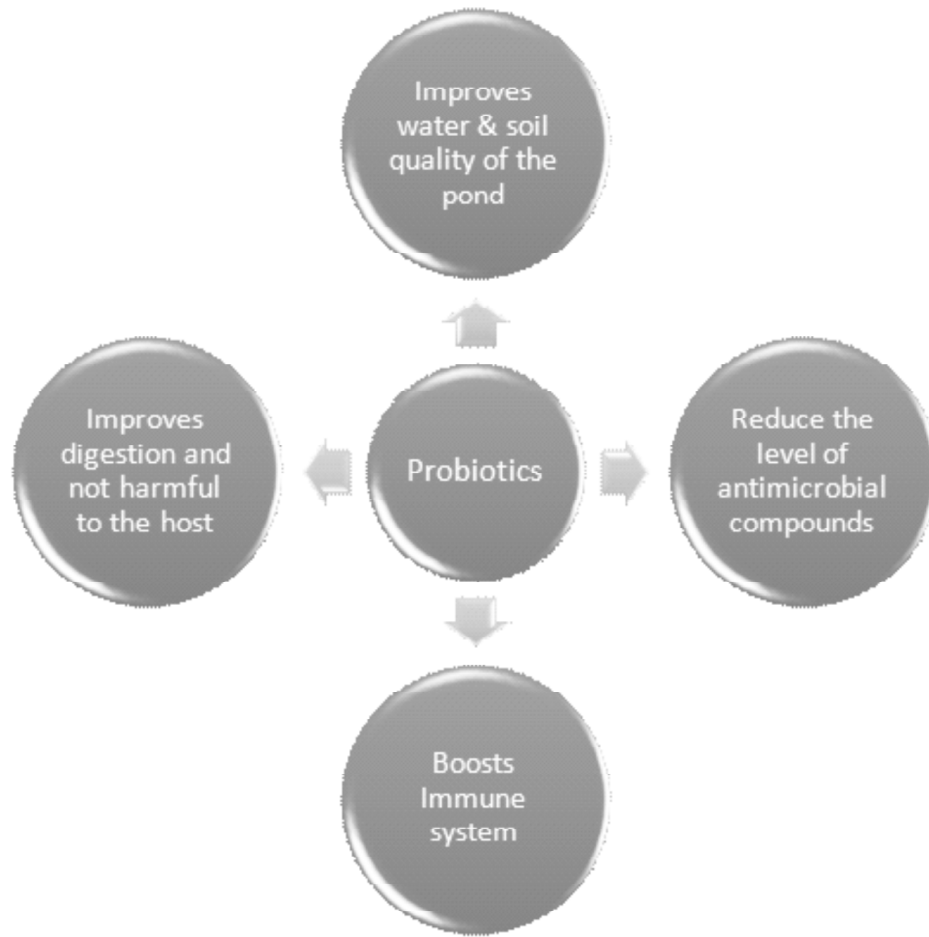


Figure 1: Benefits of Probiotics

Probiotics, although not a new concept, has only recently begun to receive an increasing level of scientific interest. Probiotics are generally defined as live microbial feed supplements that can benefit the host by improving its intestinal balance. Probiotics fall under two broad classifications, those for animal use and those for human use. Probiotics used in animal feed are considered as alternatives to antibiotics (and therefore used as growth promoters). Statistics have revealed that the global aquaculture production continue to increase rapidly without the sign of reaching its peak. According to the United Nations Food and Agriculture Organization report (FAO, 2014), the global aquaculture production achieved another all-time high of 90.4 million tons including the 66.6 million tons of food fish and 23.8 million tons of aquatic algae in 2012 in response to the rising domestic and international seafood demand. Currently, it has been reported that food fish provides an average of one-fifth of total animal protein intake for the world population estimated at 7.3 billion people (Moffitt and Cajas-Cano, 2014). However, major disease outbreaks have been reported within the aquaculture sector in many part of the world due to the increased stocking density, over-crowding and lack of sanitary management with the rapid growth of aquaculture. For instance, the viral infections (white spot syndromes, yellow head disease and taura syndrome) in shrimp industry has cost billions of dollars worldwide (Flegel, 2012; Lightner *et al.*, 2012). Also bacterial pathogens such as *Vibrio* sp. (*Vibrio harveyi*, *V. parahaemolyticus*, *V. campbellii*) caused luminous vibriosis

in shrimp farms resulted in 50–100% mortality and vibrio infections in human (Shruti, 2012; Letchumanan *et al.*, 2014; Wang *et al.* 2015).

