



Use of Probiotics in Shrimp Aquaculture in Bangladesh

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Abstract

Antibiotic resistance in aquaculture has demanded an alternative and ecofriendly disease management approaches for the sustainable development of aquaculture sector and safe food production. The use of probiotic microorganisms has emerged and established itself as a promising alternative to antibiotics because of its ecofriendly nature and efficiency in growth enhancement, increasing disease resistance, and immunomodulation. This paper reviewed the present status of using probiotics in shrimp aquaculture in Bangladesh and their efficiency in prevention of infectious diseases and immunomodulation of shrimp. Information was collected from different secondary sources, compiled systematically and arranged chronologically. This investigation reveals the application of probiotics in shrimp aquaculture in Bangladesh is relatively a new concept but increasing day by day due to its ecofriendly nature. Several probiotic products extracted from different species of bacteria and a yeast are commercially available for use in aquaculture in Bangladesh. Probiotics have gained popularity in shrimp aquaculture in Bangladesh as an alternative to the antibiotic in prevention of infectious diseases of fish. This review also shows that, probiotics have significant effect on growth rate, feed utilization capacity and the immune performance of finfish, shrimp, prawns and crabs in aquaculture.

Keywords: Probiotics; Shrimp Aquaculture; Immunomodulation; Encapsulation; FISH

Abbreviations

SGR: Specific Growth Rate; FCR: Food Conversion Ratio; PER: Protein Efficiency Ratio; WSSV: White Spot Syndrome Virus; IgM: Immunoglobulin M, APS: Astragalus polysaccharide; FISH: Fluorescent in Situ Hybridization; PL: Post Larvae; LAB: Lactic Acid Bacteria

Introduction

Aquaculture is the fastest growing safe food producing industry in the world which provide almost half of the global fish production. It creates employment opportunities for millions of poor and vulnerable people and also provide the major portion of animal protein to the growing population [1]. In Bangladesh, aquaculture production has been increased to 2,060,408 metric tons in 2016 from 712,640 metric tons in 2000 through the implementation of scientific and technological modernization [2]. Shrimp farming in Bangladesh produces 2.5% of the global aquaculture production,

an important source of export earnings for Bangladesh is facing a lot of challenges towards the sustainable expansion [3]. Among the constraints, Disease problems have emerged as major constraints in aquaculture production. The prophylactic application of antibiotics is expensive and detrimental, along with the creation of health hazards due to the antibiotic resistance [4]. In this context, an ecofriendly management approach is the prime demand of shrimp farmers and hatchery owners. Use of probiotics have gained interest as an alternative to the antibiotics in shrimp disease management in aquaculture. Probiotics are live-beneficial microorganisms which confers health benefits to the host if administered in sufficient quantity [5]. Host species cannot utilize many important nutrients, but the gut microbiota can metabolize and convert them to end products like short-chain fatty acids. So, modulation of the intestinal microbiota of aquatic organisms in a positive way is very important which can be done administering probiotics [6]. Probiotics are the bio friendly agents which can be compete with patho-

genic bacteria and promote the growth of the cultured organisms. In addition, probiotics are nonpathogenic, nontoxic in nature, and they do not have undesirable side-effects to the aquatic organisms and many other beneficial effects [7]. The use of probiotics has gained increasing scientific and commercial interest and are now considered as an ecofriendly approach in health-promoting foods to therapeutic, prophylactic and growth supplements. This review was conducted to investigate the present status of using probiotics in shrimp aquaculture in Bangladesh and their efficiency in prevention of infectious diseases and immunomodulation of shrimp. This paper also shows the challenges and the future prospects in sustainable expansion of using probiotics in the shrimp farming in Bangladesh.

Material s and Methods

This is a review article which was conducted based on the secondary data available in published literatures related to the use and efficiency of probiotics in aquaculture such as different national and international journals, research articles, review papers or mini reviews, proceedings, periodicals, reports, relevant books and other sources. Electronic media and news media were other important source for the collection of information. Information was also collected from the websites related to aquatic health management, fish disease diagnosis centers, fisheries research institutes and pharmaceutical company's etc. National and international conference, workshops, and proceedings on the use of probiotic was important source of information. All the information collected from secondary sources, have been compiled systematically and arranged chronologically in this review paper.

