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Review Article

The advancement of probiotics research and its application in fish farming industries

Goutam Banerjee [a,b,*], Arun Kumar Ray [a]

[a] Department of Zoology, Visva-Bharati University, Santiniketan, West Bengal 731235, India
[b] Center for Nature Conservation and Biosafety (CNCB Pvt. Ltd.; cncb.co.in), Cuttack, Odisha 754132, India

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ABSTRACT

Fish are always susceptible to a variety of lethal diseases caused by different types of bacterial, fungal, viral and parasitic agents. The unscientific management practises such as, over feeding, high stock densities and destructive fishing techniques increase the probability of disease symptoms in aquaculture industries. According to Food and Agriculture Association (FAO), each and every year several countries such as China, India, Norway, Indonesia, etc. face a huge loss in aquaculture production due to mainly bacterial and viral diseases. The use of antibiotics is a common practise in fish farming sectors to control the disease outbreak. However, the antibiotics are not long term friend because it creates selective pressure for emergence of drug resistant bacteria. Probiotics are live microorganisms that confer several beneficial effects to host (enhances immunity, helps in digestion, protects from pathogens, improves water quality, promotes growth and reproduction) and can be used as an alternative of antibiotics. In recent year, a wide range of bacteria have reported as potential probiotics candidates in fish farming sectors, however, *Lactobacillus* sp. and *Bacillus* sp. gain special attention due to their high antagonistic activities, extracellular enzyme production and availability. In this present review, we have summarized the recent advancement in aquaculture probiotics research and its impact on fish health, nutrition, immunity, reproduction and water quality.

Contents

* Corresponding author.
E-mail address: banerjee.goutam2@gmail.com (G. Banerjee).

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1. Introduction

The intensive fish culture enhances disease probability (that reduce production rate and flesh quality) and ultimately hampers the economy of the country. Till date, antibiotics are more popular to farmers due to its rapid action and availability; however, it badly affects the water ecosystem. The replacement of antibiotics with probiotics might be an alternative way to control the pond health, as well as the diseases in fish. The word probiotic is constructed from the Latin word "pro" (for) and "bios" (life). The probiotics are live microorganisms (bacteria, yeast and fungi), which when administered in adequate amounts confers a health benefit to the host (Havenaar and Huis, 1992; Kechagia et al., 2013; Steenbergen et al., 2015). Gatesoupe (1999) has stated that the first use of probiotics in aquaculture was started in the mid-1980s (Kozasa, 1986) and since then interest in such environment-friendly treatment has been increased rapidly. Later on, Irianto and Austin (2002) have reported the scientific use of probiotics in aquaculture sectors and claimed as the alternative of antibiotics in near future.

The water qualities (pH, dissolved oxygen, dissolved carbon dioxide and organic load and minerals) are the major determinants for enhancing production rate and maintaining good health in fish. Alteration of such parameters foster the growth of several obligate or facultative pathogenic bacterial strains such as *Aeromonas*, *Pseudomonas*, *Citrobacter*, *Proteus*, *Streptococcous*, *Edwardsiella*, *Staphlycoccous* and different species of *Vibrio*, which cause huge mortality in both freshwater and brackish water fish (Welker et al., 2005; Verma et al., 2006; Cruz et al., 2012; Sihag and Sharma, 2012). Among several pathogens, *Aeromonas hydrophila* and *Aeromonas salmonicida* are considered to be the most common pathogens in freshwater fish, while *Vibrio anguillarum* and *Vibrio Parahaemolyticus* are the most familiar bacterial pathogens in marine environment, which cause different types of fish disease like ulcer disease, carp erythrodermatitis, motile *Aeromonas* septicemia etc. (Lightner et al., 1992; Cruz et al., 2012; Silva et al., 2014; Bartkova et al., 2016; Ghomrassi et al., 2016).

