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Review Article

Effects of Probiotic *Bacillus* on Growth Performance, Immune Response and Disease Resistance in Aquaculture

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ABSTRACT

Aquaculture is one of the fastest-growing animal food-producing agricultural industries in the world and proper performance of fish in morphological, physiological and immunological aspects is important for fish production and sustainable expansion of aquaculture. But several inhibitors like disease, pathogen, and adverse environment can overpower these performances. At present, antibiotics in preventing these inhibitors have been seen as becoming favorable to those inhibitors. So, *Bacillus*, an important group of probiotic bacteria can be an alternative to these antibiotics in aquaculture. *Bacillus* has been seen used in different experiments, mainly as a supplement in feed at various concentrations. *Bacillus* showed effective results like improved growth with minimum cost, improvement in reproduction, hematology, improved immune response and disease, and stress resistance as well as better proximate composition in different fish species. Application of *Bacillus* strains has proven efficient in improving water quality by reducing ammonia and nitrite toxicity, harmful algal blooms and utilization of H+ ion. Larger application of probiotic *Bacillus* instead of the hazardous synthetic chemicals would promote eco-friendly low-input sustainable aquaculture for food and nutritional security of the increasing world population. So many more experiments should be conducted in commercially important fishes for better growth and health of fishes which will certainly increase fish production.

Keywords: Probiotics; *Bacillus subtilis*; Growth performance; Immune-hematological parameters; Stress resistance

INTRODUCTION

Aquaculture is one of the most important and fastest-growing animal food-producing agricultural industries in the world that provides 47% of the total food fish supply to the global population [1-3]. The global aquaculture production has been rapidly increasing and almost doubled within the last decade due to expansion, diversification, and intensification of aquaculture activities [4]. The rapid expansion of aquaculture also increased the occurrence of infectious diseases resulting from high stocking densities and stress conditions that favored the spread of pathogens [5]. Severe economic losses could have occurred in intensive fish production due to exposure to microbial diseases [6]. The approach of bacterial diseases depends usually on the abuse of chemicals and antibiotics that negatively affect the fish, environment, and humans, leading to the production of antibiotic-resistant pathogens, immune suppression, and disturbance to the gastrointestinal bacterial population [7-10]. In this context, the use of live beneficial microorganisms "probiotic" is expected to be an eco-friendly alternative for chemotherapeutics [11]. Probiotics can exert beneficial effects through enhancing growth performance, improving intestinal morphology, improving gut microbiota, providing digestive enzymes, competing for the pathogenic bacteria by inhibitory substances production and enhancing the immune response, and inducing the pro-inflammatory cytokines [12-18].

Bacillus sp is one of the most commonly used probiotics in aquaculture and has beneficial effects on fish performances [19]. *Bacillus* spp. are aerobic, Gram-positive, rod-shaped heat-stable and spore-forming bacteria belong to the phylum Firmicutes [20,21]. Besides, *Bacillus* sp has antibacterial activities, can survive in acidic media or high concentration of bile, able to colonize in the gut and can produce digestive enzymes [22,23]. *Bacillus* is a genus of Gram-positive bacteria and the species belongs to *Bacillus* are the

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obligate aerobes or facultative anaerobes. They will test positive for the enzyme catalase when there has been oxygen used or present [24]. Under stressful conditions, the bacteria can produce oval endospores that don't seem to be true spores but the bacteria can reduce themselves to a dormant state and remain for very long periods. These characteristics originally defined the genus, but not all such species are closely related, and many have been moved to other genera of Firmicutes [25]. Research on the live-cell preparations in aquatic organisms is being raised to sustain the aquaculture industry. *Bacillus* spp., *Lactobacillus* spp., *Bifidobacterium* spp., *Lactococcus* spp., and *Saccharomyces cerevisiae* are the common probiotics employed for growth improvement in carps [26,27]. Among *Bacillus* spp. the most widely used species include *Bacillus subtilis*, *Bacillus cereus, Bacillus coagulans, Bacillus clausii, Bacillus megaterium, Bacillus licheniformis, Bacillus circulans, Bacillus aerius* and *Bacillus polymyxa* [28-30].

LITERATURE REVIEW

This study reviewed the efficiency of *Bacillus* spp. as a probiotic, and the major beneficial roles of probiotic *Bacillus* for promoting the sustainable aquaculture considering the antagonistic, health-promoting, immunity-inducing, bioremediation and other beneficial properties for the immense welfare of fish and fish production.

Probiotics in aquaculture

Fish is the richest source of animal protein and is the fastest food-producing sector in the world. Worldwide, people obtain about 25% of their animal protein from fish and shellfish and consumer's demand for fish continues to climb [31]. The production can be maximized through intensification with the addition of commercial diets, growth promoters, antibiotics, and several other additives. Application of these products leads to high production beyond any doubt, but the most worrisome factor is that the routine use of these measures causes severe complications and even a stage has come where its sustainability is in the stake [32]. In aquaculture practices, probiotics are used for a long time however within the most recent couple of years probiotics turned into a basic part of the aquaculture practices for improving growth and disease resistance. This strategy offers plentiful advantages to overcome the limitations and side effects of antibiotics and other drugs and also leads to high production through enhanced growth and disease prevention [33-36].

