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Mst. Nahid Akter
Dept. of Aquaculture, Faculty of Fisheries, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh

Imran Parvez
Dept. of Fisheries Biology and Genetics, Faculty of Fisheries, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh

Zubaida Parveen Patwary
Dept. of Aquaculture, Faculty of Fisheries, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh

Correspondence
Mst. Nahid Akter
Dept. of Aquaculture, Faculty of Fisheries, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh

Beneficial effects of probiotics in aquaculture

Mst. Nahid Akter, Imran Parvez and Zubaida Parveen Patwary

Abstract
With the growing claim for environmentally pleasant aquaculture, the application of non-antibiotic eco-friendly agents such as probiotic which is being considered one most significant tool for health management in the field of aquaculture. Generally, probiotics are live micro-organisms, which, when administered in adequate amounts, confer health benefits to the host. These microorganisms are able to colonize and multiply in the intestine of the host and therefore show numerous beneficial effects by modulating various biological systems in the host. Probiotics or their secreted products found to use in aquaculture to control disease, and replace the use of chemotherapeutic agents. A large quantity of beneficial Gram-positive as well as Gram-negative bacteria, microalgae, and yeasts have been evaluated as probiotics in aquaculture. Probiotics application in poultry and swine rearing are well documented, while little has been concentrate to include them into aquaculture. This review provides a concise current knowledge on probiotics, modes of their application, beneficial effects on aquaculture and its application in Bangladesh.

Keywords: Probiotic, aquaculture, disease outbreak, chemotherapeutic agents

1. Introduction
Presently the world is facing numerous interrelated challenges because of the negative effects of the ongoing economic crisis caused by the remarkable climate change. Simultaneously, another momentous anxiety is the requirement to meet up the nutrition needs of the progressively growing global population. Globally, the fisheries sector provides immense opportunities to accomplish food security, create economic growth to lessen poverty, as well as to confirm better utilization of all types of fisheries assets. Generally, world aquaculture production has grown more rapidly compared to the world population growth and aquaculture has been considered as the most significant food production sectors. With the increased intensification and commercialization of aquaculture practice, fish health has turned into a most important issue to aquaculturists [11]. In aquaculture, application of antibiotics was generally used as the most familiar technique for dealing with the incidence of bacterial diseases [27]. The indiscriminate use of these antibiotics for maintaining bacterial infection has been accountable for the development of antibiotic resistant bacteria, that has significant effect on the reduction of the efficiency of a treatment option and may be liable for long term unpleasant impacts in the aquaculture environment [13, 53] such as accumulation of those antibiotics in fish body tissues, reduction of beneficial microbiota and immune suppression of fish [43]. Among all of the mentioned unpleasant impacts, the development of antibiotic resistant bacteria [24] has paying more attention globally. Due to the threat related with the application of antibiotics, the improvement of a non-antibiotic eco-friendly agent is being considered as the most significant factors for proper health maintenance in aquaculture. Therefore, many aquaculturists have proposed a number of alternative approaches in order to build up environmental friendly aquaculture, one of them is the use of probiotics as bio-control agents in place of the use of chemotherapeutics in aquaculture [16, 39].

2. Selection of Probiotics
The main objective of using probiotics is to re-establish a favourable association between beneficial and harmful microorganisms that make up the microbiota of the intestine of fish. A well-known probiotic should have few specific characteristics in order to exert a positive impacts.

2.1 Characteristics of Probiotic

A high-quality probiotic should have the following mentioned characteristics [19]:

- Should be a strain which is capable of exerting a beneficial effect on the host animal, e.g. increased growth or resistance to disease.
- Should not have any side effect; should neither be pathogenic nor toxic, not only with regard to the host species but also with regard to aquatic animals in general and human consumers.
- Should be viable under normal storage conditions and able to survive during industrial process.
- Should be capable of surviving and metabolizing in the gut environment, e.g. resistant to bile and low pH due to organic acids enrichment.
- Possess high ability to multiply in the intestine.
- Possess strong adhesive ability with the gut of the fish.
- Should have strong antagonistic activity against pathogenic microorganisms.