Review of Findings

History of Using Probiotics

The Persian version of the Old Testament (Genesis 18:8) states that "Abraham owed his longevity due to the consumption of sour milk." A study reported that the intake of *Lactobacilli* rich yogurt results in a reduction of toxin producing bacteria in the gut. There is another claim that the administration of *Bifidobacteria* to diarrhea suffering infants can supersede the disease causing bacteria. Using probiotics to animals started in 1920's and the term Probiotic was introduced in the 1970s to describe microbial feed supplements given to humans and animals [8]. Probiotics are alive or dead whole microorganism or part of a microorganism or extract of microorganism which confers health benefits to the host when administered at appropriate dose and improve disease resistance,

growth performance, feed conversion ratio (FCR) and stress resistance [9]. The origin of using of probiotics in aquaculture is not clear from the historical perspective and available literatures but, evidence exists on usage of probiotics in extensive finfish and invertebrate culture in China and India. It has been reported that, the word probiotics was first used by Lilly and Stillwell in 1965 to denote health promoting bacteria which was then defined by Fuller as a live microbial feed supplement having ability to improve the microbial balance of host animal in 1989 [10,11].

Source of Probiotics

Westerdahl, *et al.* (1991) preferred the host-derived microorganisms as probiotics to be used against pathogen. Probiotic microorganisms may be derived from intestine or guts of healthy fish, water of rearing environment, sediments of culture tank, other animals, and different fermented food products. The efficacy of commercial probiotic is not clear from the literature stock, but host-derived probiotics were found beneficial to host microbiota isolated from the gastrointestinal tract of aquatic [12]. Those microorganisms are beneficial which lives inside a healthy host and they are argued to be the essential part of natural defense system [13]. Terrestrial microorganisms were also proven ineffective as probiotic for marine organisms.

Kongnum and Hongpattarakere (2012) isolated two hundred and two lactic acid bacteria (LAB) strains of from the digestive tracts several species of adult shrimp including *Litopenaeus vannamei*, *Metapenaeus brevicornis* and *Penaeus merguensis* [14]. Swain, *et al.* (2009) isolated *Streptococcus phocae* and *Enterococcus faecium* from brackish water shrimp and proved increased survival rate against *Vibrio harveyi* and disease resistance to *Vibrio parahaemolyticus* [15].

Application of Probiotics

Immunomodulatory activity of probiotics depends on various factors like source of probiotic, dose of probiotic, method of administration and the duration of supplementation [12]. Proper administration method is a key factor to use the probiotics in aquaculture [16].

1. Direct use of Probiotics in the form of spore with feed pellets is the most common administration technique.
2. The widely used administration strategy is encapsulation. Encapsulation helps by improving nutritional values and proper delivery of the microbe to the host.

3. Immobilization of Probiotic is a new technique, extensively used in dairy and pharmaceutical industries which has been reported to be advantageous [16].

Effects of Probiotics

Several beneficial effects have been reported to provide by probiotics in shrimp aquaculture such as increased survival rate of shrimp [17], improve the population density of beneficial bacterial flora [6], reduce concentrations of nitrogen and phosphorus [18], and increase yields of shrimp [14], and improve water quality [18]. Probiotic treatment with cell-free extracts of *Bacillus subtilis* BT23 significantly reduced the average mortality rate and controlled the growth of disease causing pathogen *Vibrio harveyi* [19]. A study reported that the use of probiotics significantly increased DO concentration, reduced dissolved phosphorus and total inorganic nitrogen, and chemical oxygen demand. This indicates that the addition of the commercial probiotics had a noticeable influence on water quality of shrimp ponds and shrimp production [18]. Probiotics increased the specific amylase activity, increased survival rate and wet weight gain. The feed conversion ratio, specific growth rate, and final production were significantly higher in shrimp receiving the probiotic [20]. Feeding of potential probiotics showed the best feed conversion ratio, effectively reduced the occurrence of disease in shrimp by *Vibrio parahaemolyticus* [21]. Another study showed that the probiotics is a suitable alternative to the use of antibiotics. Obviously, minimizing the risk of vibriosis demands a multi-disciplinary approach, including good hygiene and sanitation measures to reduce the input of potential pathogens, as well as a suitable farm management [4]. Swain, *et al.* (2009) fed the *Penaeus monodon* with four probiotics and concluded that the probiotic strains effectively inhibited the pathogens, increased survival rate to *Vibrio harveyi* and disease resistance against *Vibrio parahaemolyticus*. He proved that the *Streptococcus phocae* and *Enterococcus faecium* isolated from brackish water shrimp is highly potential to control pathogenic vibriosis in shrimp culture [15]. A LAB strain *Lactobacillus plantarum* MRO3.12 showed the highest efficiency in reducing *Vibrio harveyi* pathogen. Supplemented diet containing *Lactobacillus plantarum* showed (L.) significantly increased relative growth rate (RGR), feed conversion ratio (FCR) and survival rate of *Litopenaeus vannamei* [14]. Nimrat, *et al.*, (2012) examined the effectiveness of mixed *Bacillus* probiotics and mode of action on growth, bacterial numbers and water quality in *Litopenaeus vannamei* and found that the Post larvae treated with probiotic ex-

