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Probiotics: For sustainable development of aquaculture

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Abstract
The growing demand towards the environment friendly culture practices is motivating researchers to focus on certain experiments on the probiotics and its application in aquaculture. Probiotics are the microbial feed supplements or water additives that improve the culture media and the health of the cultured organisms. Researchers found the beneficial effects of probiotic micro-fauna as a promoter of growth, survival, health, etc. to the host. It has been proven that the bacterium which is a pathogen for a species may bring benefits to the other species. They produce anti-microbial substances such as anti-bacterial, anti-viral, anti-fungal, etc. which provide protection to the host from the pathogens. It replaces the use of antibiotics in aquaculture. So, this study aims to give a clear idea on the beneficial effects of probiotics in aquaculture.

Keywords: Aquaculture, environment, experiments, pathogen, probiotics

Introduction
Aquaculture is one of the most important sectors to contribute to nutritional security [1]. It is the culture of aquatic organisms including fish, crustaceans, mollusks, and aquatic plants such as seaweeds. It is one of the fast growing and rapidly expanding industry and its contribution to global animal intake is appreciable [5]. India produced 12.61 Million Metric tons (MMT) of fish out of which 8.92 MMT and 3.68 MMT were contributed by inland and marine fisheries respectively in the year 2017-18 [4] and occupied a unique position in world fish production.
In order to increase fish production, the present farmers are changing the management practices in terms of low stocking density to high stocking density, excess use of feed and antibiotics. Due to the intensification of fish culture which leads to deteriorate water quality parameters. The emergence of a wide array of pathogens causing infectious diseases in the culture system becomes the limiting factor for most of the aquaculture practices. Every year, diseases are causing a huge economic loss in millions over the worldwide [10]. The aquatic environment also supports pathogenic bacteria to grow and cause diseases, especially in the larval and fry stages [6]. To avoid the occurrence of diseases and economic loss, the number of chemicals and antibiotics have been using in the culture system [7]. Excessive use of these substances results in the contamination of aquatic livestock and developed antibiotic-resistant pathogenic organisms [6]. The accumulation of these chemicals in aquatic organisms is not safe for final consumers. So, the development of eco-friendly alternatives has become important to produce healthier aquatic fauna. Most of the researchers revealed probiotics as an environment-friendly alternative to solve the above problems of aquaculture. Probiotics are considered safe additives that can improve the physiological condition of the host, control diseases, and improve water quality [9].

History of Probiotics
The word probiotics are derived from the Greek words “pro” and “bios” which means “for life”. The use of probiotics has been started with the discovery of milk preservation for longer periods in 2000 BC [43] and used as fermented dairy products to cure some diseases for two reasons; they contain living microorganisms which can combat certain infections and have high nutritive value. According to Sicard and Legras (2011) [45], yeast is used by the people for production of beverage as earlier than 2000 BC.
According to the World Health Organization (WHO), the most widely accepted definition of probiotics is defined as “live microorganisms that when administrated in adequate amounts,

confer a health benefit to the host" [38]. The term "probiotics" also can be defined as the substances secreted by one kind of microorganism which stimulates the growth of other microorganisms [7]. Probiotics are microorganisms used orally that alter the microbial composition of the host which creates some beneficial effects on the health of the host [23]. The basic principle of probiotics is "microorganisms against microorganisms" [44]. According to Salminen *et al.* (1999), these are microbial cell preparations that have a positive effect on the health and well-being of the host [46]. It implies that probiotics do not need to be viable; some non-viable organisms can also show a beneficial effect on the host.

