PROBIOTICS IN FINFISH AQUACULTURE: AN INDIAN PERSPECTIVE

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Abstract

With the ever-expanding global population, there has been an increasing demand for fish and fishery products. To meet this ever-increasing demand for fish, aquaculture practices have been expanded and intensified over the decades. Intensive aquaculture practices cause stress to the reared animals, leading to increase susceptibility of the host to infectious diseases, which ultimately cause economic loss to the fish farming communities. Generally, antibiotics and chemotherapeutics are used to control infectious diseases in aquaculture. The use of probiotics is increasingly being recognized as an alternative to antimicrobial agents, as the development and emergence of drug and antibiotic resistance has become a growing concern. Probiotics are live microbial adjuncts that confer health benefits to the host. Besides enhancing the resistance of fish towards infectious diseases, probiotics also benefit the fish by promoting growth, stimulating immunity, providing nutrition, and improving the water quality of the rearing environment. This review aims to signify the necessity of using probiotic for sustainable aquaculture and to enlist the probiotic research in the recent past in India with their results on growth and health status of finfish.

Key words: Aquaculture, Growth, Immunity, India, Probiotics

Introduction

Aquaculture is one of the fastest-growing animal production sectors to cater the demands for protein-rich diet of today's growing population. Currently, the aquaculture sector contributes 46% of the total fish production, and the consumption of global food fish increased at an average annual rate of 3.1 percent from 1961 to 2017, a rate higher than that of all other animal protein foods, which increased by 2.1 percent per year (FAO, 2020). Over the years, diversification, expansion and intensification of aquaculture have taken place globally, to cater to the increasing demand of fish. With the increasing intensification and commercialization of aquaculture practice, diseases have become a major stumbling block in the fish farming industry. To tackle such significant concern,

several vaccines are available; however, the solution may not be viable (due to protection against a single pathogen, availability, cost-efficacy, mass application) for farmers in developing country like India (Sommerset *et al.*, 2005). Under such circumstances, the farmers are only left to treat diseases with a plethora of chemical additives and medicines, especially antibiotics (Huang *et al.*, 2015).

However, there has been an increasing concern pertaining to the use of antimicrobial agents as a disease preventive measure due to the evolution of antimicrobial resistance among pathogenic bacteria (Balcázar *et al.*, 2006). Many solutions have been suggested as an alternative, which includes natural immunostimulants, herbal

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extracts, antimicrobial peptides, bacteriophages, probiotics, prebiotics, synbiotics etc. Among all these novel approaches, probiotics have received particular interest owing to its efficacy in augmenting immune response, enhancing growth, natural availability, abundance and convenience in the application (Hoseinifar *et al.*, 2018).

What is a probiotic?

The term 'probiotic' was coined by Lilly and Stillwell (1965) to designate unknown growth-promoting substances produced by ciliated protozoans. A joint working group of Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) stated that probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/ WHO, 2001).

Probiotics have gradually gained popularity as an alternative to antimicrobial agents to promote growth and prevent diseases in aquaculture. Probiotics confer beneficial effects in the fish host when they are applied through the feed. Dietary administration of probiotics helps to modify the microbial balance of the gastrointestinal tract, stimulates the immune system, and provides nutritional benefits (Kesarodi-Watson *et al.*, 2008). Besides the health and growth promoting potentials, probiotics have an extended application in aquaculture. Given the intricate relationship between an aquatic animal and its surrounding environment, the concept of probiotic application in aquaculture has been broadened to include improvement of water quality by directly applying probiotics into the pond water. This extended application has been suggested to describe probiotics as microbial "water additives" (Moriarty, 1998). It is presumed that the bacteria which improve water quality may also improve the health status of the fish, and in fact, several commercial products, marketed as "probiotics", have sought to take

advantage of this idea (Gatesoupe, 1999). In the last two decades, research and application of probiotics in aquaculture have expanded enormously. Recent findings have revealed that members from approximately 20 bacterial genera have been recognised as potential probiotic candidates, where the majority of potential species are represented by *Bacillus* spp. and lactic acid bacteria (LAB) group (Knipe *et al.*, 2020).