The Greek word probiotic means "for life", was introduced by Parker [31]. Probiotics are living organisms and substances, which contribute to intestinal and microbial parity. A more extensive meaning of the term probiotic is characterized as a live microbial aide who beneficially affects the host by modifying the host-associated microbial community and the related microbial network, by ensuring improved use of the feed or upgrading its dietary benefit, by enhancing the host reaction towards disease, or by improving the condition of its surrounding environment [37,38]. According to the currently adopted definition by FAO/WHO, probiotics are live microorganisms, which when administered in sufficient amounts confer a health advantage on the host animal [39].

A probiotic organism must have resistance to the acidic stomach environment, bile and pancreatic enzymes, accession to the cells of the intestinal mucosa, capable of colonization for a long period of time they can stay alive so that they can colonize the host efficiently, capable of producing antimicrobial substance against the pathogenic bacteria, and the absence of translocation and should be non-pathogenic and non-toxic [40]. Probiotic bacteria have become the fore of current research in fish culture because of their excellent effects in improving fish growth and health while ensuring environmental safety [41]. Several works [42-44] have reported growth enhancement following probiotic application in fish culture. In addition, increased of the activities of lysozyme, peroxidase, superoxide dismutase (SOD), catalase (CAT), protease, and anti-protease and immunoglobulin M (IgM) levels have been reported in serum and mucosal surfaces which are important defense molecules against many infectious pathogens in mass-cultured fish [45-47].

Probiotics are microorganisms that are claimed to supply health benefits when consumed [48]; the term became more common after 1980 and therefore being widely-used from 1989. The definition of the probiotics may be a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance [49]. The introduction of the concept (but not the term) is mostly attributed to laureate bacteriologist Elie Metchnikoff who postulated that yogurt-consuming Bulgarian peasants lived longer lives [50]. The dependence of the intestinal microbes on the food made it possible to adopt measures to change the microbiota in our bodies and replace the harmful microbes by using useful microorganisms [51].

Probiotics are initially defined as organisms or substances that contribute to intestinal microbial balance [37]. The term probiotic has emerged from the Greek words "Pro" and "bios" meaning "for life" and is commonly recognized as a support for living organisms that helps naturally to enhance the health condition of the host animal. According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are live microorganisms that, when administered in an appropriate amount provides health benefits to the host [52,53].

Probiotics are often defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance [49]. It can even be defined as a viable micro-organism which when ingested in a sufficient quantity confers a beneficial effect on the host due to an improvement of the intestinal microbial balance [54]. A decent definition for probiotics in aquaculture is, "live, dead or component of a microbial cell that when administered via the feed or to the rearing water benefits the host by improving either disease resistance, health condition, growth performance, feed utilization, stress response or general vigor, which is achieved a minimum in partially via improving the hosts microbial balance or the microbial balance of ambient environment" [55]. Another definition of probiotics is employed in aquaculture as "live microbial cultures added to feed or environment (water) to extend viability (survival) of the host" [56].

Nevertheless, aquaculture has been an expansion undertaken by FAO/WHO to incorporate a wide range of Gram-positive and Gram-negative bacteria, bacteriophages, microalgae and yeast with the appliance through the aqueous/water route, additionally as by supplying the feed. The essence of the definition of FAO/WHO is that probiotics are living organisms, which are administered orally having some tangible health benefits, and are being widely used for the control of diseases in aquaculture, especially in developing countries [57-59]. In practice, only limited evidence is there that the viability of probiotic cultures checked as soon as they are added to feed, and therefore the health benefits may be inaccurately described. Of course, it's going to be a control on the viability of the processing of diet, namely pelleting/granulation and extrusion. For example, the administration of the probiotic extruded diet led to an improvement in non-specific immunity of Nile tilapia compared with granulated/pelleted diet [60].