hibited higher growth, high survival of shrimp, increased beneficial bacteria and enhanced water quality [22].

Use of Probiotics in Bangladesh

Bacterial infectious disease is a great threat to the Penaeids shrimp especially Vibriosis, the fatal cause of mass mortality in shrimp hatcheries [38]. Several studies have been conducted on the applicability and acceptability of probiotics in shrimp aquaculture in Bangladesh, even on the isolation of potential probiotics from Shrimp including Rahman, *et al.* (2009), Rahman, *et al.* (2010), Hossain, *et al.*, (2013), Uddin, *et al.* (2013), Ali, *et al.* (2015) and many others. Use of probiotics on poultry in Bangladesh have been studied more than in aquaculture. But use of probiotic in shrimp aquaculture is gaining more attention of shrimp farmers and hatchery owner in Bangladesh due to its ecofriendly nature and efficiency in improving water quality, growth performance and others [39]. Majority of the probiotics used in Bangladesh have been found effective against *Vibrio harveyi*, which causes luminous vibriosis in *Penaeus monodon* [40]. The average body length, and survival rate of *Penaeus monodon* were significantly increased by using probiotics supplemented feed in Bangladesh [41]. Use of probiotics for sustainable shrimp culture in Bangladesh is increasing steadily. A study reported that the use of probiotics in shrimp farming pond increased average final body weight, average survival rate, and the average daily gain (ADG), and average production along with improving water and soil quality, and the health status of shrimp [35]. Another study reported that a Microencapsulated organic acid blend (OAB) in commercial feed significantly increased survival rate, feed conversion ratio, nutrient utilization capacity, pathogenic resistance and enhanced the protein and phosphorous utilization rate of the tiger shrimp (*Penaeus monodon*) [42].

Research Gaps and Future Prospects

Using probiotics with plant product is a promising disease control approach which can improve growth performance, hematological parameters, immune response and disease resistance of fish. But, little information is available on the effect of plant-probiotic mixture in aquaculture [10]. Probiotic products activate the defense mechanism and innate immunity of host, influence the virulence of disease causing pathogen and stimulate the response to stressors, but these aspects have been investigated very few for shrimp [39]. The possible mechanism of action of probiotics largely depend on probiotic-host interactions. But, there is uncertainties