OBJECTIVES OF STUDY

- To understand the use of probiotics in Indian shrimp aquaculture
- To study the mechanism of action and application of probiotics in aquaculture
- To Explore the selection and functions of probiotic in aquaculture
- To study the prospects and challenges of probiotic in India
- To identify the other approaches to disease prevention
- To explicate the commercial probiotic strains used in shrimp culture in India

LIMITATIONS OF STUDY

- The study is conducted based on the secondary data available which is only an explorative research and no empirical results are available.

METHODOLOGY

- The study is conducted based on secondary data base and it is explorative research study. The first step in an exploratory study is a search of the secondary literature. Studies made by others for their own purposes represent secondary data. Secondary data has been gathered from marketing research data, magazines, old reports and other sources where the relevant information has been stored.

Use of Probiotics in Shrimp aquaculture

Shrimp farming is one of the most important aquaculture in worldwide especially in Asia due to their economic value. Recently, it is estimated that approximately more than 5 million metric tons of shrimp are annually produced but the current global demand for both the wild and farmed shrimp is approximately more than 6.5 million metric tons per annum. So, in recent times there are many shrimp farms are being created throughout the world to solve this increasing food demands (FAO 2012). However, fast development of these shrimp industry has produced various ecological, economical and social issues. In general, intensive shrimp farming is the main aquaculture activity which has been frequently affected by bacterial pathogens especially in India. Among that, vibriosis is the common bacterial disease responsible for mortality of cultured shrimp (Sivakumar *et al.*, 2014). Using antibiotics and chemotherapeutic agents to be an important disease controlling measures has developed drug resistance microorganisms (Verschuere *et al.*, 2000). In recent times, an alternative that has been widely engaged in the aquaculture industry is the dietary supplementation with probiotic bacteria, because probiotic bacteria are a “live microbial cells administered to cultured organisms to colonize the digestive tract and improve their immune response” (Vine *et al.*, 2006).

Researchers also have demonstrated about the use of probiotic bacteria in aquaculture to improve the water quality and immune system by balancing bacterial flora in water and reducing pathogenic

bacterial load (Kesarcodei-Watson *et al.* 2008). Among the probiotic bacteria used in aquaculture, the lactic acid bacteria are found to be great due to their easy multiplication, production of antimicrobial compounds (bacteriocins, hydrogen peroxide, organic and lactic acids) and the stimulation of the non-specific immune response of the host (Gatesoupe, 2008). Some, studies also have demonstrated about the beneficial effect of lactic acid bacteria in several aquatic species culture by their nutritional benefits and strong antimicrobial activity against pathogenic microorganisms (Gilliland *et al.*, 1985; Rossland *et al.*, 2003; Ajitha *et al.*, 2004; Gatesoupe, 2008; Qi *et al.*, 2009; Ismail and Soliman, 2010 and Sivakumar *et al.*, 2012).

The application of probiotics in aquaculture has been widely used as a means of controlling disease, enhancing immune response, providing nutritional and enzymatic contributions to the digestion of the host, and improving water quality (Qi *et al.*, 2009). Probiotics are also regarded as an environmentally friendly treatment method. The probiotics may be added to feed as live microorganisms to create a balanced indigenous micro floral community in the gastrointestinal tract (Rengpipat, 2005). Moreover, probiotics are being considered for use as therapeutic agents and some farmers are already using them preferentially over antibiotics (Fuller, 1989). The use of probiotics, which control pathogens through a variety of mechanisms, is increasingly viewed as an alternative to antibiotic treatment (Verschuere *et al.*, 2000).

The benefits observed in the supplementation of probiotics in aquaculture include:

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.

PROBIOTICS IN AQUACULTURE

Mechanisms of action

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

- (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.

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, B12, D3) that enhance the action of beneficial microorganisms.