Mode of action of probiotics

Although, probiotics have a generalised mode of action, they certainly play a vital role in the overall health responses of the host, particularly in immunological responses. In the recent past, intensive studies on probiotic characterization and application have shed light on the significance of probiotics, their mode of action, and led the future avenues for interdisciplinary work. Globally, researches have proposed several ways of action of probiotics, and the well-accepted modes of actions are presented in Fig. 1.

Fig. 1. Mode of action of probiotics

Competitive exclusion: Most of the pathogenic microbes require adherence site to mucosal surfaces of the host to initiate the disease

development (Adams, 2010). Competitive exclusion is a phenomenon where a probiotic prevents or reduces the colonization of other bacteria such as pathogens, by competing for the site of adhesion, nutrition, etc. (Lara-Flores, 2011). For survival and proliferation of any microbial community, they require sufficient source of adhesion surface, nutrients, or energy (Verschuere *et al.*, 2000). While struggling for basic needs for proliferation, colonized probiotics can out-compete the harmful pathogens by utilizing all the available resources. The colonization of probiotic bacteria leads to the production of certain antagonistic substances, which prevent the adhesion of any other challenging bacteria and ultimately eliminate its colonization (Verschuere *et al.*, 2000). Although competition for adhesion sites has been suggested as a mode of action of probiotics, there is little evidence in the literature to demonstrate the competitive exclusion principle, particularly in the *in vivo* situation (Kesarcodi-Watson *et al.*, 2008). In one study, a competitive exclusion effect was demonstrated by Vine *et al.* (2004), and they found that the attachment of the pathogen on the fish intestinal mucus was inhibited by the presence of probiotic bacteria.

Antagonism: Probiotics are responsible for the secretion of certain substances (e.g. bacteriocins) that may have a bactericidal or bacteriostatic effect on other harmful microbial populations (Verschuere *et al.*, 2000). Antagonism of pathogens through probiotic may correspond to the production of various antimicrobial substances such as acetic acid, lactic acid, hydrogen peroxide, siderophores, lysozyme, short-chain fatty acids, *etc.* (Hoseinifar *et al.*, 2017). Moreover, some probiotics may have the capacity to produce volatile fatty acid (butyric, acetic and propionic acid) and some organic acids, which results in reduced pH to inhibit the proliferation of opportunistic pathogens (Tinh *et al.*, 2008). Antagonistic activity against pathogenic

bacteria is probably the most studied mode of action of probiotics. In fact, the initial screening for potential probiotics essentially include *in vitro* (as well as *in vivo*) antagonism assays against different pathogens. A large number of published literatures have demonstrated antagonistic activity of potential aquaculture probiotics against a wide variety of fish pathogens (Balcázar *et al.*, 2006; Kesarcodi-Watson *et al.*, 2008).

Contribution to nutrition and digestive processes: Some probiotics are responsible for exerting a beneficial effect on the digestion of bio-molecules owing to their capacity to produce some extracellular enzymes, such as proteases, lipases, amylases and cellulase (Balcázar *et al.*, 2006). Besides extracellular enzymes, probiotics have also been reported to secrete certain fatty acids, vitamins, essential amino acids, growth factors *etc.*, which ultimately facilitate optimum feed utilization and enhance growth (Balcázar *et al.*, 2006; Tinh *et al.*, 2008; Lara-Flores and Aguirre-Guzman, 2009). For example, potential probiotics isolated from the intestine of *Catla catla* have been demonstrated to produce several extracellular enzymes including protease, amylase, lipase, cellulase, phytase and xylanase (Mukherjee and Ghosh, 2016).