3. Modes of Application of Probiotics in Aquaculture

Probiotics can be applied through feeding, injection or immersion or directly by adding in the water [22].

3.1 Application in Feed

Usually, probiotics are used by adding directly in the feed ingredients or by spraying in the prepared feed. In aquaculture, commonly used probiotics are *Lactobacillus* sp., *Bacillus* sp. or *Saccharomyces cerevisiae* [39]. According to the guidelines of Food and Agriculture Organization (FAO) and (World Health Organization (WHO), probiotics should have the capability of surviving while passing through the gut as well as resisting the gastric juices and bile [45]. Additionally, probiotics should have ability to flourish and settle in the gut, which should be safe and effective for the host species.

3.2 Application through Immersion or Injection

Probiotics also can be applied by injecting or immersion [5, 55]. A previous study also demonstrated the effect of administration of a well-known probiotic *Micrococcus luteus* by intra peritoneal injection to *Oreochromis niloticus* and observed only 25% mortality while 90% mortality with *Pseudomonas*.

3.3 Direct Application to Culture Water

Probiotic application directly in ponds and tanks water also exhibited beneficial effect on fish health by modulating microbial composition and water qualities of water and sediments [52]. Probiotic bacteria, particularly *Bacillus* spp. and some other species including *Aerobacter* sp., *Nitrobacter* sp. and *Saccharomyces cerevisiae* (yeast), played a remarkable task in the water quality improvement. As a previous study reported that the Gram-positive bacteria such as *Bacillus* spp. are able to change the organic matter into CO_2 efficiently, on the other hand the Gram-negative bacteria are capable to alter a larger amount of organic substance into bacterial biomass or slim [24, 29]. Besides, some probiotic strains are able to provide algicidal activity against some microalgal species, specifically the red tide plankton [18]. The application of nitrifying bacteria into the culture system also effectively removed the excess of ammonia and nitrite from the water which are considered as a foremost water quality problem [26].

4. Beneficial Effects of Probiotics

4.1 Effect of Probiotics on Intestinal Ecosystem

The gastro-intestinal (GI) tract of fish has been recognized as significant harbours of a multiple microbial community, particularly two discrete groups, such as autochthonous (indigenous) and allochthonous (exogenous) [31]. Autochthonous microorganisms have significant effects in the improvement of GI tract of fish, including development and maturation of the intestine and immune system [9, 35, 31] and resistance to infectious pathogenic microbiota [10, 38]. These microorganisms are also able to obstruct the colonization of some other bacteria using several mechanisms such as competition for space and food, secreting antimicrobial compounds as well as being receptors at mucosal surfaces [31]. In that way, probiotic species provide the most favourable positive impacts on the host body by modulating its intestinal microbial balance [23]. Few studies have been carried out to assess the beneficial impacts of probiotics administration on the intestinal autochthonous microflora of fish [31, 28]. As previous studies only have emphasised on the cultivation-based techniques in order to know about intestinal microbial population, which distinctly only permit the study of culturable bacteria, while a large number of non-culturable bacteria are lack behind, which may account a enormous effect in the intestine of fish [31, 28, 56].

4.2 Effect of Probiotics on Digestive Enzymes

The digestive organs of an animal have an immense influenced on the composition of food and are accountable for immediate changes in the digestive enzymes activities [47], which is simultaneously related with the fish health as well as growth. Even, the effective utilization of feed in fish also seriously influenced by the existence of various digestive fluids and enzymes. The digestion of carbohydrate, fat and protein are mainly dependable on various types of enzymes such as amylase, lipase and protease respectively. There is evidence of increasing feed utilization and growth of Nile tilapia after feeding probiotic diets by increasing the digestion of starch, fat and protein [14]. Increased digestive enzyme activities including amylase, lipase and protease were also noted in *Labeo rohita* fed with a combination of three probiotics such as *Bacillus subtilis, Lactococcus lactis* and *Saccharomyces cerevisiae* [29]. Bacteria and their secreted enzymes have played a significant influence on the digestion process of fish [30] by increasing their total digestive enzyme activities in the intestine [54]. The enzymes that are secreted by the probiotic bacteria are recognized as exogenous enzymes. These exogenous enzymes can help to induce the production of endogenous enzymes [29] that are able to endure a broad range of pH compared to those endogenous enzymes, which ultimately able to make delay the digestion phase [14], in that way they assured the higest utilization of digested nutrients. Nevertheless, it is complicated to differentiate between endo enzymes secreted by fish and exo enzymes produced by the probiotics [14]. There is also evidenced that probionts secreted exoenzymes are capale of increasing the digestive utilization of feed [29]. Probiotics not only have shown a positive effect on the digestive systems of fish, but also in the absorption of digested food components [22].