Apart from both gram-positive and gram-negative bacteria, probiotics also include some other organisms such as yeast, bacteriophage, etc can also show benifical effect. Several microorganisms are used in aquaculture as probiotics such as lactic acid bacteria (*Lactobacillus, Leuconostoc, Lactococcus, Enterococcus, and Carnobacterium*)., *Bacillus spp., Vibrio spp., Pseudomonas spp., Saccharomyces cerevisiae., Aeromonas spp., etc. Lactococcus lactis* subsp. *lactis* 1FT, 1FWand 3FT; *Lactobacillus plantarum* 1MTK, 4BC and 13BC, and *Lactobacillus brevis* 1BT shown strong anti-microbial activity against pathogenic organisms such as *Escherichia coli*, *Listeria monocytogenes*, *Salmonella sp*., *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio sp* [6]. *Lactobacillus plantarum* is gram-negative rod-shaped non-pathogenic bacterium which is highly versatile and found in fish, meat, vegetables, gastro-intestinal tract of human, aquatic and terrestrial animals and possess valuable health-promoting features [8]. *Bacillus* species are reported to enhance antioxidant enzyme activity, digestion, immune-related gene expression, and improving the ability to fight against pathogenic microbes [9]. Application of Gram-positive bacteria shown a beneficial effect in improving the water quality of the system [10]. Increase in the gonado-somatic index, fecundity and viability of *Poeciliareticulata* (Peters), *Poecilia sphenops* (Valenciennes), *Xiphophorushelleri* (Heckel) and *Xiphophorus maculatus* (Gunther) have been documented using a strain of *B. subtilis* isolated from the intestine of *Cirrhinus mrigala* [47].

Source and types of probiotics

Lactic acid fermentation plays an important role in preserving food items. Fermented fruits and vegetables are the potential sources of lactic acid bacteria such as *Lactobacillus plantarum, L. pentosus*, *L. brevis, L. acidophilus, L. fermentum, Leuconostocfallax,* and *L. mesenteroides* [48]. Microbiota having haemolymph may participate in the protection of bivalve and confer health benefits to the organism. Bivalve haemolymph microbiota is a great source for aquaculture probiotics [49].

Probiotics are commonly used in aquaculture as growth supplements, prophylactic and most importantly as therapeutic purpose and also to maintain the water quality of the system. Microbiota which is associated with the healthy host is considered as a part of the defense system of the host. Generally, probiotic organisms are indigenous to the environment and can survive spontaneously and function physiologically at an optimum level when the organisms get to be exposed [18]. *C. butyricum* which is an obligatory anaerobic bacterium reported from the intestine of healthy chicken and successfully evaluated as an immune-modulatory effect on fish [50]. Most inhibitory bacteria against the fish pathogen *Vibrio anguillarum* HI 11345 were found in the rinse and mucus of the gastrointestinal tract of *Scophtalmus maximus* [51]. The bacterial strains obtained from the intestinal tract of *Gadus morhua* (Atlantic cod) and were tested against *Aeromonas salmonicida* and *Vibrio anguillarum* at 13 and 20^{0}C incubation temperature and found antagonistic activity against targeted pathogens [52].

According to Prosenjit *et al.* (2019) [12], probiotics are classified into two categories- Gut probiotics and Water probiotics. Gut probiotics can be used orally or with feed to increase the microbial balance in the gut. Probiotic organisms are added to the feed using cod liver oil and egg which act as a binder in feed to minimize the environment deterioration; bio-encapsulation is also an efficient method to use [20]. Probiotic strains such as *Bacillus subtilis, B. licheniformis, B. coagulans*, *Lactobacillus acidophilus,* etc. are used as feed probiotics in aquaculture. Water probiotics are directly used in the water and consume all the necessary nutrients from the water body so that all the pathogenic bacteria get eliminated through starvation. They convert organic matters into smaller units such as amino acid and glucose that are used as food for beneficial bacteria and also improve the water quality [14]. *Bacillus spp., Saccharomyces spp., Streptomyces spp.,* lactic acid bacteria, etc. are the water probiotic strains. Commercial probiotics are coming in powder form which can be added in the fish feed and liquid form which is mostly used in the water [11].