Enhancement of the immune response and disease resistance: The probiotic application can lead to improve non-specific immune response (Balcázar *et al.*, 2006). The administration of probiotic modulates the immune-competence of aquatic animals by stimulating cellular and humoral responses (Biswas *et al.*, 2013). The probiotic application promotes phagocytic response, production of cytokines, TNF- α (tumour necrosis factor α), interleukins (IL-1 IL-6, IL-10, IL-12, IL-1 β), IFN- γ (interferon gamma) and chemokines, along with the increased expression of certain immune component molecules, such as antibodies, lysozyme, complements, superoxide ion, acid phosphatase, serum proteins, and several

antimicrobial peptides (Balcázar *et al.*, 2007; Lara-Flores and Aguirre-Guzman, 2009; Mohapatra *et al.*, 2012a,b; Das *et al.*, 2013; Giri *et al.*, 2013). All these probiotic attributes to the immuno-physiological response of the host ultimately correspond to elevated immune response and increased resistance against the pathogenic micro-organisms (Lakshmi *et al.*, 2013). These immunomodulating responses by probiotic bacteria lead to the resistance of host against an array of pathogens, including *Aeromonas* spp., *Edwardsiella* sp., *Photobacterium* sp. *Flavobacterium* sp. and *Vibrio* spp. (Taoka *et al.*, 2006). Additionally, several reports suggest that probiotics can also rouse the protective response against the viral (Chen *et al.*, 2017), and fungal (e.g. *Saprolegnia parasitica*) pathogens (Atira *et al.*, 2012).

Improvement of water quality: The application of probiotics maintains the optimum water quality parameters such as pH, dissolved oxygen, ammonia, and hydrogen sulphide (Aguirre-Guzman *et al.*, 2012). It was revealed that gram-positive bacteria such as *Bacillus* spp. are more efficient in converting organic matter into CO₂, slime, or bacterial biomass than the gram-negative bacteria (Chauhan and Singh, 2019). Moreover, during the production cycle, elevated levels of gram-positive bacteria (*Bacillus* spp.) can control the accumulation of dissolved and particulate organic carbon and facilitate improved water quality, survival, and growth rate (Balcázar *et al.*, 2006). Several probiotics have been reported to have an algicidal property, thereby improving water quality (Fukami *et al.*, 1997). Moreover, probiotic bacteria help in the bioremediation of ammonia and nitrite toxicity. Probiotics have also been related to the augmentation of beneficial microbes in the aquatic environment (Mohapatra *et al.*, 2012a).

Interference of quorum sensing: Quorum sensing (QS) is a mechanism in which bacteria regulate various phenotypes such as biofilm formation, bioluminescence, virulence factors and swarming, and these have been shown to

contribute to bacterial pathogenesis (Miller and Bassler, 2001). Since pathogenicity traits of bacteria are controlled by QS, disruption of QS (known as quorum quenching) has been suggested as a new anti-infective strategy to control pathogenic bacteria in the field of aquaculture and animal husbandry (Defoirdt *et al.*, 2004; Boyen *et al.*, 2009). In a recent research finding, bacteriocin from *Bacillus* sp. QSI-1 has been found to be an efficient quorum quencher and prevents biofilm formation of fish pathogen *Aeromonas hydrophila* (Chu *et al.*, 2014). Some other probiotic bacteria, such as *Lactobacillus* and *Bifidobacterium* degrade the signal molecules of pathogenic bacteria by enzymatic secretion (Brown, 2011).

Application of probiotic in Indian finfish aquaculture

Intensive and semi-intensive farming practices in the Indian aquaculture sector have emerged as one of the most convenient and promising options to meet the nutritional requirements of a constantly increasing population. Furthermore, the involvement of modern approaches, such as the application of probiotics, has improved the overall capability and quality of production (Bandyopadhyay *et al.*, 2015a,b). In recent time, most frequently used probiotic microorganisms in aquaculture belong to *Bacillus* spp., *Lactobacillus* spp., *Bifidobacterium* spp., *Enterococcus* spp., *Streptomyces* spp., *Carnobacterium* spp. and yeast (Van Doan *et al.*, 2019). Numerous studies have been carried out to evaluate the effect of probiotics on fish growth, physiology, and health status (Bandyopadhyay and Mohapatra, 2009; Das *et al.*, 2013; Giri *et al.*, 2013; Gupta *et al.*, 2014). Several studies have also been conducted in Indian finfish aquaculture with *Bacillus* spp. and *Lactobacillus* spp. being the most studied probiotic species. The effect of several probiotic strains on immunity, growth, haematological and biochemical responses of finfish is worth-mentioning. A summary of the application of probiotics in finfish aquaculture of India is presented in Table 1.