| Probiotic Strain | Isolated from | Applied to Species |
|--|--|---|
| <i>Bacillus</i> S11 | <i>Penaeus monodon</i> | <i>Penaeus monodon</i> , to know the growth and survival |
| <i>Bacillus subtilis</i> BT23 | Shrimp culture ponds | <i>Penaeus monodon</i> , against the growth of <i>Vibrio harveyi</i> |
| <i>Pseudomonas</i> sp. PM11 <i>Vibrio fluvialis</i> PM17 | Gut of farm reared sub-adult shrimp | <i>Penaeus monodon</i> , Immuno-modulation of shrimp |
| <i>Arthrobacter</i> XE-7 | <i>Penaeus chinensis</i> | <i>Penaeus chinensis</i> , to protect the PL from <i>Vibrio</i> spp. |
| <i>Bacillus</i> spp. | Shrimp digestive tracts | <i>Fenneropenaeus indicus</i> , digestive enzyme activity, survival and growth |
| <i>Bacillus subtilis</i> <i>B. megaterium</i> | Marine environment | Production of digestive enzymes |
| <i>Paenibacillus</i> spp., <i>B. cereus</i> , <i>Pa. polymyxa</i> | Seawater, sediment and marine fish-gut | Against pathogenic <i>Vibrio</i> species |
| <i>Synechocystis</i> MCCB 114 and 115 | Seawater | <i>Litopenaeus vannamei</i> , against <i>Vibrio harveyi</i> |
| <i>Bacillus licheniformis</i> | Shrimp pond | <i>Litopenaeus vannamei</i> , to increase the immunity |
| <i>Lactobacillus plantarum</i> | Shrimp isolate | <i>Litopenaeus vannamei</i> , to increase immune response against <i>Vibrio</i> sp. |
| <i>V. alginolyticus</i> UTM 102, <i>B. subtilis</i> UTM 126, <i>R. gallaeciensis</i> SLV03 <i>P. aestumarina</i> SLV22 | <i>Litopenaeus vannamei</i> | <i>Vibrio parahaemolyticus</i> , survival against <i>Vibrio parahaemolyticus</i> |
| <i>Bacillus subtilis</i> UTM 126 | Shrimp culture pond | <i>Litopenaeus vannamei</i> , for Protection against vibriosis |
| <i>Bacillus</i> sp. | Digestive tract | <i>Penaeus vannamei</i> , growth performance and enzyme activity |
| <i>Pediococcus acidilactici</i> | Strain MA 18/5M, CNCM | <i>Litopenaeus stylirostris</i> Survival against <i>Vibrio</i> sp. |
| <i>B. subtilis</i> , <i>B. natto</i> , and <i>B. licheniformis</i> | Not available | <i>Litopenaeus vannamei</i> , for growth and enzyme activity |
| <i>Streptococcus phocae</i> PI80, <i>Enterococcus faecium</i> MC13 | Marine fish and shrimp intestine | <i>Penaeus monodon</i> , resistance against Vibriosis |
| <i>Lactobacillus plantarum</i> | Digestive tract of wild shrimp | <i>Litopenaeus vannamei</i> , growth and survival against <i>Vibrio harveyi</i> |
| <i>Bacillus</i> | <i>Penaeus monodon</i> intestine and cultured environments | <i>Litopenaeus vannamei</i> , enhance bacterial numbers, water quality and growth |
| <i>S. faecalis</i> | <i>Penaeus monodon</i> intestine | <i>Penaeus monodon</i> , growth and Survival |
| <i>Bacillus</i> NL 110, <i>Vibrio</i> NE 17 | <i>Penaeus monodon</i> intestine | <i>Macrobrachium rosenbergii</i> , growth performance |
| <i>Lactobacillus plantarum</i> , <i>L. fermentum</i> , <i>Bacillus subtilis</i> , <i>B. licheniformis</i> , <i>B. megaterium</i> , <i>Nitrobacter</i> sp., <i>Nitrosomonas</i> sp. | Shrimp intestine and culture water | <i>Litopenaeus Vannamei</i> , Boone growth, mortality, and feed conversion ratio |

Table 1: Potential Probiotic microorganisms evaluated for use in shrimp aquaculture [6,14-15,19-37].

| Sl. No. | Composition | Effects on Host Species | Trade Name |
|---------|--|--|--------------|
| 1 | <i>Rhodopseudomonas</i> sp. | Increase growth rate and disease resistance | Aqua gold |
| 2 | <i>Bacillus subtilis</i> <i>Rhodopseudomonas</i> sp. | Control toxic gas formation in the sediment and increase plankton growth | Aqua photo |
| 3 | <i>Bacillus Subtilis</i> <i>Saccharomyces cerevisiae</i> | Increase immunity, help in digestion | Bio-zyme |
| 4 | Coated Vitamin C | Increase disease resistance | C-150 |
| 5 | <i>Bacillus Subtilis</i> , <i>Bacillus amyloliquefaciens</i> , <i>Bacillus pumilis</i> and <i>Bacillus megaterium</i> | Control vibriosis and luminescent bacteria | Eco marine |
| 6 | <i>Bacillus</i> sp. | Control vibriosis, luminescent bacteria | Ecomax |
| 7 | <i>Yeast</i> , <i>Bacillus subtilis</i> and <i>Lactobacillus</i> sp. | Waste purification and ensure maximum use of feed | Golden Bac |
| 8 | Major vitamin and minerals | For better health | Mutagen |
| 9 | <i>Bacillus</i> sp. | Improve water quality and control pH | pH fixer |
| 10 | <i>Bacillus</i> sp. <i>Rhodococcus</i> , and <i>Rhodobacter</i> | Control unwanted gas, sediment and arrests the pathogens | Procon-PS |
| 11 | <i>Bacillus</i> sp. | Reduce pathogenic bacteria in water | Super Biotic |
| 12 | <i>Rhodobacter</i> sp. <i>Rhodococcus</i> sp | Improve soil quality and reduce toxic gas from bottom | Super PS |
| 13 | <i>S. faecalis</i> and other bacteria | Inhibit pathogenic bacteria | Zymetine |