Different strains of probiotics have different roles and some have multiple roles. According to Cruz *et al*. (2012), *Bacillus sp., Carnobacterium divergens, Alteromonas* CA2, *Lactobacillus helveticus, Lactobacillus lactis* AR21, *Streptococcus thermophiles, Streptomyces, Bacillus coagulans, Bacillus* NL 110, *Vibrio* NE 17 etc. are applied as a growth promoter. Whereas *Bacillus sp., Enterococcus faecium SF* 68, *L. rhamnosus* ATCC53103, *Micrococcus luteus* A1-6, *Pseudomonas fluorescens, Roseobacter sp*. BS. 107, *Saccharomyces cerevisiae, S. exiguous, Vibrio alginolyticus*, *Lactobacillus acidophilus*, etc. has pathogen inhibition capacity; *Bacillus* NL 110, *L. helveticus, Vibrio* NE 17, *Carnobacterium sp*. Hg4-03, *Lactobacillus acidophilus*, *Shewanella putrefaciens* Pdp11 has nutrient digestibility ability; *Bacillus sp*. 48, *Lactobacillus acidophilus*, *B. coagulans* SC8168, *Bacillus sp., Saccharomyces sp, Vibrio sp.* NE 17 can improve water quality; *Bacillus subtilis, L. rhamnosus, L. acidophilus, L. casei, Enterococcus faecium, Bifidobacterium thermophilum* improves the reproduction of the host [2].

Probiotics which are used in aquaculture should have some properties and these are:(a) probiotic organisms should not be pathogenic to the host, (b) should survive the acidic environment of the gut of the host and must resistant to the enzyme, (c) safe for use as feed ingredients and must promote growth, (d) should have the ability to destroy pathogenic organisms, (e) should remain viable for longer period of time and in storage condition, etc. [11]

Prebiotics and Synbiotics

Prebiotics are the non-digestible food ingredients that are metabolized by health-promoting bacteria or probiotics [54]. Prebiotics convert the different microbial fauna to beneficial bacteria, such as *Bifidobacterium spp*. and *Lactobacillus spp*. [56]. Prebiotics reduces fish mortality due to invasion of pathogen and enhances biological responses to the host. The use of Mannanoligosaccharides (MOS), insulin which are obtained from the cell wall of yeast, *Saccharomyces cerevisiae*, and Fructooligosaccharide (FOS) prebiotics as immunostimulants improve the immune response and disease resistance to the fish [55]. According to Ganguly *et al*. (2009), prebiotics should have some characteristics: (a) easy to incorporate in feed, (b) should have low calorific value, (c) must be effective at low concentration, (d) must be non-carcinogenic, (e) should have the capacity to destroy harmful micro-organisms, etc. [56]. Some of the potent oligmers having greater role in aquaculture such products includes lactulose, lactosucrose, galacto-oligosaccharides, isomalto-oligosaccharide, fructo-oligosaccharide, soybean-oligosaccharides, gluco-oligosaccharides, xylo-oligosaccharides, palatinose, etc. [53].

Prebiotics are mainly selected based on their ability to enhance the growth of lactic- acid-producing bacteria. FOS prebiotics used in some fishes and observed improved growth rate, survivability, non-specific immunity, feed intake, feed conversion, etc. [54].

Synbiotic is a procedure of microflora management where prebiotics and probiotics are used in combination which improves the survival of probiotics. Administration of dietary synbiotic in the feed of common carp (*Cyprinus carpio*) fingerlings cultivated in aquaponics has shown a positive effect on the growth rate, feed utilization, and feed conversion ratio [42].