Table 1. Studies using probiotics in finfish aquaculture in India

Probiotic	Fish species	Doses and duration	Observations [#]	References
Probiotic application in IMCs				
<i>Bacillus subtilis</i>	<i>Labeo rohita</i>	0.5 × 10 ⁷ , 1 × 10 ⁷ and 1.5 × 10 ⁷ CFU g ⁻¹ of diet for 2 weeks	WG, TLC, Hb, TSP, GLB ↑ ALP, AST, ALT ↔ DR ↑	Kumar <i>et al.</i> (2006)
<i>B. subtilis</i>	<i>L. rohita</i>	1 × 10 ¹⁰ CFU g ⁻¹ feed for 90 days	WG, SGR, RNA : DNA, FCR, DR ↑	Bisht and Pandey (2013)
<i>B. subtilis</i>	<i>L. rohita</i>	10 ⁸ CFU g ⁻¹ of diet for 60 days	Ab/Immunoglobulin production ↑ TSP, GLB, DR ↑	Nayak <i>et al.</i> (2007)
<i>B. subtilis</i>	<i>L. rohita</i>	0.5 × 10 ⁷ , 1 × 10 ⁷ and 1.5 × 10 ⁷ CFU g ⁻¹ of diet for 2 weeks	RBA, SBA, DR ↑	Kumar <i>et al.</i> (2008)
<i>B. subtilis</i>	<i>Catla catla</i>	1 × 10 ⁸ CFU g ⁻¹ feed for 60 days	TEC, TWC, differential leucocyte count and Hb, RBA, LZY, MPC, bactericidal activity ↑	Kumar <i>et al.</i> (2015)
<i>B. subtilis</i> KADR1	<i>L. rohita</i>	10 ⁶ , 10 ⁸ and 10 ¹⁰ CFU g ⁻¹ for 28 days	LZM, TP, RBA, Ab, SOD, ACP, phagocytosis, DR ↑	Ramesh and Souissi (2018)
<i>B. subtilis</i> ATCC 6633	<i>C. catla</i>	Synergistic application of mannan-oligosaccharides (MOS) @0.4% and probiotic at 10 ⁶ CFU g ⁻¹ feed for 60 days	WG, SGR, ALT, AST, TSP, protease, amylase and lipase, PCV ↑ FCR ↓ Muscle LDH, MDH ↓ Liver LDH and MDH level ↑	Gupta <i>et al.</i> (2020)
<i>B. subtilis</i> and Mannan Oligosaccharides (MOS)	<i>Cirrhinus mrigala</i>	[<i>B. subtilis</i> 150 × 10 ⁷ CFU mL ⁻¹ + MOS 0.2%], [<i>B. subtilis</i> 50 × 10 ⁷ CFU mL ⁻¹ + MOS 0.6%] [<i>B. subtilis</i> 150 × 10 ⁷ CFU mL ⁻¹ + MOS 0.6%] for 60 days	TEC, TWC, Platelets, RBA, LZM, DR ↑ Hb, (↑↓), SOD (Liver ↑, Gill ↑↓),	Kumar <i>et al.</i> (2018a)
<i>B. subtilis</i> and MOS	<i>C. mrigala</i>	<i>B. subtilis</i> [150/ 50 × 10 ⁷ CFU g ⁻¹] + MOS [2 g/6 g kg ⁻¹] for 60 days	SGR, FER, WG, FCR, DR, Digestive enzyme (Amylase, protease and lipase) ↑ Hepatosomatic index and intestinal somatic index ↑↓	Kumar <i>et al.</i> (2018b)