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.

Commercial Probiotic Preparations

At present, there are several commercial preparations of probiotics that contain one or more live microorganisms, which have been introduced to improve the cultivation of aquatic organisms. Probiotics can be used as a feed additive added directly to the culture tank or mixed with feed.

Currently, commercial products are available in liquid or powder presentations, and various technologies have been developed for improvement. Some commercial aquaculture products included prebiotics in their formulation, such as mannans, glucans, and yucca extract that further increase the beneficial effects of the product. More recently, systems have been developed for immobilization of probiotics, especially using microencapsulation. Microbial cells at high density are encapsulated in a colloidal matrix using alginate, chitosan, carboxymethyl cellulose, or pectin to physically and chemically protect the microorganisms. The methods commonly used for microencapsulation of probiotics are the emulsion, extrusion, spray drying, and adhesion to starch. Focused on the application to aquaculture, Rosas *et al.* have effectively encapsulated cells of *Shewanella putrefaciens* in calcium alginate, demonstrating the survival of encapsulated probiotic cells through the gastrointestinal tract of sole (*Solea senegalensis*). Encapsulation in alginate matrices protects bacteria from low pH and digestive enzymes; this protection helps to release the probiotic into the intestine without any significant damage. Currently, the lyophilized commercial preparations have advantages for storage and transport. However, conditions for reconstitution of these preparations such as temperature, degree of hydration, and osmolarity of the solution are vital to ensure the viability of bacteria. It is important to emphasize that these products must provide a health benefit to the host; for this, it is necessary that contained microorganisms have the ability to survive storage conditions, and after that in the digestive tract of aquatic species, remaining viable and stable, and finally improving production. According to the opinion of the producers, these preparations are safe to use and effective in preserving the health of aquatic animals.

Some commercial probiotic strains are wide used in Indian shrimp aquaculture are shown in Table 1

Table 1
Microorganisms recognized as safe and used as probiotics in animals. Source: [22]

Aspergillus	A. niger, A. orizae
Bacillus	B. coagulans, B. lentus, B. licheniformis, B. subtilis
Bifidobacterium	B. animalis, B. bifidum, B. longum, B. thermophilum
Lactobacillus	L. acidophilus, L. brevis, L. bulgaricus, L. casei, L. cellobiosus, L. fermentarum, L. curvatus, L. lactis, L. plantarum, L. reuterii, L. delbrueckii,
Pediococcus	P. acidilacticii, P. cerevisiae, P. pentosaceus, P. damnosus
Saccharomyces	S. cerevisiae, S. boulardii
Streptococcus	S. cremoris, S. faecium, S. lactis, S. intermedius, S. thermophilus, S. diacetylatis

APPLICATION OF PROBIOTICS IN AQUACULTURE

Application in feed

Probiotics have been supplied directly through feed, in some cases using binders for stabilization (Kolndadacha *et al.*, 2011). Supplementing feed with probiotics is common in aquaculture; the aim of this method is to introduce live cells of probiotics to the host animal gut in order to establish a balanced gastrointestinal microbial flora and to improve digestive function or immune system responses. Probiotics, including bacterial strains, yeast and extracted substances, are generally supplied by this method of application. Probiotics are diverse and are usually derived from the intestines of the host animals (Tovar *et al.*, 2002; Chantharasophon *et al.*, 2011; Chu *et al.*, 2011; Sun *et al.*, 2012), cultured in diverse environments (Wang and Xu, 2006), and come have been developed into commercial products which are also introduced and used (Abraham *et al.*, 2008; Fernandez *et al.*, 2011). Some probiotics that have been supplemented in animal feed include bacterial species, such as *Lactobacillus* spp., *Enterococcus faecium*, *Bifidobacterium thermophilum*, *Streptomyces* spp., *Micrococcus* spp., *Pseudomonas fluorescens*, as well as yeast, such as *Saccharomyces cerevisiae*, as well as herbs and extracted substrates, such as azadirachtin.