Mode of action

Anti-microbial activity: Probiotic strains of bacteria produce different kinds of anti-microbial substances such as antibacterial, antifungal, antiviral substances, etc. Metabolites of probiotics include hydrogen peroxide, lysozyme, siderophores, organic acids, bacteriocins, proteases, antibiotics, etc [16]. In the presence of oxygen, some strains of probiotics capable to produce hydrogen peroxide and it oxidize the cell wall of pathogenic bacteria [16]. Streptococcus Pneumoniae produce a large amount of H_2O_2 in aerobic conditions and showed an inhibitory effect on other pathogenic bacteria [57]. Siderophores are different types organic compounds produced by some plants and microorganisms such as hydroxamate type (i.e. ferrioxamine B), carboxylate type (i.e. rhizobactin), catecholate type (i.e. enterobactin), mixed type (i.e. ferrichrome, pyoverdine, etc.) are the microbial siderophores and mugineic acid is an example of phyterosides [58].Important aspect of Siderophores control the fish pathogens by limiting the Fe that is important for bacterial and virulence interaction. Acetic acid and lactic acid are the main organic acid produced by probiotics [59] where as lactic acid function to disrupts the outer membrane of gram-negative bacteria and shows an inhibitory effect against pathogens [60]. Bacteriocins are toxins peptides produced by probiotic bacteria to inhibit the growth of pathogenic bacterial strains.

Some probiotic bacterial strains (e.g. *Vibrio*, *Pseudomonas, Aeromonas*, etc.) have antiviral effects on Infectious Hematopoietic Necrosis Virus (IHNV) [15]. They enhance the immunity and disease resistance power of the host and resist and prevent viral infection [61]. Fungal infection or aflatoxin contamination is one of the major problems in fisheries. *Lactobacillus rhamnosus* L60 and *L. fermentum* L23 strains show inhibitory activity on *Aspergillus flavi* [62]. *Lactobacillus* strains KCC-26, KCC-25, KCC-27 and KCC-28 showed potential antifungal activity against *Penicillium chrysogenum, P. roqueforti, Aspergillus fumingatus, Botrytis elliptica* and *Fusarium oxysporum* [63].

Competitive exclusion: Competitive exclusion is a strategy of probiotics which reduce the colonization of pathogenic bacteria in the gastrointestinal tract. It is mainly based on bacteria to bacteria interaction due to competition for adhesion sites and nutrients. Dietary application of Probiotic strains utilize the available nutrient in the gastrointestinal tract and occupy space and modify the gut environment by producing antimicrobial substances (e.g. acetic acid, lactic acid, etc.) to make less suitable for other pathogenic microorganisms to grow [64] and prevent colonization.

Adhesion: One of the important criteria for the selection of probiotics in aquaculture is the adhesion capacity of the probiotic strains to the intestinal mucosa. Non-pathogenic bacteria occupy the gastrointestinal mucosa of the host and binding sites of the pathogenic micro-fauna. The mechanism of both competitive exclusion and blocking of adhesion sites eliminate the pathogenic organisms from the host.

Improve epithelial barrier: For epithelial integrity, the intestinal barrier plays a major defense mechanism. Antimicrobial peptides, mucous layer, antibody, and the epithelial junction adhesion complex form the epithelial barrier which protects the organisms from the outer environment. This barrier prevents the entry of pathogenic bacteria into sub-mucosa to form inflammatory responses. Non-pathogenic bacteria or probiotics can contribute to maintaining the intestinal barrier to safe the organism from the action of pathogenic bacteria and food antigens [65].

Modulation of the immune system: The gut micro-biota produces of molecules with anti-inflammatory and immune-modulatory functions to stimulate the immune system. Interaction of probiotic bacteria with dendritic cell and epithelium cell and with monocyte and lymphocyte results in immune-modulatory effect of probiotic [66]. Gut probiotic produces intestinal mucosa and induce the responses of B and T- cell and complement system after entering any pathogen inside a body [13].

Uses of probiotics in aquaculture

The aim of aquaculture is to increase the growth of the cultured organisms in shorter period of time and meet demand of fish in the country. For sustainable aquaculture development, probiotics gaining utmost importance for its multiple application.

Table 1: Effects of probiotics in some targeted aquatic animals.