Mechanism of action of probiotics

Probiotic selection depends on their colonization, antagonism to pathogens and therefore the production of beneficial compounds like vitamins, fatty acids and digestive enzymes [61]. For the successful application of probiotic strains as microbial ingredients in fish, other characteristics seem to be essential, like high viability during processing, storage and after gastro-intestinal transit [62]. A probiotic dosage is often bringing positive and negative results to different receivers, whose responses to different dietary probiotic levels are observed [63,64]. Major Probiotic mechanisms of action include enhancement of the epithelial barrier, increased adhesion to the intestinal mucosa, competitive exclusion of pathogenic microorganisms and production of anti-microorganism substances is as follow [60]:

- **Enhancement of the epithelial barrier:** The intestinal barrier is a major defense mechanism used to maintain epithelial integrity and to protect the organism from the environment.
- **Increased adhesion to intestinal mucosa:** Adhesion to intestinal mucosa is regarded as a prerequisite for colonization and is important for the interaction between probiotic strains and the host.
- **Competitive exclusion of pathogenic microorganisms:** Probiotics bacteria bind with the binding sites in the intestinal mucosa, forming a physical barrier, preventing the connection by pathogenic bacteria;
- **Production of anti-microorganism substances:** Organic acids, in particular acetic acid and lactic acid, have a strong inhibitory effect against Gram-negative bacteria, and they have been considered the main antimicrobial compounds responsible for the inhibitory activity of probiotics against pathogenic microbes.

Role of *Bacillus* in aquaculture

The beneficial roles of *Bacillus* as probiotic microorganism have been broadly studied during the most recent decade. *Bacillus* can contribute a lot to the inward physiological, morphological, hematological, and immunological status of fish by being available in the fish through the administration at an optimum level and improves water quality in aquaculture. The findings of the roles of *Bacillus* in fish as a probiotic are being illustrated below:

Bacillus in growth and reproductive performance

Probiotic microbes are able to colonize in the gastrointestinal tract and adhere to the intestinal mucosa of fish and exert their multiple benefits [52]. Dietary supplementation of probiotics in aquaculture offers an eco-friendly prophylactic measure in fish growth performance and health. Gobi et al. [58] reported that the dietary supplementation of probiotic *Bacillus licheniformis* to *Oreochromis mossambicus* significantly improved the growth performance, immune parameters, enzyme activities and resistance against *Aeromonas hydrophila*. Analysis of growth parameters shows that the final weight, Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) of *Oreochromis mossambicus* increased significantly [61]. Weight gain and specific growth rate of fish fed with 10^7 CFU g^{-1} (D2) was significantly higher than those fed with 10^5 CFUg^{-1} (D1) and control diet. Fish fed with a control diet showed the lowest weight gain and specific growth rate (Figure 1). There was no significant difference in FCR for fish fed diets, between D2 and D1.

Adorian et al. [59] reported that the dietary supplementation with probiotic *Bacillus* (*Bacillus licheniformis* and *Bacillus subtilis*) in Asian sea bass *Lates calcarifer* showed significantly better growth than those fed the basal diet. The treatments were supplemented with 10^3, 10^6, and 10^9 CFUg^{-1} probiotics B3, B6, and B9 respectively. Highest SGR was found in B9 treatment while increasing the dose resulted in growth depression. Lowest FCR value was found in B9 treatment rather than others (Table 1). Based on this experiment, the relationship between different diets and SGR can be graphically illustrated in Figure 2. The addition of *Bacillus* sp. in the Guppy (*Poecilia reticulata*) increased fry production and reduced the fry mortality during spawning [63]. The probiotic test feeds were prepared by gently delicately showering the necessary measure of bacterial suspension on the control diet and blending it part by part in a drum blender to obtain a prominent concentration of 5×10^8 cells g^{-1} and 5×10^7 cells g^{-1} control feed respectively. Significant differences were found in average fecundity per female, the average number of fry survived per female, average weight and length of fry per female (Table 2). The average fecundity/female, the average number of fry survived/female, average weight and length of fry/female were high in *Bacillus* sp. inoculated group than in control. The percentage survival of fry was more in *Bacillus* inoculated group than the control group thereby reflecting its positive influence on the reproductive performance of Poecilia reticulates (Figures 3 and 4).

Saputra et al. [63] reported that the dietary supplementation of *B. amyloliquefaciens* significantly improved the growth performance of Nile tilapia (*Oreochromis niloticus*) and immunity against *Aeromonas hydrophila*. Growth and feeding parameters including final weight (FW), specific growth rate (SGR) and feed conversion ratio (FCR) were significantly influenced by all probiotics with better growth performance and survival [64-75]. In another study, it has been found that the dietary administration of *Bacillus aerius* strain B81e significantly influenced weight gain, specific growth rate and feed utilization efficiency of *Pangasius bocourti* [76]. Ghosh et al. [75]

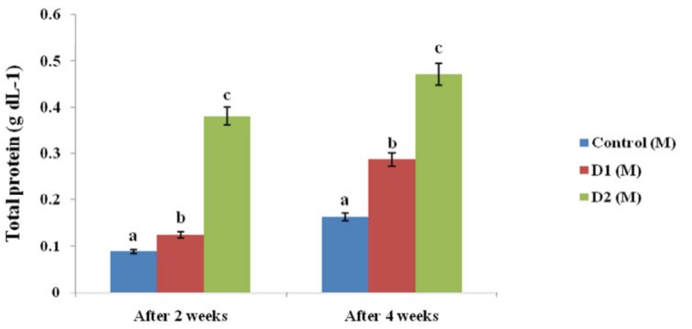

Figure 1: Graphical representation of weight gain of *Oreochromis mossambicus* fed with *Bacillus licheniformis* bacteria.