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Probiotic	Fish species	Doses and duration	Observations [#]	References
Probiotic application in IMCs				
<i>B. subtilis</i> FPTB13 and chitin	<i>C. catla</i>	10 ⁹ cells g ⁻¹ and chitin (2%)	ALP, TP, MPC, TP, DR ↑ Skin mucosal lysozyme ↑	Sangma and Kamilya(2015)
<i>B. subtilis</i> , <i>Terribacillus saccharophilus</i>	<i>L. rohita</i>	1 × 10 ⁷ CFU g ⁻¹ of diet for 60 days	Humoral immune response ↑ Phagocytosis, RBA ↑	Kalarani <i>et al.</i> (2016)
<i>B. subtilis</i> , <i>Lactococcus lactis</i> and <i>Saccharomyces cerevisiae</i>	<i>L. rohita</i>	10 ¹¹ CFU kg ⁻¹ feed for 60 days	WG, SGR, PER, PA ↑ Dry matter digestibility (ADC _{DM}) ↑ Protein digestibility (ADC _{Protein}) ↑ Lipid digestibility (ADC _{Lipid}) ↑	Mohapatra <i>et al.</i> (2012b)
<i>B. subtilis</i> (VSG1), <i>Lactobacillus plantarum</i> (VSG3), <i>Pseudomonas aeruginosa</i> (VSG2)	<i>L. rohita</i>	<i>B. subtilis</i> (1 × 10 ⁸ CFU g ⁻¹), <i>L. plantarum</i> (0.5 × 10 ⁸ CFU g ⁻¹) and <i>P. aeruginosa</i> (0.5 × 10 ⁸ CFU g ⁻¹) combinations for 60 days	WG, SGR and FCR, LZ, complement, Ig, phagocytic activity, RBA, SOD, DR ↑	Giri <i>et al.</i> (2014)
<i>B. subtilis</i> , <i>L. lactis</i> and <i>S. cerevisiae</i>	<i>L. rohita</i>	1 × 10 ¹¹ CFU kg ⁻¹ for 30 days	WG, RBA, TSP, ALB, ALP, ACP, MPC, Ab, Glucose, HSP70 ↑	Mohapatra <i>et al.</i> (2014)
<i>B. circulans</i> PB7	<i>C. catla</i>	2 × 10 ⁵ cells 100 g ⁻¹ of diet for 60 days	DR, WG, ALP, PER, CCA, CCL, PA, PHR, ACP, GOT, GPT ↑ Phagocytic activity ↑; AMY ↔	Bandyopadhyay and Mohapatra (2009)
<i>B. circulans</i>	<i>L. rohita</i>	10 ⁸ bacterial cells g ⁻¹ of diet for 21 days	RNA: DNA ↑ SGR ↑	Ghosh <i>et al.</i> (2004)
<i>B. amyloliquefaciens</i> FPTB16	<i>C. catla</i>	1 × 10 ⁷ , 1 × 10 ⁸ and 1 × 10 ⁹ cells 100 g ⁻¹ of feed and fed up to 8 weeks	SOD, LZ, TSP, DR ↑ Myeloperoxidase content (MPC) ↑	Das <i>et al.</i> (2013)
<i>B. amyloliquefaciens</i> CCF7	<i>L. rohita</i>	1 × 10 ⁵ , 1 × 10 ⁷ , 1 × 10 ⁹ CFU g ⁻¹ of diet for 98 days	AST, ALT, SOD, Ab, LZ, DR ↑ Liver malondialdehyde and catalase ↑	Nandi <i>et al.</i> (2018)
<i>B. amyloliquefaciens</i> KU556165, <i>B. methylotrophicus</i> KU556164 and <i>B. licheniformis</i> KU556167,	<i>L. rohita</i>	Application was singly or conjointly with count of 0.5 to 1 × 10 ⁷ CFU g ⁻¹ of diet up to 60 days	Haematological indices, LM, ACP, RBA, phagocytosis, Ab, antiprotease and Peroxidase activities, DR ↑ Stress enzymes (glutamic oxaloacetic transaminase and glutamic pyruvic transaminase) ↔	Mukherjee <i>et al.</i> (2019)