The use of antibiotics is common practise in aquaculture, however, it creates a selective pressure for emerging drug resistant bacteria, which might be transmitted through food chain from fish to human (SCAN, 2003; Kim et al., 2004; Cabello, 2006; Sørum, 2006; Da Costa et al., 2013; Tanwar et al., 2014). Furthermore, antibiotics inhibit or kill beneficial microbial flora in gut and disturb the natural ecosystem that affects fish nutrition, physiology and immunity (Rekecki et al., 2009; Rawls et al., 2007; Maynard et al., 2012). To avoid such adverse situation, probiotics candidates have been introduced in fish farming industries for better health management practise (Cruz et al., 2012). Along with diseases control, probiotics strains are also responsible for other beneficial purposes such as, extracellular enzyme production (Ray et al., 2012; Banerjee et al., 2013a,b), growth promotion (Cruz et al. 2012), maintaining water quality and immune modulation (Nayak, 2010; Montalban-Arques et al., 2015). In recent year, several microorganisms such as bacteria (Rengpipat et al., 2000; Patil et al., 2007; Vijayabaskar and Somasundaram, 2008; Nouri et al., 2010; Ibrahem, 2015; Banerjee et al., 2016a,b) and yeast (Chiu et al., 2010; Pinpimai et al., 2015; Caruffo et al., 2015) have been used randomly as promising probiotic candidates in fish industries. However, in this present review, we have focused only on bacterial probiotics and their vast applications in aquaculture sectors. Until now, this area is not so explored and a few probiotics are commercially available to market. In this present review, we have summarized the progress of probiotic research in fisheries science.

2. Selection criteria of probiotics

The successful probiotic candidate has to possess several important criteria (Fig. 1). In last few years, plenty of reports have been published regarding the screening, selection and characterization of fish probiotic bacterial strains (Vázquez et al., 1996; Gatesoupe, 1999; Vijayabaskar and Somasundaram, 2008; Harikrishnan et al., 2010; Liu et al., 2012), however, few are available for commercial uses. The selection procedures (both *in vivo* and *in vitro*) of probiotic bacteria are laborious and differ slightly from one organism to another, as the mode of action of probiotic candidate varies from aquatic to terrestrial animals (Huis in't Veld et al., 1994; Hou et al., 2015; Song et al., 2015). However, the general selection parameters are almost same and have been discussed below.

2.1. Non-pathogenic

The selected bacterial strain must be non pathogenic to fish (Zokaeifar et al., 2012). The degree of pathogenicity depends on toxin producing capability and it varies from one strain to another strain. For example, *Aeromonas hydrophila* is considered to be a deadly pathogen in fish (Mohideen and Haniffa, 2015), however few strains of *Aeromonas hydrophila* are used as probiotic candidates in fish (Gunasekara et al., 2010). Several *in vitro* techniques such as haemolytic activity, manitol utilization ability and other biochemical tests have been introduced to check the bio-safety of the selected bacterial strains. *In vivo* tests (fish fed with probiotic bacteria) also should be performed to confirm the non-pathogenic activity of the selected candidates. Pathogenicity of any bacterium is determined on the basis of disease symptoms (both internal and external) and mortality rate.

2.2. Drug resistant gene

The emergence of multi-drug resistant bacteria is a big treat for animals, including fish. In general, the drug resistant property of bacteria comes from the plasmid encoded genes (Jackson et al., 2011). The successful probiotic strain must not possess any plasmid-encoded antibiotic resistance gene or gene cluster (Gueimonde et al., 2013). In stress condition (presence of antibiotics), bacteria evolve very fast due to their high mutation rate and this unique property can be transferred from one species to another through lateral gene transfer mechanism. Thus, before selecting a bacterial isolate as promising probiotic candidate, few experiments (broad spectrum antibiotic sensitivity and PCR detection of multi drug resistant gene) should be performed.

2.3. Tolerance to pH

Probiotic candidate is administrated along with food and thus, it has to face a changing environment in gut in term of pH level. The environment of gut provides a favourable ecological niche for endosymbionts (Ray et al., 2012); however, at different physiological conditions such as during metabolism the pH level of the gut varies greatly. Even, during metabolism, different types of bile salts are also secreted (Buchinger et al., 2014). So, the probiotic candidates must have the ability to tolerate a wide range of pH (low acidic to high alkaline) and high concentration (>2.5%) of bile salts.