Table 1: Effect of different levels of *Bacillus* as probiotics supplement on growth performance in *Lates calcarifer* juvenile under laboratory conditions (B3=10^3 $CFUg^{-1}$, B6=10^6 $CFUg^{-1}$, B9=10^9 $CFUg^{-1}$)

Growth Parameters	Control	B3	B6	B9
Initial weight (g)	23.50c	32.57b	40.83a	37.27ab
SGR (% d^{-1})	4.58c	5.13b	5.50a	5.35ab
FCR	2.10a	1.52b	1.21b	1.32b
Weight Gain (g)	1466.7c	2071.1b	2622.2a	2384.4ab

Letters signify statistically significant values

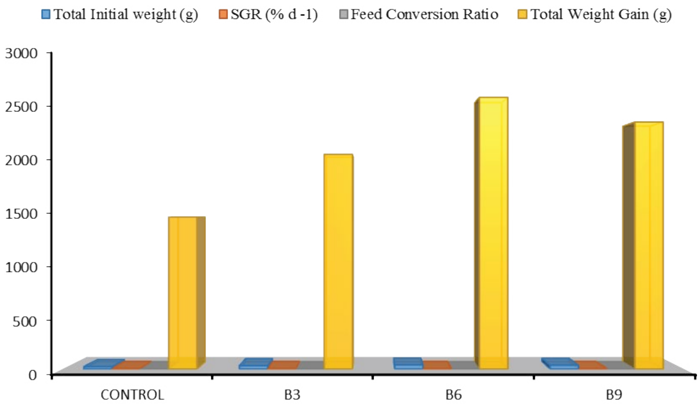

Figure 2: Relationship between dietary probiotics supplement level and specific growth rate in juvenile *Lates calcarifer*.

Table 2: Effects of probiotic supplements on the reproductive performance of *Poecilia reticulate*.

Experiment Groups	T1	T2	C
Average Fecundity	20.23 ± 10.29a	19.9 ± 9.46ab	15.23 ± 7.39c
Average Fry survival	19.19 ± 9.39a	18.66 ± 8.94ab	12.91 ± 5.15c
Average Dead fry	1.04 ± 1.49a	1.24 ± 1.72a	2.32 ± 2.69b
Average Fry Weight (g)	0.0024 ± 0.0004a	0.0023 ± 0.0003a	0.0019 ± 0.0002b
Average Fry Length (mm)	6.36 ± 0.54a	6.29 ± 0.58ab	5.82 ± 0.76d
Gonadosomatic Index (%)	9.25 ± 0.56a	9.4 ± 0.5a	8.13 ± 0.44b

Letters signify statistically significant values

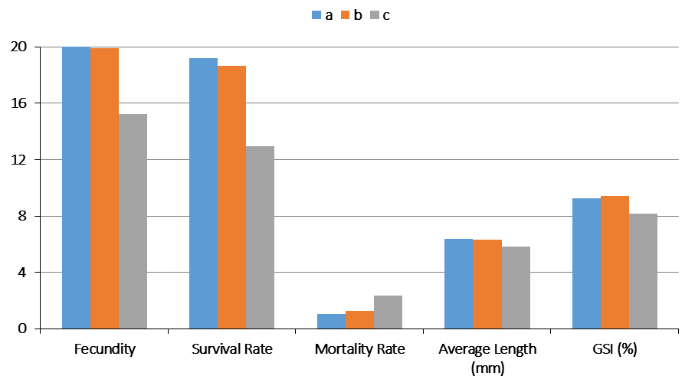

Figure 3: Relationship between dietary probiotics supplement level and gonadosomatic index in juvenile *Poecilia reticulate* (a = 5 . 10^8; b = 5 . 10^7, c = 5 ͺ 10^6).

reported that *B. circulans* significantly improve growth, reduce feed conversion ratio, and increase protein efficiency ratio in *Labeo rohita* when fed at a rate of 1.5 × 10^5 CFU 100 g^{-1} feed at 3% of the bodyweight of fish [77,78]. Elsabagh et al. [79] reported that the growth performance, feed conversion ratio, and blood profiles in tilapia fed on *Bacillus* treated diets were notably increased. John et al. [80] demonstrated that the weight of *Macrobrachium malcolmsonii* fed on the *Bacillus subtilis* coated diet increased 3.5 times more compared to the control diet.