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Probiotic	Fish species	Doses and duration	Observations [#]	References
Probiotic application in IMCs				
<i>B. cereus</i>	<i>C. mrigala</i>	2×10 ⁶ cells 100 g ⁻¹ of feed for 90 days	WG, SGR, DR ↑	Bhatnagar and Lamba (2015)
<i>B. pumilus</i>	<i>L. rohita</i>	1 × 10 ⁷ CFU g ⁻¹ of feed for 60 days	Hb, Total erythrocyte count (TEC), DR ↑	Rajikkannu <i>et al.</i> (2015)
<i>B. acillus</i> sp. PP9	<i>C. mrigala</i>	2 × 10 ⁴ CFU 100 g ⁻¹ of feed for 60 days	Growth, RNA: DNA, Hb, TSP, albumin: globulin and SBA ↑ Protease and α-amylase ↑	Bandyopadhyay <i>et al.</i> (2015b)
<i>Bacillus</i> spp.	<i>L. rohita</i>	1×10 ⁸ CFU g ⁻¹ of feed for 14 days	LY, TSP, RBA, Ab, SOD, ACP, phagocytosis, DR ↑	Ramesh <i>et al.</i> (2015)
<i>B. aerophilus</i>	<i>L. rohita</i>	10 ⁶⁻⁹ CFU g ⁻¹ of diet for 6 weeks	LZY, TP, B, SOD, ACP, DR ↑ Phagocytosis ↑	Ramesh <i>et al.</i> (2017)
<i>B. coagulans</i>	<i>C. catla</i>	1x10 ³ , 2x10 ³ , 3x10 ³ and 5x10 ³ CFU g ⁻¹ of diet for 90 days	Growth, TEC, TLC, phagocytosis, DR, NBT ↑	Bhatnagar and Raparia (2020)
<i>P. aeruginosa</i> VSG-2	<i>L. rohita</i>	1 × 10 ⁵ , 1 × 10 ⁷ , and 1 × 10 ⁹ CFU g ⁻¹ of feed and fed up to 60 days	LZY, ACR, SOD, RBA, DR, Phagocytosis ↑	Giri <i>et al.</i> (2012)
<i>L. plantarum</i> VSG3	<i>L. rohita</i>	1 × 10 ⁶ , 1 × 10 ⁸ , 1 × 10 ¹⁰ CFU g ⁻¹ of feed and fed up to 60 days	SGR, LZY, ACR, PHR, SOD, DR, RBA, Ab ↑	Giri <i>et al.</i> (2012)
<i>L. plantarum</i> , <i>B. megaterium</i>	<i>C. catla</i>	10 ⁷ cells g ⁻¹ diet for 60 days	Growth, PER, SGR, TEC, TP, DR ↑ FCR, TLC ↓	Parthasarathy and Ravi (2011)
<i>Lactobacillus</i> spp. (<i>L. acidophilus</i> , <i>L. bulgaricus</i> , <i>L. casei</i> , <i>L. plantarum</i>) +	<i>L. rohita</i>	1, 1.5, and 1 g kg ⁻¹ of diet	WG, SGR, DR ↑	Jha <i>et al.</i> (2015)

Table 1., Cont. ...

Cont. Table 1.