Table 2: Probiotics used in Shrimp culture in the southwestern coastal zone of Bangladesh [35-41,43-44].

about the longevity of protection conferred by probiotics [31]. To date, aquaculture studies on probiotics in combination with plant products and b-glucan, have not investigated properly, the effect on epithelial barrier function, gut integrity and architecture, disease resistance against pathogenic bacteria [27]. Successful formulation of probiotics in combination with plant products and b-glucan is a complex issue but, very little information is available from aquaculture studies [40]. Only few commercial probiotics have been licensed for use in shrimp aquaculture in Bangladesh, but in near future use of probiotics in aquaculture will be rapidly expanded. So it an urgent need to isolate new indigenous probiotics to fulfil the specific requirement in Bangladesh [39]. Probiotics in aquaculture have provided numerous beneficial effects on fish health and sustainable aquaculture management by reducing in the risk of disease occurrence, but the large scale application of probiotics in the development of commercial aquaculture has been constrained due to problems associated with handling, pelleting and storage [8]. Probiotics are highly sensitive to environmental factors, so new technology is required for fermentation. The available probiotics are produced using conventional batch fermentation and suspended culture System. So novel cultivation approaches should be developed for process optimization [45]. Proper identification

strategies should be developed because there was a misidentification in case of complex genera *Lactobacillus* and *Bifidobacterium*. More than 28% of commercial bacteria culture for use as probiotic were misidentified at the genus or species level due to the lack of proper microbiological procedure and label correctness of probiotic products [46]. Combined use of probiotics and plant products is in its infancy, so the topic deserves further attention. An aspect that deserves attention is the dietary supplement, combination of probiotic bacteria and plant-based replacers in aqua feeds, as the continued growth and intensification of aquaculture has increased inclusion of plant based replacers in diets [27]. Further investigations on these issues alongside an environmental impact assessment of probiotics are key requirements to realize desirable outcomes in aquaculture. It has been widely accepted that 16S rDNA sequencing analysis may be universally regarded as the best tool for the taxonomic positioning of probiotic cultures. But, it has a limited resolution for the discrimination of several closely related lactic acid bacterial species used in probiotic production [45]. Probiotic strains derived from the terrestrial environment are seldom successful in shrimp aquaculture due to their lack of competency in aquatic environment Therefore, exploring the marine bacterial endosymbionts would be a prospective approach for developing novel shrimp probiotics.

Conclusions

The probiotics in aquatic environment is still a controversial concept due to lack of authentic evidence or real environment demonstrations on the successful use of probiotics and their mechanisms of action. The present review highlights the potential sources of probiotics, mechanism of action, diversity of probiotic microbes and challenges of probiotic usage in shrimp aquaculture. Probiotic is an alternative to antibiotics and chemicals in aquaculture which provide better health benefits, higher growth rate, increased survival rates and produce safe organic fish products to meet the protein requirements of future generations. Further research on probiotics should focus on molecular biotechnology tools to gain a greater understanding of the modes of action because the exact mode of action of probiotics is not totally known in fish. Using immunohistochemistry, gene expression and proteomics can be used to explore the mechanisms of actions of probiotics. Researches on the interaction between probiotics and carbohydrate-to-lipid ratio can reduce pressures on feed nutrition in the intensive aquaculture industry. The FISH technique is a potential tool to characterize the dynamics of potential probiotic bacteria and their efficiency in the control of pathogenic bacteria in pathogen detection.

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Conflicts of Interest

The author declares that there is no financial interest or conflicts of interest regarding the publication of this paper.

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