Direct to culture water/pond

Probiotics have been applied directly to culture ponds to improve water quality (Boyd and Cross, 1998) and the survival of cultured animals (Moriarty, 1998). The effectiveness of probiotics can be explained by bioaugmentation or biocontrol mechanisms by which the microbial ecology of the water and sediment is improved (Rengpipat, 2005). Several biological products, such as live bacterial inocula, enzyme preparations, and plant substrates extracts have been used as water and soil quality condition improvement factors in aquaculture ponds (Boyd and Cross, 1998). Probiotics include numerous strains of bacteria.

Application through Injection

The method of injecting probiotic products into aquatic animals has been used to stimulate the immune response of fish against bacterial pathogenic infection (Anderson and Siwicki, 1994; Sahoo and Mukherjee, 1999). Freeze dried probiotics can also be used as vaccinations in fish (Austin *et al.*, 1995) because the host animal probiotics stimulate the immune system by promoting the activity of antibodies. However, it

is difficult to inject probiotics into cultured fish, especially into small animals, and to treat large numbers of fish in this way.

Selection of Probiotics

The research done so far indicates the efficiency of various probiotics use in aquaculture. It is very important to characterize and identify the mode of action of the potential candidate probiotics and its efficiency on the pathogen and safety to the aquatic animals and aquaculture environment. This can be done through several in vitro and in vivo experiments. The selection criteria of Gomez-Gil *et al.* are based on:

- (1) collection of background information;
- (2) acquisition of potential probiotics;
- (3) evaluation of the ability of potential probiotics to outcompete pathogenic strains;
- (4) assessment of the pathogenicity of the potential probiotics;
- (5) evaluation to the effect of the potential probiotics on the host; and
- (6) economic cost/benefit analysis.

Recently, the United Nations has recommended a number of specifications that should be considered when a probiotic product is selected and approved. These include the following:

- (1) viability of the probiotic to survive when passage through the GIT should be demonstrated;
- (2) colonization by the probiotic should occur when present in the GIT of the host;
- (3) competition of the probiotic culture against pathogenic bacteria for the attachment on intestinal surfaces should happen successfully;
- (4) the probiotic should be efficient in inhibiting pathogenic bacteria according to in vitro tests;
- (5) probiotics should show resistance against other sanitary agents or disinfectants;
- (6) identity of probiotic products should be indicated on the label by genus and species name according to international nomenclature;
- (7) dosing and expiration date should be indicated on the label, and
- (8) data indicating that the product will not infect immunocompromised animal is desirable.

FAO/WHO (2001) guidelines recommend that in the cause of selecting probiotics, the probiont should be evaluated for a number of parameters such as, antibiotic susceptibility patterns, toxic production, metabolic and hemolytic activities, infectivity in immuno-compromised host and side-effects.

Characteristics of Good Probiotics

Filler (1989) listed the following as features of good probiotic bacteria.

- Its should be a strain, which is capable of exerting a beneficial effect on the host animal e.g.increased growth or resistance to disease
- It should be non-pathogenic and non-toxic

- It should be present as viable cells preferable in large numbers
- It should be capable of surviving and metabolizing in the gut environment e.g. resistance to low pH and organic acid.
- It should be stable and capable of remaining viable for periods under storage and field conditions.

A probiotic agent with all these features has considerable advantage over antibacterial supplements such as antibiotics currently in use. They do not induce resistance to antibiotics which will compromise therapy. They are not toxic and therefore will not produce undesirable side effect when being fed and in the case of food animal will not produce toxic residues in the carcass. They may stimulate immunity whereas the immune status remains unaffected by antibiotics. An essential determinant in the choice of a probiotic microorganism is its ability to reach, survive and persist in the environments (Charles *et al.* 1998).

Functions of Probiotics in Aquaculture

The need for sustainable aquaculture has promoted research into the use of probiotics on aquatic organisms. The initial interest was focused on their use as growth promoters and to improve the health of animals; however, new areas have been found, such as their effect on reproduction or stress tolerance, although this requires a more scientific development.

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 if, by their nature, improve digestibility. Some people are inclined to think that it could be both factors; furthermore, it would be important to determine whether probiotics actually taste good for aquaculture species. According to Balcázar *et al.*, probiotic microorganisms are able to colonize gastrointestinal tract when administered over a long period of time because they have a higher multiplication rate than the rate of expulsion, so as probiotics constantly added to fish cultures, they adhere to the intestinal mucosa of them, developing and exercising their multiple benefits. This also depends on factors such as hydrobionts species, body temperature, enzyme levels, genetic resistance, and water quality.