Bacillus in hematological and lysozyme contribution

Several studies have reported significant stimulations of serum lysozyme and phagocytic activities in *Oreochromis niloticus* [81-90], *Rachycentron canadum* [25], *Oncorhynchus mykiss* [53,58,59,78,91], *Paralichthys olivaceus* [40,48,65], *Litopenaeus vannamei* [70,82], and *Labeo rohita* [17,73,77,79,92] fed with *Bacillus* and *Lactobacillus* supplemented feeds. The hematology parameters monitored of *Lates calcarifer* fingerlings fed diets supplemented with and without *Bacillus* at the end of the 8-weeks culture period are presented in Table 3 [62,86]. Significant differences were observed in the hematology parameters between the four treatments. The supplementation of probiotic *Bacillus* significantly altered the fish hematological parameters (Table 4). Diets containing 10^6 and 10^9 $CFUg^{-1}$ probiotics led to a higher count of red blood cells when compared to other treatments. For white blood cells and hematocrit independent of level probiotic supplementation, increased cell count and hematocrit percentage were compared to the control. A significantly higher percentage of hemoglobin was observed in the blood of a fish fed diet supplemented with 10^6 $CFUg^{-1}$ probiotics. Lysozyme activity of plasma differs significantly between the treatments tested. Lysozyme activity was higher in animals fed the diet containing 10^6 $CFUg^{-1}$ probiotics (Figure 5). The treatments were supplemented with 10^3, 10^6, and 10^9 $CFUg^{-1}$ probiotics B3, B6, and B9 respectively. *Paralichthys olivaceus* individuals treated with a commercial probiotic demonstrated significantly higher plasma LYS activity [48]. Abarike et al. [65] reported that the dietary supplementation of commercial probiotic *Bacillus subtilis* and *Bacillus licheniformis* significantly improved the protease, lysozyme, anti-protease activities, catalase activities and immunoglobulin in

Table 3: Hematological data for *Lates calcarifer* fed diets containing *Bacillus* strains.

Hematological parameters	Control	B3	B6	B9
RBC (cells ×10^7 mL^{-1})	1.12c	1.34b	1.92a	1.76a
WBC (cells ×10^5 mL^{-1})	1.63b	2.16a	2.55a	2.44a
Hematocrit (%)	20.3b	24.2a	26.37a	26.7a
Hemoglobin (g %)	0.62b	0.63ab	0.82a	0.78ab

Letters signify statistically significant values

Table 4: Antibacterial activity of *Bacillus* strains against two bacterial fish pathogens determined by an agar well diffusion assay.

Bacterial Strains	Inhibition zone (mm)	
	A. hydrophila FW52	*S. agalactiae* F3S
B31m	14.7 ± 0.1c	15.6 ± 0.1f
B47b	16.8 ± 0.9d	12.0 ± 0.2a
B78e	12.1 ± 0.1b	13.1 ± 0.1b
B79a	11.1 ± 0.2a	14.4 ± 0.8de
B81e	12.5 ± 0.9b	18.1 ± 0.3h

Letters signify statistically significant values

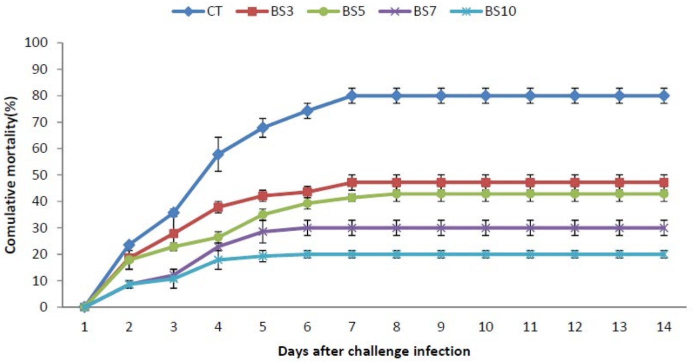

Figure 4: Cumulative mortality of *Oreochromis niloticus* fed a commercial probiotic *Bacillus subtilis* and *Streptococcus agalactiae*.

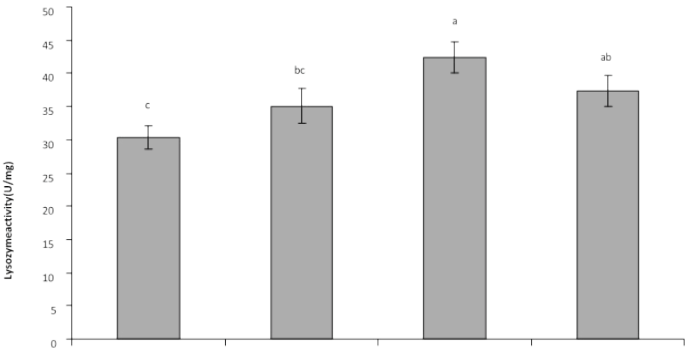

Figure 5: Lysozyme activity of plasma of *Lates calcarifer* fed diets containing *Bacillus*; $B_3=10^3$ $CFUg^{-1}$ probiotic *Bacillus*; $B_6=10^6$ $CFUg^{-1}$ probiotic *Bacillus* and $B_9=10^9$ $CFUg^{-1}$ probiotic *Bacillus*.