Probiotic	Fish species	Doses and duration	Observations [#]	References
Probiotic application in IMCs				
<i>Bifidobacterium</i> <i>bifidum</i> + <i>S. cerevisiae</i> + <i>S. faecium</i> + <i>Torulopsis</i> + <i>Aspergillus oryzae</i>	<i>L. rohita</i>			
<i>L. sporogenes</i> , <i>S. cerevisiae</i>	<i>L. rohita</i>	Application was singly or conjointly with 2-4 % of feed and fed up to 120 days	SGR, feed efficiency↑ FCR↓	Das <i>et al.</i> (2020)
<i>S. cerevisiae</i>	<i>L. rohita</i>	0.5, 0.75 and 1% lyophilized whole yeast (<i>S. cerevisiae</i>) cells for 60 days	RNA: DNA, PER, GOT, GPT, Hb, TEC, TLC, DR↑ Intestinal enzyme (protease and α-amylase) activities ↑	Bandyopadhyay <i>et al.</i> (2015a)
Probiotic application in other food fishes				
G-probiotic (commercial product)	<i>Oreochromis</i> <i>mossambicus</i>	5, 7.5 and 10 g kg ⁻¹ diet for 90 days	SGR, PER↑	Naik <i>et al.</i> (1999)
<i>B. subtilis</i>	<i>Poecilia</i> <i>reticulata</i> , <i>P. sphenops</i> , <i>Xiphophorus</i> <i>hellerii</i> , <i>X. maculatus</i>	5×10 ⁵⁻⁸ cells g ⁻¹ diet for 90 days	DR, Protease and amylase ↑	Ghosh <i>et al.</i> (2008)
<i>B. subtilis</i>	<i>P. reticulata</i> , <i>P. sphenops</i> , <i>Xiphophorus</i> <i>shelleri</i> , <i>X. maculatus</i>	5 × 10 ⁵ cells g ⁻¹ , 5 × 10 ⁶ cells g ⁻¹ , 5 × 10 ⁷ cells g ⁻¹ and 5 × 10 ⁸ cells g ⁻¹ for 1 year	Gonadosomatic index, fecundity Fry production ↑	Ghosh <i>et al.</i> (2007)
<i>L. acidophilus</i> and/or <i>S. cerevisiae</i>	<i>Cyprinus</i> <i>carpio</i>	10 ⁶ CFU g ⁻¹ diet for 45	WG, SGR, NPU ↑ Feed intake↑	Dhanaraj <i>et al.</i> (2010)
<i>Enterococcus faecium</i> MC13	<i>C. carpio</i>	10 ⁸ CFU g ⁻¹ diet for 60 days	NBT, DR↑	Gopalakannan and Arul(2011)

Table 1., Cont. ...

Cont. Table 1

Probiotic	Fish species	Doses and duration	Observations [#]	References
Probiotic application in other food fishes				
<i>B. coagulans</i> , <i>B. mesentericus</i> and <i>B. infantis</i>	<i>Puntius conchoniis</i>	6×10 ¹⁰ CFU ml ⁻¹	Probiotic bacteria established themselves in gut and significant reduction in other pathogenic gut inhabitants	Divya <i>et al.</i> (2012)
<i>B. subtilis</i>	<i>C. carpio</i>	4 × 10 ⁶ and 4 × 10 ⁸ cells 100 g ⁻¹ of feed	Growth, RNA: DNA, SGR, FCE↑	Bisht <i>et al.</i> (2012)
<i>B. coagulans</i> (MTCC 9872), <i>B. licheniformis</i> (MTCC 6824), <i>Paenibacillus polymyxa</i> (MTCC 122)	<i>C. carpio</i>	10 ⁹ CFU g ⁻¹ diet for 80 days	Growth, SGR, PER, LZY, RBA, MPC, DR↑	Gupta <i>et al.</i> (2014)
<i>B. licheniformis</i> Dabb1	<i>Pangasius hypophthalmus</i>	Oral administrated of 10 ⁵ and 10 ⁷ CFU mL ⁻¹	Growth, MPC, BRA, ACP, LZY↑ Antioxidant responses (glutathione-S-transferase, reduced glutathione, total glutathione) ↑	Gobi <i>et al.</i> (2016)
<i>Micrococcus</i> MCCB 104 and <i>Bacillus</i> MCCB 101	<i>Etroplus suratensis</i> , <i>O. mossambicus</i>	10 ³ CFU animal ⁻¹ day ⁻¹ for 28 days 10 ⁵ , 10 ⁷ cfu g ⁻¹ for 28 days	Alpha-amylase, lipase, pepsin, total alkaline protease activity ↑	Sankar <i>et al.</i> (2017)
<i>B. licheniformis</i> Dabb1	<i>O. mossambicus</i>		Growth, WG, SGR, TP, ALP, MPC, LYZ, SOD, DR, ROS, RNS, GPx ↑	Gobi <i>et al.</i> (2018)