The effect of probiotics has been tested on phytoplankton (microalgae), which forms the basis of aquatic food chains, due to its nutrient-producing photosynthetic machinery that in most cases, higher organisms are unable to synthesize such is the case of polyunsaturated fatty acids and vitamins. Within groups of microalgae used in aquaculture are distinguished central diatoms as *Chaetoceros* spp., which have proven to be a good live food; however, production has limitations due to the complexity of their nutritional requirements. Gómez *et al.* assessed the growth of *Vibrio alginolyticus* C7b probiotic in the presence of the microalgae *Chaetoceros muelleri*, organisms can be grown together to achieve high density and fed to shrimp.

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, as well as an imbalance in the gastrointestinal microbiota of aquatic species, which affected their health. In fact, 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 for preventing diseases rather than treating them. Thus, the use of probiotics is a viable alternative for the inhibition of pathogens and disease control in aquaculture species.

Probiotic microorganisms have the ability to release chemical substances with bactericidal or bacteriostatic effect on pathogenic bacteria that are in the intestine of the host, 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/or hydrogen peroxide, as well as alteration of the intestinal pH due to the generation of organic acids.

Gomez *et al.* reported the use of *Vibrio alginolyticus* strains as probiotics to increase survival and growth of whiteshrimp (*Litopenaeus vannamei*), also by using probiotics in Ecuadorian shrimp hatcheries, production increased by 35%, while with the use of antimicrobials it decreased by 94%.

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 as well as provide growth factors such as vitamins, fatty acids, and amino acids. Therefore, nutrients are absorbed more efficiently when the feed is supplemented with probiotics.

In white shrimp *Litopenaeus vannamei* Boone and *Fenneropenaeus indicus*, various strains of *Bacillus* have been used as probiotics to increase apparent digestibility of dry matter, crude protein, and phosphorus. Results showed higher sizes when the diet is supplemented with 50 g of probiotic kg⁻¹ of food. Other research has suggested the importance of managing the probiotic in all ontogenetic stages of the shrimp to generate a constant effect on the production of digestive enzymes.

Water quality improvement: The susceptibility of cultured aquatic species to high concentrations of nitrogenous compounds, such as ammonia, nitrite and nitrate, is generally species-specific but high concentrations of these compounds affect animals in aquaculture and likely cause high mortality. The application of gram-positive *Bacillus* spp. is generally more efficient than the application of gram-negative bacteria species for converting organic matter back to CO₂, which results in the conversion of a greater percentage of organic carbon to bacterial biomass or slime (Verschuere *et al.*, 2000). The effectiveness of aerobic gram-positive endospore-forming bacteria, such as *Bacillus* spp., for improving water quality by affecting the composition and abundance of waterborne microbial populations associated with farmed species was evaluated (Bandyopadhyay and Mohapatra, 2009). *Bacillus* spp. were associated with improvement of water quality, reduction of pathogenic vibrios in culture environment, enhancement of survival and growth rate, and the improved health status of juvenile *Penaeus monodon* (Dalmin *et al.*, 2001; Ngan and Phu, 2011). In parallel, other beneficial bacterial species in the genera *Nitrobacter*, *Pseudomonas*, *Enterobacter*, *Cellulomonas* and *Rhodospseudomonas*, and probiotics derived from plant sources, including yucca extract, potassium ricinoleate, tannic acid and citrus seed extract were also reported to have been used in culture systems noted to have considerable improvement in water quality (Boyd and Cross, 1998; Verschuere *et al.*, 2000). The requirement for the use of candidate probiotics in aquaculture ponds is the enhanced decomposition of organic matter, reduced nitrogen and phosphorus concentrations, improved algal growth, improved availability of dissolved oxygen, suppressed cyanobacteria blooms,

controlled ammonia, nitrite, and hydrogen sulfide concentrations, lower incidences of disease, greater survival, and improved production (Boyd and Cross, 1998).