Nile tilapia, *Oreochromis niloticus*. Another study reported that the probiotic-treated *L. vannamei* postlarvae showed enhancement of antibacterial activity with a considerable increment of LYS [70]. A recent study reported that the isolated gut probiotic *B. subtilis* with formulated diet insignificantly increased the hematological parameter values of the hemopoietic stimulation in freshwater fish *Labeo rohita* [77].

Bacillus in innate immunity and disease resistance

Probiotics in aquaculture enhance the immunity and disease resistance against bacterial pathogens. Using probiotics in aquaculture to control and prevent diseases has gained interest increasingly [64]. There have been reports of using probiotic bacteria to protect fish from bacterial infections [17,65,66]. The administration of probiotics can improve immunity and protect against several pathogens in many fishes such as *Labeo rohita*, *Oreochromis niloticus*, *Epinephelus bruneus*, *Oncorhynchus mykiss*, and *Cyprinus carpio*. Das et al. [15] remarked that the *B. amyloliquefaciens* can be utilized in aquaculture to improve health status and disease resistance in Catla (*Catla catla*) with an optimal dietary supplementation of 10^9 $CFUg^{-1}$. Beck et al. [37] supplemented the mixed probiotics of *Lactococcus lactis* BFE920 and *Lactobacillus plantarum* FGL0001 to olive flounder, *Paralichthys olivaceus* and reported that the growth performance, innate immunity, and disease resistance were significantly improved. Mixed probiotic showed better performance than the single probiotic agent in improving lysozyme activity and phagocytic activity of innate immune cells. In addition, neutrophil activity was also improved significantly compared to the control group.

Probiotics can be also used as immunostimulants in aquaculture. Selim and Reda [17] demonstrated that the dietary supplementation of *Bacillus amyloliquefaciens* improves innate immunity and disease resistance in *Oreochromis niloticus* with significant increases in IL-1 and TNF α mRNA levels in the kidneys. Fish that were fed *Bacillus amyloliquefaciens* exhibited better relative survival percentages when challenged by Yersinia ruckeri or Clostridium perfringens also appeared to enhance *in vitro* serum bactericidal activities against *Aeromonas hydrophila*. Dietary supplementation of different levels of *Bacillus* probiotic in juvenile Asian sea bass (*Lates calcarifer*) under laboratory conditions for 56 days significantly reduced the occurrence of disease in fish culture [62]. In another study, dietary administration of *Bacillus* (B47b) for two months yielded significantly higher survival rates against *A. hydrophila* FW52. This evidence stated that *Bacillus* spp are quite effective against *A. hydrophila* and *S. agalactiae*. Many of these strains were inhibited at varying levels by *Bacillus* spp, probably due to the production bacteriocin-like inhibitory substance. *Bacillus* bacteria have been known to inhibit many gram-positive and gram-negative bacteria mainly through the production of organic acids, hydrogen peroxide and bacteriocin-like inhibitory substances (BLIS) [72]. *Bacillus* spp. have disease resistance capacity. The protection activity of each strain against h infection of *Oreochromis niloticus* was tested [66,68]. *O. niloticus* were fed with (*Bacillus*) Control (CT): commercial diet, BS3, BS5, BS7 and BS10 fed with commercial diet containing commercial probiotic BS at 3 $g\text{-}kg^{1}$, 5 $g\text{-}kg^{1}$, 7 $g\text{-}kg^{1}$, and 10 $g\text{-}kg^{1}$ doses respectively for 14 days. All these strains decreased the cumulative mortality of *O. niloticus* compared with the control group, while BS10 showed the highest protection. Meidong et al. [72] isolated *Bacillus aerius* (strain B81e) from the intestine of healthy catfish and examined its probiotic properties both *in vitro* and *in vivo*. They found that this bacterium exhibited a broad-spectrum antibacterial activity against the fish pathogens especially *Aeromonas hydrophila* and *Streptococcus agalactiae* along with inhibiting both Gram-positive and Gram-negative bacteria.