Probiotic stain	Targeted aquatic species	Effect on targeted species	Ref
Vibrio tasmaniensis	Sea cucumber	Increased phagocytic activity and increased survival rate of 87.60%-97.97%.	[5]
V. alginolyticus	Salmonids	Disease control	[24]
V. gazogenes	*Litopenaeus vannamei*	Decrease pathogenic Vibrio count and improve immunity	[16]
Bacillus additive	Grass carp	Immunity parameters including IgM, alkaline phosphatase and complement	[25]

	(*Ctenopharyngodon idellus*)	C3 significantly increased (p<0.05) by 30.77, 107.79 and 34.62% respectively.	
Bacillus coagulans	*Cyprinus carpio koi*	Increased specific growth rate, lysozyme activity, total leucocyte count, phagocytic activity, respiratory burst activity, and disease resistance to *A. veronii* (P<0.05).	[26]
B. subtilis	*Labeo rohita*	Variable response were found in haemoglobin, total protein, albumin-globulin ratio, total erythrocyte count, total leucocyte count etc.	[27]
B. subtilis	*Macrobrachium rosenbergii* larvae	Increased survival rate and a faster rate of metamorphosis.	[28]
B. subtilis	Cobia (*Rachycentron canadum)*	Increased specific growth rate, increased serum lysozyme activity	[29]
Bacillus subtilis DCU	Mud crab (*Scylla paramamosain*)	Immunostimulatory, increased survival Against *V. parahaemolyticus*	[16]
Bacillus subtilis E20	*Litopenaeus monodon*	Enhance humoral immune response	[20]
B. coagulans	*Litopenaeus vannamei*	Increased (P < 0.05) final weight and daily weight gain, and survival rate with No significant differences appeared in the muscle compositions of moisture, crude protein, and ash.	[30]
Bacillus subtilis, B. licheniformis, B. firmus	*Penaeus vannamei*	Increased survibility	[22]
Lactobacillus	*Clarias orientalis*	the size and weight of the fish statically increased	[31]
Lactobacillus	*Macrobrachium rosenbergii* post larvae	inhibitory effects has been shown against the gram-negative bacterial flora present in the gut, increased growth, feed efficiency ratio, protein efficiency ratio.	[32]
Lactobacillus casei	*Tor grypus*	significantly (P< 0.05) increased serum lysozyme, serum bactericidal, complement, respiratory burst activities, along with enhance the immune responses and gene expression	[33]
Enterococcus faecium	*Pterophyllum scalare*	improved growth and viability of fish *in vivo*	[34]
Enterococcus casseliflavus	*Oncorhynchus mykiss*	Improved serum IgM, respiratory burst activity of blood leukocytes, neutrophil count.	[35]
Pseudomonas aeruginosa	*Cyprinus carpio*	Immune related factors, such as protein level, lysozyme, alkaline phosphatase, and alternative complement pathway levels increased.	[36]
Pseudomonas aestumarina	Pacific white shrimp	Disease resistance against *V. parahaemolyticus*, increased growth rate, survivality.	[38]
Lactococcus lactis subsp. *Lactis*	*Litopenaeus vannamei*	Growth rate, survival, and body protein level were increased, decreased the *Vibrio* count	[37]
Lactobacillus plantarum	*Oreochromis niloticus*	Improve the growth, immunity, antioxidant and tolerance of Nile tilapia to *A. sobria* infection.	[41]
Lactobacillus plantarum	*Pangasius bocourti*	Improved specific growth rate (SGR), feed conversion ratio (FCR), serum lysozyme activity, phagocytosis, respiratory burst activities.	[39]
Yeast *Kluyveromyces lactis* M3	*Sparus aurata*	Enhanced bactericidal activity against *Vibrio parahaemolyticus* N16, *V. harveyi* Lg 16/00, and *V. anguillarum* CECT 43442. Nitric oxide production, skin mucus lectin union levels, and peroxidase activity strongly increased.	[40]

Growth promoter: The use of probiotics attracts the attention of fish farmers as a means of improving growth performance.

Numbers of researchers Conducted experiment and revealed that the use of probiotics in aquaculture improves the growth and health of the cultured organisms and influences on survival rate [3, 11].