Prospects and challenges

The use of probiotics in aquaculture is becoming more popular. As described herein, the use of probiotics confers many advantages, such as improved growth, feed efficiency, enhanced immune system response, as well as improved water quality. Further studies are needed to thoroughly understand the mechanisms of probiotics. Probiotics are more effective when used in the early stages of culture. As aquatic animals are in direct contact with their environment, supplementation to the water can be an effective. For example, exposure to probiotics in the feed at the larval stage may lead to the development of a positive transient intestinal flora that may become established at later stages. Further, regular application of probiotics through feed to animals reared in captivity can be used to maintain the microbial population in the gastrointestinal tract at a level that can express sufficient functionality.

In principle, probiotic bacteria were directly isolated from the gastrointestinal tract and were then applied to this host species, but recently, many commercial probiotics have been developed and used. Probiotics have the potential to positively or negatively impact both the animals in aquaculture and the surrounding environment. The identity of the bacteria strain and host is extremely important and determines the characteristics of the relationship. Therefore, the selection and source of probiotics play an important role; in particular, optimization of the probiotic application is important to avoid unnecessary expense. Moreover, mutations may occur in the natural environment; thus, dominant populations of supplied probiotics may become pathogenic and may be harmful to host animals, which are stressed or weakened state of health. A number of probiotic products have been researched as evidenced by their efficacy in aquaculture. Beneficial bacterial inocula that are species-specific probiotics have become widely available to the aquaculture industry. These preparations have been refined to have more effective function as applied probiotics. Further, the quality control of probiotic products should be thoroughly considered. The application of new analysis methods, including molecular methods, for the evaluation of probiotic products and for *in vivo* validation, is expected to significantly improve both the quality and functional properties of probiotics.

Other approaches to disease prevention

The approaches to disease prevention that have evolved in India after the outbreaks of shrimp disease is given below. The guidelines developed are exhaustive and only important aspects are described herein.

Crop Holiday

The widespread disease outbreak in Andhra Pradesh, Odisha and West Bengal necessitated the declaration of a crop holiday. The idea behind the declaration of crop holiday was to allow a period long enough for the culture system and the water source with which it is associated to recover to a degree to permit successful shrimp farming. It was also envisaged that the farmers would dry their ponds thoroughly. This method is proving to be successful, as many farmers restarted farm operation without any problem after observing a crop holiday.

Adequate Pond Preparation

Removal of the accumulated black top soil, drying until cracks develop, plowing and reflowing the ponds two or three times, and sun drying after each crop are now suggested to farmers as approaches to disease prevention. The importance of application of soil & water probiotics for disinfecting the soil & water has been emphasized. Farmers are advised to keep the application of organic fertilizers to a minimum.

Regulating Stocking Density

The ADB/NACA/GOI study conducted in 1995 found that stocking density has a strong influence on the performance of shrimp culture farms (Pathak and Palanisamy 1995). The survey results show some clear differences in the environmental problems in farms related to stocking density. The study also showed that both profit and production increase with increasing stocking density. However, the shrimp farmers faced increased occurrence of disease, environmental problems and conflicts with higher stocking densities. A significant increase in problems is noted when stocking densities exceed 20 PL/m². This information appears to strongly support the government's policy of not promoting intensive shrimp culture (Government of India 1995).

Effluent Treatment Systems

Before the outbreaks of disease, no shrimp farm had put up an effluent treatment system, which was a great omission. It has been estimated that nearly 60% of the feed given turns into biological waste. Therefore, one can imagine the extent of organic load that is being discharged with the effluents in a given area. Such effluent treatment systems have now been made an integral part of farming activity.

CONCLUSIONS

In conclusion, the specific functions of probiotics in aquaculture may not be denied. Probiotics confer benefits of increased disease resistance, improved nutrient digestibility and growth in the host animals, and they also improve culture water quality. Although numerous reports have demonstrated the efficiency of probiotics, most of these studies were conducted and evaluated under laboratory conditions. Therefore, the application of probiotics under culture conditions is necessary in order to accurately evaluate their use. In particular, the consideration of species, source, quality and application methods will be needed to evaluate the use of probiotics.

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