Bacillus in stress resistance and water quality improvement

Probiotics are also known to play significant role in developing innate immunity in fishes, and thus help them to fight against any pathogenic microbes and also against environmental stressors. Taoka et al. [45] reported that Japanese flounder, *Paralichthys olivaceus* had greater heat tolerance after being treated with a commercial probiotic supplied in the diet and in the rearing water compared to the control. Abdollahi-Arpanahi et al. [66] studied the efficacy of probiotics *Bacillus subtilis* and *Bacillus licheniformis* on growth performance, immuno-physiology and resistance response of juvenile white shrimp (*Litopenaeus vannamei*) and concluded that the shrimps fed with probiotics showed higher resistance to environmental stressors including low and high salinities, formalin, chlorine, high and low water temperatures, and ammonia. However, commercial probiotics fed shrimps demonstrated a better resistance than the individuals fed with the indigenous probiotic group. Such resistance is obviously due to better immunological functions of the animal, as higher levels in immune defensive cells, lysozyme activity, total protein as well all a lower cortisol level were measured in the commercial group compared to indigenous one. Liu et al. [67] reported an improved stress tolerance of *Litopenaeus vannamei* post-larvae after being treated with the probiotic *B. subtilis* E20, which may be due to the regulation of physiological response of shrimp by probiotic *B. subtilis* to adapt acute environmental stresses. Rollo et al. [92] fed *Sparus aurata* fry with two probiotics (*Lactobacillus fructivorans* and *Lactobacillus plantarum*) using *Brachionus plicatilis* and subjected the fries to acute pH stress. They reported a significantly lower cortisol level, higher gene expression of heat shock protein 70, and significantly lower cumulative mortality.

The use of probiotics in lentic water bodies could improve fish wellbeing by boosting up many water quality parameters since they modify the bacterial arrangement of the water and dregs. The water temperature, pH, dissolved oxygen, ammonia, and hydrogen sulfide were accounted for to be of more excellent when *Bacillus* were included the shrimp hatchlings culture in the green water system (Tables 5-7). *Bacillus* spp. has advantageous effects in water quality improvement in aquaculture with better efficiency of transforming organic matter than Gram-negative bacteria [58]. Ammonia and nitrite concentration increase as the culture turns into an intensive operation. Ammonia and nitrite toxicity can be removed by the use of nitrifying bacteria into the fish environment [77]. *Nitrosomonas* spp. can be used in treating them to keep water quality in a suitable range, which may be conceivable in light of different roles played by the probiotic microscopic organisms. A buffering framework to dodge wide pH vacillation is fundamental for aquaculture [78]. *Bacillus* spp. have known to utilize different nitrogen sources, including both NH_3 and NH_4^+ for catabolism of proteins and subsequently use H+ particle [74]. Zink et al. [71] reported that the exchange of HCO_3^- as a waste product

Table 5: Digestive enzyme activity (µmol product liberated min-1 mg protein-1 at 370C) at different levels of probiotic supplement.

Diets	Amylase	Protease	Lipase
Initial values	0.865	0.115	0.005
CD	0.912	0.142	0.009
ED1	1.135	0.195	0.016
ED2	1.258	0.219	0.019
ED3	1.196	0.174	0.012

Table 6: Proximate carcass composition of common carp fingerlings fed commercial probiotic diets.

Parameters	Initial values	Control diet (CD)	Experimental diets		
			ED1	ED2	ED3
Protein (%)	10.25	11.84	12.78	13.12	12.45
Lipid (%)	5.84	6.18	6.24	6.26	6.19
Ash (%)	3.21	3.95	4.37	5.12	4.25
Moisture (%)	65.71	65.98	65.32	64.87	65.16

Table 7: *Bacillus* used in aquaculture to promote growth, increase survival, fish nutrition, enhance immunity and antimicrobial activity.

Probiotics	Host species	Effects	References
B. circulans	*Labeo rohita*	Improved growth, reduced feed conversion ratio, and increased protein efficiency ratio	[79]]
Bacillus spp.	*Cyprinus carpio*	Better digestive enzyme activities; better growth performance and feed efficiency	[81]
B. subtilis	*L. vannamei*	Increased survivability against *Vibrio harveyi* infection	[26]
Bacillus spp.	*Oncorhynchus mykiss*	Better growth performance and survival	[59]
Bacillus sp.	*Acipenser persicus*	Increased specific growth rate and decreased food conversion ratio	[81]
B. subtilis	*L. vannamei*	Significantly increased survival rate	[82]
Bacillus sp.	*Oreochromis niloticus*	Increased growth performance, decreased feed conversion ratio	[83]
B. subtilis	M. *malcolmsonii*	Increased weight gain	[84]
Bacillus sp.	*S. aurata, L.*	Increased protease activities; improved nutritional condition in larvae	[85]
Bacillus sp. Strain DDKRC1	*P. monodon*	Provided better growth, digestibility, FCR, survival, and immune response	[86]
B. subtilis strain ANSB060	*C. carpio*	Improved digestive enzyme activities of and decreased aflatoxin B1 residues in hepatopancreas and gonad	[87]
B. licheniformis strain fb11	*C. chitala*	Modulated intestinal microflora and significantly improved digestion	[88]
B. coagulans and B. subtilis	*Artemia nauplii*	Produced antimicrobial activity against the pathogenic *Vibrio* species	[89]
Bacillus	*I. punctatus*	Good potential to mitigate the enteric septicemia of catfish (ESC)	[90]
B. subtilis	*E. coioides*	Increased the innate immunity and intestinal microbial population	[91]
B. subtilis and B. licheniformis	*O. mykiss*	Increased resistance to *Yersinia ruckeri*	[92]
B. subtilis	*Gilthead seabream*	Stimulated cellular innate immune response	[93]
B. subtilis AB1	*O. mykiss*	Controlled *Aeromonas* infection	[94]
B. subtilis (ATCC 6633)	*O. niloticus*	Stimulated and enhanced the gut and immune system and health status	[95]