By the application of Meracid aquaculture feed probiotics for 3 months in *Labeo rohita* culture system at the rate of 1.0%, 1.5%, and 2.0% reported the increase of average growth at the rate of 21.50 cm, 24.74 cm and 19.60 cm respectively against the growth of 16.30 cm in the control system [17].Improved specific growth rate (SGR) has been observed in cobia (*Rachycentron canadum*) with dietary supplementation of *Bacillus substilis* and chitosan in 8 weeks of feeding trial [29].with addition to that Pangasius catfish (*Pangasius bocourti*) used as experimental species and fed with Jerusalem artichoke (*Helianthus tuberosus*) at the dose of 5 g/kg or *Lactobacillus plantarum* with diet at the rate of 10^8 cfu/g observed significantly higher Feed Conversion Ratio (FCR) and SGR over control in 12 weeks of experiment [39]. Same results were obtained with Increased SGR, FCR and survival percentage has been observed in koi (*Cyprinus carpio koi*) with oral administration of chitosan oligosaccharide (COS) and *Bacillus coagulans* [26]. Probiotic bacteria are able to increase the appetite and also increase the digestibility [21].

Disease resistance: Probiotics become the best alternative of antibiotics in aquaculture to control diseases. For a longer period of time, probiotic organisms colonized in the gastrointestinal tract and they have a high multiplication rate in the gastrointestinal tract, they develop multiple beneficial effects on the host.

Probiotic bacteria release some chemical substances in the gastrointestinal tract of the host that inhibit the proliferation of opportunistic pathogens [2] and act as an antibacterial, antifungal, and antiviral agents. *Labeo rohita* fed with the live bacterial cell of *Bacillus substilis* KADR1 orally at 1 x 10^6, 1 x 10^8 and 1 x 10^{10} CFU/g showed resistance against *Aeromonas hydrophila* infection and also observed highest post-challenge survivability (p<0.5) in the diet containing 1 x 10^8 CFU/g of bacteria [19].While in case of Common carp (*Cyprinus carpio*) fed with dietary heat-killed *Pseudomonas aeruginosa* strain VSG2 showed higher tolerance against *Aeromonas hydrophila* infection [36]. Bacterial isolates obtained from the intestine of Turbot (*Scophtalmus maximus*) showed inhibitory effects against *Vibrio anguillarum* HI 11345 [51].

Improved water quality: Water quality parameters such as Dissolved Oxygen (DO), CO_2, pH, hardness, alkalinity, ammonia, nitrate, etc. are the most important factor in intensive or semi-intensive culture system for disease-free production. Over-feeding and high stocking density leads to deterioration of water quality and enhances the susceptibility towards infectious and non-infectious diseases [13]. Probiotics strains of bacteria improve the water quality and make suitable for the growth of cultured aquatic fauna. It acts as bioremediation and eco-friendly bio-control agent in aquaculture [13] and improves health status and performance level of cultured fauna [21]. They control ammonia, nitrite, hydrogen sulphide, etc. and enhanced the decomposition of organic matter [3]. Gram-positive Bacillus spp. is more efficient in converting the organic matter into CO_2 in comparison to gram-negative bacteria but gram-negative bacteria can convert organic matter into bacterial biomass or slime [11, 15].

Improved immunity: Immune system modulation mainly stimulation of non-specific immunity is one of the most important functions of probiotics with addition to that it improves immunity by the interaction between the intestinal epithelial cells and bacterial cells in the host gut [21]. Higher phagocytic activities and serum IgM levels were observed in *Labeo rohita* within 4 weeks of feeding trial with *Bacillus substilis* KADR1 [19]. Higher lysozyme activity (10^8 and 10^{10} CFU/g) and serum protein level (10^8 CFU/g) were noticed in the same experiment than that of control. Li. W. F *et al.* (2012) reported an increase of total serum protein, albumin, globulin, IgM level, alkaline phosphatase (AKP) and decrease of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lysozyme in grass carp given a diet containing Bacillus additives [39]. Increased total leucocyte count (WBC) was observed in koi (*Cyprinus carpio koi*) given a feed added with *B. coagulans* and chitosan oligosaccharides [26]. Mohammadian. *et al.* (2019) also observed increased serum lysozyme activity, complement activity, respiratory burst activity in *Tor grypus* fed on *Lactobacillus casei* supplemented commercial diet [33]. Dietary administration of heat-killed *Pseudomonas aeruginosa* strain VSG2 in *Cyprinus carpio* showed increased the activities of IgM, lysozyme and Alkaline phosphatase, as well as the protein content, in both mucus and serum [36].