4.3 Effect of Probiotics on Intestinal Morphology

Probiotics could colonize and implement their beneficial activity in the intestinal region of animals which is being considered as a complex harbour of non-pathogenic, pathogenic and commensal microorganisms [31]. Intestinal

development can be performed by the existance of beneficial endogenous intestinal microbiota which can play as vital components in the regulation of mucosal development and tolerance [9]. Probiotics are capable of retaining the healthy intestinal condition by reducing the amount of harmful pathogenic microorganisms that persist within the GI tract of fish and which can develop the structure of the intestinal epithelial layer by lowering the mucosal damage and increasing of absorption of nutrients [28]. Supplementation of probiotic (*L. acidophilus*) added diet to the Senegalese sole [8] represented significantly increased villus length, which recommends that an enlarged surface area capable of better assimilation of digested nutrients [12]. The probable mechanism is might be during moving through the stomach, the probiotic bacteria able to propagate in the intestine of fish and can utilize the sugar in diet in the hind gut as their main growth factors, thus making a component, such as short chain fatty acids (SCFAs) during the fermentation process, which might play a momentous responsibility in rising the villus height [34].

4.4 Effect of Probiotics on Haematological Parameters

It is well documented that haematological parameters strictly influenced the physiological state of a fish which also gives very important clue on the health condition of cultured fish [20]. Several researches have been reported the optimistic outcome of probiotic on the haematological parameters including white blood cell (WBC) count, red blood cell (RBC) count, packed cell volume (PCV) and haemoglobin (Hb) content in fish [33]. Haematological parameters, specially PCV, total and differential leukocyte count in the blood, provide an indication of the health status of the fish [42]. Two weeks after feeding with Gram-positive as well as negative probiotic bacteria at 10^7 CFU/g of diet, the amount of total RBC augmented immensely [22]. A previous study [15] also reported increased number of RBC, WBC and Hb content in rainbow trout supplemented with LAB added diets. WBC predominantly macrophages and neutrophils have shown direct effect on the health condition of fish by rising the innate immunity of the fish. Several researches also have been reported that probiotics are capable of actively increased the production of both B and T lymphocytes in fish [1] which may act as an important function to recognize the specific antigens by the formation of antibodies, or destroying the infected cells that possibly will cause damage.

4.5 Effect of Probiotics on Immune Response

The immune functions of a fish has two basic parts such as the innate or nonspecific, and the adaptive or specific immune system unlike higher vertebrates. A series of humoral as well as cellular factors are involved in the stimulation of both immune systems. Actually, probiotics afford defence against pathogenic organisms through the stimulation of fish immune responses by destroying the negative impact of antibiotics and chemotherapeutic agents. The components produced by bacteria are able to augment the immune response of fish and crustacean [40]. Additionally, probiotics such as *L. acidophilus* and *B. subtilis* can be applied efficiently to improve the health status, thus increasing the disease resistance capability of Nile tilapia and improving the growth response by rising the non-specific immune response [2]. An improved growth performance by increasing feed utilization, immune response and survival against *Vibrio harveyi* were also evidenced in Cobia fed with compound probiotic diets, might be due to increase the non-specific immunity of Cobia after feeding probiotic supplemented diets [17]. Many antimicrobial peptides are exist in the blood serum of fish and among them the most important are immunoglobulin and lysozyme, which may play as the first line of defense. Consequently, they are capable of preventing the colonization of pathogenic microorganisms thus leading to the prevention of disease [3].