resulting from respiration diminished the pH, which prompted physiological pressure. Probiotic *Bacillus* produces substances that hinder the growth of harmful algae by algicidal compound and forestall blooms. Algal bloom can possibly cover the surface of the water and prevent the daylight to enter into the water to provide the vitality to phytoplankton production [75-76]. Probiotic *Bacillus* also improves the decay of organic matters; diminishes nitrogen and phosphorus availability, and controls ammonia, nitrate, and hydrogen sulfide [65].

In situ bioremediation has also been widely used in aquaculure through bio-augmentation abuse endemic or exogenous probiotics, which modifies water quality. Devaraja et al. [94] isolated indigenous *E. pumilus*, *B. licheniformis*, and *B. subtilis* from marine water and soil and investigated these microscopic organisms for their bioremediation capacity in *P. monodon* culture, and prescribed *Bacillus* spp. as potential candidates for bioremediation for *P. monodon* culture systems. They also reported that *Bacillus* spp. improved water quality, health status, and survival and growth rates of juvenile *P. monodon* and diminished the number of infective vibrios. For palatable fish, *Bacillus* spp. could lessen the load of high concentrations of nitrogen in trout aquaculture and decrease the total ammonia in tilapia production in recirculating aquaculture systems [95]. Moreover, commercially available probiotics made from *E. licheniformis* and *B. subtilis* were utilized in Nile tilapia (*Oreochromis niloticus*) farming to enhance the dissolved oxygen concentration [42]. This may because of the characteristic capacity of catfish, permitting the fish to withstand themselves even as far as possible. Bacterial species belonging to the genera *Bacillus*, *Eubacterium, Pseudomonas, Acinetobacter, Cellulomonas, Rhodopseudomonas*, and *Nitrosomonas* are accounted for to be potent and powerful in bioremediation for organic wastes. These probiotic bacteria regulate the microflora of development water and control infective microorganisms to upgrade the disintegration of bothersome organic substances inside the water and sediment because of the improved environmental atmosphere of cultivation [95].

Bacillus in enzyme activity and body composition improvement

According to Zink et al. [71], *Cyprinus carpio* juveniles were supplemented with different treatment of *Bacillus* circulans probiotic under laboratory conditions with following dosages: The treatments were CD (control), ED1 (2×10^2 $CFUg^{-1}$ *B. circulans*), ED2 (2×10^4 $CFUg^{-1}$ *B. circulans*) and ED3 (2×10^6 $CFUg^{-1}$ *B. circulans*). In general, the total and specific activity of digestive enzymes (Protease, amylase, and lipase) remained significantly higher in the fish fed diet containing *B. circulans* at ED2 diet in comparison with other treatments and control (Table 5). The body composition of the fish can also be affected by the probiotics concentrations in diets. According to an experiment on *Cyprinus carpio* [76], a specific amount of probiotics can positively result in the improvement of body composition. The accumulation of carcass protein, lipid and ash were significantly higher in groups fed diets ED2. Carcass moisture contents remained significantly low at ED2 (Tables 6 and 7).

CONCLUSION

The Gram-positive probiotic *Bacillus* is thought to be an important tool for promoting sustainable aquaculture because of they have significant advantageous effects for aquaculture industry. *Bacillus* exerts beneficial effects via multiple mechanisms including promoting growth performance of aquaculture organisms, suppress irritation and disease infestation by induction of immunity, promote nutrition, and improve aquatic ecosystems for growth and reproduction of fishes and shrimps. The species of *Bacillus* can increase the Specific Growth Rate (SGR) of *Oreochromis mossambicus* and *Lates calcarifer* fry and decrease the Feed Conversion Ratio (FCR) of the mentioned fishes to the desired level. *Bacillus* can be increasing the fecundity, survival rate, Gonadosomatic Index (GSI), hemoglobin, Red Blood Cells (RBC), White Blood Cells (WBC) in blood, stress resistance, and disease resistance. The proximate composition of a fish body can be brought under the optimum condition for fish physiology and morphology if optimum supplementation with *Bacillus* can be administered. Administration of *Bacillus* probiotics can enhance the activity of the different intestinal enzymes which will contribute to efficient digestion in fish. Current biotechnological approaches provide opportunity to understand the underlying molecular mechanisms of the beneficial effects of the probiotic *Bacillus*. Larger application of probiotic *Bacillus* instead of the hazardous synthetic chemicals would promote eco-friendly low-input sustainable aquaculture for food and nutritional security of the increasing world population.

CONFLICTS OF INTEREST

The author declares there is no conflict of interest.

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