Reproduction: The manipulation of high-quality diets, hormone injection and environmental parameters can improve the efficiency of reproduction performance and productivity [67]. Probiotics enhance gastro-somatic index, fecundity, embryo survival, hatching rate and changes GnRH, leptin and vitellogenin in female fishes [68]. Zebra fish is a protogynous hermaphrodite species [69] and has asynchronous ovary and considered as one of the best fish species as a model organism to study due to its ability to spawn throughout the year under laboratory condition. Under the influence of estradiol (E_2), vitellogenin (vtg) produce in the liver in the growth phase of the ovary. Probiotic administration in the diet of Zebra fish induced to elicit the expression of both estradiol receptor (era) and vtg gene [70].
Increased total fry production per female, fry survival, relative fecundity, GSI, average weight and length of fry and reduction in fry deformity were noticed in livebearer ornamental fish(Sword tail), with a dietary mixture of equal proportion of *Lactobacillus casei, L. acidophilus, Enterococcus faecium* and *Bifidobacteriumthermophilun* strains as probiotics [71]. In another case study noted higher GSI, fecundity, embryo survival, hatching rate, length of hatchling, and bodyweight of hatchling in killifish (*Fundulus heteroclitus*) than that of control has been recorded by the administration of *Lactobacillus rhamnosus* IMC 501®, supplied by Synbiotec S.R.L., Camerino – Italy [72]. Again, the effects of dietary probiotics, *Pediococcusacidilactici* and nucleotide on reproductive performance in goldfish were studied by Mehdinejad *et al.* (2018) [73] and they result showed to increase sperm density and spermatocrit value of male goldfish over control.

Effect of probiotics in crustacean
Several beneficial effects in shrimp aquaculture have been reported by providing probiotics in the culture system such as the increase of density of beneficial micro-organisms, improve water quality, increased survival rate, reduce phosphorus, and nitrogen concentration in the culture system, etc. [17]. There are some microorganisms authorized as probiotics in feeding stuff under council directive 70/524/EEC, these are *Bacillus cereus var. toyoi, B. licheniformis, B. substilis, Enterococcus faecium, Lactobacillus casei, L. farciminis, L. plantarum, L. rhamnosus, Pediococcusacidilactici, Saccharomyces cerevisae* and *Streptococcus infantarius* [20].
Several experiments demonstrated the increase of innate immunity by using probiotics which includes cellular components such as phagocytosis, encapsulation, and humoral components such as anticoagulant protein, agglutinins, antimicrobial peptides, phenoloxidase enzymes, etc. [21]. The effect of probiotics in water and in feed on *Macrobrachium rosenbergii* was studied by Prasad and Reddy in 2016 [23] and found the increment of growth rate, survival percentage, and decrease of fed conversion ratio (FCR) and protein efficiency ratio (PER) in both male and female-targeted species. An increased growth rate, survival rate, protease activity, lipase activity; decreased moisture, crude fat and ash content in the muscle of white shrimp (*Litopenaeus vannamei*), while in case of crude protein content increased and decreased when viable and dead probiotic cells of *Bacillus coagulans* were used [30].

Conclusion
Aquaculture is the fast growing sector in India that produces both finfishes and shellfishes. With increasing technologies to produce more fish in less water, disease occurrence in the culture system is become challenge for fish producers. So, prebiotics, probiotics, and synbiotics become important part of fish culture practices that has the ability to improve growth, culture media, immunity, health condition, and cure diseases.

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