Lysozyme is a protein that is found in various vertebrates including fish, which act as most defensive activity against invasion of Gram-positive and certain Gram-negative bacteria, by breaking of the β-1, 4 glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan layer of bacterial cell walls [3, 6] can be increased after feeding with probiotic supplemented diets. Lysozyme activity in fish has been reported to increase either when they were challenged with pathogenic bacteria and/or as a result of natural bacterial infection [7]. Similar results have also been reported from a different experiment where, higher lysozyme content were obtained in the serum of snakehead fish fed LAB supplemented diet [49].

Another most significant humoral immune factor in fish body is immunoglobulin, which performs a considerable function in the prevention of the harmful microorganisms [3]. There is evidenced that probiotics are able to improve the innate and specific immune response of fish by the improvement of total immunoglobulin production [32]. A significantly higher total immunoglobulin content were also reported in the serum of African catfish [1] when fed with *L. acidophilus* added diet.

4.6 Effect of Probiotics on Growth and Feed Utilization

One of the most expected consequence of using probiotic bacteria is the direct effect of probiotic on the growth performance of fish either by direct increment in nutrient uptake [22], or by providing the nutrients [24, 41]. Several studies have demonstrated the positive effects of one most vital group of probiotic bacteria such as *Lactobacillus* sp., on the growth response of Nile tilapia [25] freshwater prawn [51], gilthead sea bream [48], African catfish [1], Persian sturgeon, beluga fry [4]. The reason of improved growth performance of cultured fish after feeding with probiotic diets might be due to improved quality and feed efficiency of diet [1], which ultimately stimulate the appetite of fish [22].

5. Present Status of Probiotics Use in Shrimp Farms in Bangladesh

The beneficial outcome of using certain probiotics in poultry sector has been documented in Bangladesh. However, in compare to the poultry sector, the use of those probiotics in Bangladesh aquaculture is relatively new concept. At present the application of probiotics has gaining more interest in Bangladesh aquaculture specifically in shrimp hatcheries and grow out ponds with the demand to make it environment friendly which have initiated several research projects to document the current status on probiotic use to stimulate survival and growth performance of shrimp, improving water and soil quality.

An experiment reported that the growth of a few number of *Vibrio* spp. can be maintained by a large number of indigenous microflora and majority of the probiotics found effective against *V. harveyi*, which causes luminous vibriosis in the hatcheries and grow-out ponds of *Penaeus monodon* [37]. The identified probiotic bacteria, *Bacillus licheniformis* displayed *in vitro* vibriocidal activity against 60% of the isolated *Vibrio* species from the similar water sources. Further characterization of the isolate unveil that higher activity was recorded in the neutral and slightly alkaline pH at 30 ˚C and

the antagonistic property was refractory to the surfactants used [44]. Similarly *Lactobacillus* spp. isolated from curd samples clearly outcompete the growth of pathogenic bacteria: *Vibrio* spp. and *Shigella* spp. that cause mass mortality in post larvae of *Macrobrachium rosenbergii* in Khulna and Satkhira region [36]. Another study reported that the average final body weight of harvested *Penaeus monodon* was significantly increased ($P < 0.01$) and survival rate was 19.67% more in probiotic used ponds as compared to controlled ponds in Gazi Fish Culture Limited located in Dacope, Khulna, Bangladesh [21]. Another study evaluated that the average length, muscle gut ratio and final survival rate were significantly improved with reduced ammonia concentration, less fouling organisms and not as much of size variation of PL_{15} (*Penaeus monodon*) in comparison with probiotics unsupplemented tanks during the eight production cycles at Prime Shrimp Hatchery Limited, south-eastern coastal region of Bangladesh [50].