[#] SGR-Specific growth rate; WG-Weight gain; TLC-Total leukocyte count; Hb-Haemoglobin; TSP-Total serum protein; GLB-Globulin; ALP-Alkaline phosphatase; AST-Aspartate aminotransferase; ALT-Alanine amino transferase; DR-Disease resistance; Ab-Antibody; RBA-Respiratory burst activity; SBA-Serum bactericidal activity; PER-Protein efficiency ratio; CCA-Carcass composition crude protein; CCL-Carcass composition lipid; PA-Protease activity; PHR-Phagocytic ratio; ACP-Acid phosphatase; GOT-Glutamate-oxaloacetate transaminase; GPT- Glutamate pyruvate transaminase; AMY-Amylase activity; LZY-Lysozyme; ACR-Alternative complement response; SOD-Superoxide dismutase; EC-Erythrocyte count; HSP70- Heat shock protein 70; PCV-Packed cell volume; LDH- Lactate dehydrogenases; MDH-Maleate dehydrogenases; NBT-Nitroblue tetrazolium; NPU-Net protein utilization; TNF-Tumour necrosis factor; TGF-Transforming growth factor; IFN-Interferon; Ig-Immunoglobulin; FCE-Feed conversion efficiency; ROS-Reactive oxygen species; RNS-Reactive nitrogen species; GPx-Glutathione peroxidase; ↑- Increases; ↓- Decreases; ↔- No change

Conclusions and future prospects

In recent years, the application of probiotics as a biocontrol agent has improved overall aquaculture sector performance. Over the period, the isolation and characterisation of potential probiotic have leapt, and now efforts are required to streamline the entire range of probiotic strains and categorize them as per their specific action. After the sequential probiotic characterisation, the cutting-edge approaches should involve in designing the specific probiotic or cocktail of probiotics to target specific fish species to make them very efficient and economical. The determination of an optimal environment for probiotic interaction with their host and surrounding environment also holds a wide scope for further investigation. The fate of probiotic in host fish and time span for its efficacy in host organism are uncertain areas, which require further elaborated investigation.

Even after the great extent of work about the efficacy and mode of action of probiotics, there are still several uncertainties that require further clarification for advancing the probiotic application such as safety concerns, antibiotic resistance, production of enterotoxin, etc. Real-

time evaluation of adherence and colonization of probiotic with the help of modern tools like hybridisation with green fluorescence protein (GFP) or some nontoxic radioactive compounds or fluorescence *in situ* hybridization (FISH) will be an interesting area to explore the relevance to probiotic research. Furthermore, interpretation with modern tools such as next-generation sequencing (NGS), genomics, transcriptomics, proteomics in the identification of unique genes and proteins associated with potential probiotics might reveal novel avenues for identification and application. Besides, the gnotobiotic approach can also be explored as they help in unveiling the function of beneficial symbionts on numerous biological processes of the host. Several synergistic strategies, such as application of immunostimulants, non-digestible fibres (prebiotics) etc. to enhance proliferation of gut microbiota, could be an area to explore. In conclusion, the possibilities are enormous, however systematic approaches alongside modern tools need to be undertaken to identify potential probiotics, which might offer novel possibilities to design the feasible future strategies in the health management of finfish aquaculture in India.

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