Major companies involved in producing and marketing of probiotics in Bangladesh are Organic Pharmaceuticals Ltd., ACI Animal Health, C.P. Bangladesh Co. Limited, Univet Limited, Rals Agro Ltd., Fishtec BD Limited. In an experiment conducted in south west coast of Bangladesh found 13 branded probiotics were available in the market to use mainly in shrimp culture to control vibriosis, luminescent bacteria, improve water and soil quality and control pH [46] (Table 1). The species currently being used as probiotics are varied and many. These are mainly different concentration of beneficial bacteria which include *Bacillus* sp., *Rhodobacter* sp., *Rhodococcus* sp., *Streptococcus faecalis*, etc.

Table 1: Probiotic used in shrimp culture in Khulna region [46]

Trade name	Composition	Purpose of use	Dose	Source	Price (Tk)
Aqua gold	*Rhodopseudomonas* sp.	Increase growth rate and disease preventive power	2 ml/100 dec.	Organic Pharmaceuticals Ltd.	–
Aqua photo	*Bacillus subtilis* and *Rhodopseudomonas*	Control unwanted gas, sediment and increase growth of plankton	50–70 ml/100 dec.	ACI Animal Health	350/L
Bio-zyme	*Bacillus Subtilis, Saccharomyces cerevisiae*	Increase immunity, help in digestion	500 g/100 kg feed	Bio	–
C-150	Coated Vit-C	Increase resistant power for shrimp	5 g/kg feed	CP Aquaculture	1050/kg
Eco marine	*Bacillus Subtilis, B. pumilis, B. amyloliquefaciens, B. megaterium*	Control vibriosis and luminescent bacteria	3–4 tablet/acre	Organic Pharmaceuticals Ltd.	–
Ecomax	*Bacillus* sp.	Control vibriosis, luminescent bacteria	6–8 tablet/acre	Organic Pharmaceuticals Ltd.	–
Golden Bac	Yeast, *Bacillus subtilis, Lactobacillus* sp.	Waste purification and ensure maximum use of feed	1.5–2 kg/acre	Univet	–
Mutagen	Major vitamin and minerals	For better health	5 g/kg	CP aquaculture	230/kg
pH fixer	*Bacillus* sp.	Improve water quality and control pH	1–2 kg/acre	CP Aquaculture	325/kg
Procon-PS	*Bacillus* sp. *Rhodococcus,* and *Rhodobacter*	Control unwanted gas, sediment and arrests the pathogens	5 L/hac (1 m depth)	Rals Agro Ltd.	450/L
Super Biotic	*Bacillus* sp.	Reduce pathogenic bacteria in water	1–2 kg/acre	CP Aquaculture	550/kg
Super PS	*Rhodobacter* sp. *Rhodococcus* sp.	Improve soil quality and reduce toxic gas from bottom	4–6 L/acre	CP Aquaculture	200/L
Zymetine	*S. faecalis* and other bacteria	Inhibit pathogenic bacteria	5 g/kg	CP Aquaculture	650/kg

In Bangladesh sustainable shrimp culture with probiotics is increasing day by day and still further research is needed to test their efficacy in Bangladesh environment to identify the mechanisms of probiotics on *P. monodon* growth, digestibility and survival and its effect on immunity and disease resistance.

6. Conclusion

The evaluation of probiotics used in the aquaculture of Bangladesh is still in its prelinminery phase. Probiotics application in Bangladesh is only given improtance in the farming of shrimp, while a large number of fin fishes are lack behind. Beside, only few commercial probiotics are available and licensed for using in Bangladesh. In near future, probiotics will gain more acceptance in the aquaculture of Bangladesh, and their application will be expanded rapidly. Therefore, there is an urgent necessity to isolate new probiotics from the indigenous environment to fulfil the specific requirement in Bangladesh. The application of probiotics in aquaculture has revealed numerous useful impacts on fish health by reducing the risk of diseases, which also considered as an important step in maintaining sustainable aquaculture. Although the benificial effect of probiotics are well established, the large scale application of probiotics in the development of commercial aquaculture has been constrained due to problems associated with handling, pelleting and storage. Moreover, the quality control of a probiotic in the aquaculture of Bangladesh will be a vital concern.

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