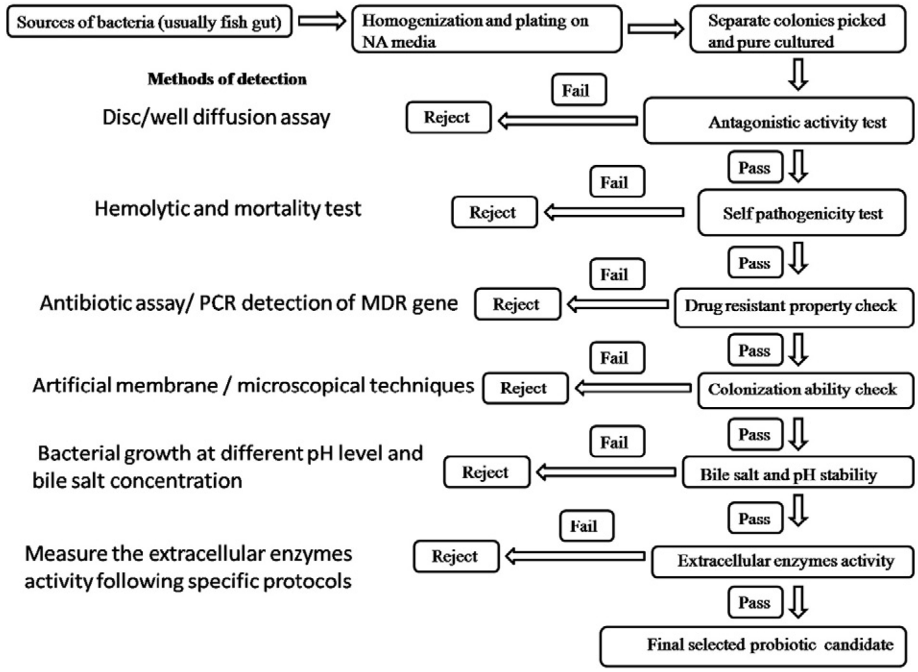

Fig. 1. Demonstrates the stepwise selection procedure and criteria of probiotics.

2.4. Colonization ability

Based on the colonization property on epithelial mucosal surface, the gut bacteria can be divided into two broad categories; autochthonous bacteria (able to colonize) and allochthonous bacteria (free living) (Ringø et al., 1995). It is now well established that autochthonous bacteria are more crucial in maintaining animal health in terms of nutrition, physiology and immunity. Thus, adhesion property of probiotics bacteria on gut epithelial mucosal surface is an important selection criterion ((Panigrahi and Azad, 2007). A wide range of sophisticated instruments such as, scanning and transmission electron microscope (SEM and TEM), fluorescence microscope, confocal microscope and few other *in vitro* techniques such as PCR, qPCR have been introduced to check the bacterial colonization ability (presence of mucus binding receptors) in gut surface (Mukherjee and Ghosh, 2014; Banerjee et al., 2016a).

2.5. Antagonistic activity

The antagonistic or inhibition ability of probiotic candidate against a variety of pathogens is the most important property. Probiotic bacteria produce a wide range of bacteriocins (small peptide to larger protein) or anti-microbial compounds to inhibit pathogens or the competitors. Several researchers have reported the *in vitro* antagonistic assay procedures (well diffusion method, disc assay and double layered molten agar chloroform assay) to measure the inhibition ability of the probiotic candidates (Patzer et al., 2003; Balcazar et al., 2007; Mukherjee and Ghosh, 2014). However, the degree of inhibition depends on other several factors and might be changed inside the body. Thus, *in vivo* experiment (challenged with pathogens) also should be performed with different fish species exposed to a wide range of pathogens. A successful probiotic candidate must have to show a broad spectrum antagonistic activity against different pathogens.

2.6. Extracellular enzymes production

Apart from antagonistic activity, extracellular enzymes (protease, amylase, cellulose, phytase, chitinase, lipase, phytase etc.) production ability is a positive sign of a successful probiotic candidate. In general, fish do not produce any vitamins. Endosymbionts/probiotics are the primary producers of vitamins and make it available to the host. Thus, the probiotic candidate must have to supply a sufficient amount of enzymes and vitamins for nutritional support to host. Several biochemical assays have been developed to quantify the extracellular enzymes production ability of the bacterial isolates (Ray et al., 2012; Banerjee et al., 2013a,b). Thus, before selecting a probiotic candidate, these biochemical assays should be done to measure its enzyme production efficiency.

2.7. Indigenous in nature

In general, the probiotic candidates are screened and isolated from the gut to avoid the colonizing difficulties and other immune related problems. In an investigation on brook charr (*Salvelinus fontinalis*), Boutin et al. (2013) stated that the indigenous probiotic candidates are better choice than exogenous sources in response to gut microbia communities. Furthermore, results of their study also have detected that indigenous probitotic strain (*Rhodococcus* sp.) did not disturb the natural skin microbial community. Thus, indigenous nature of probiotics has advantages over exogenous sources.

3. Bacterial probiotics and fish diseases

Due to intensive fish farming practise, the health management of cultured species is important for the sustainable development of the industry (Rombout et al., 2014). Regular monitoring of environmental factors and maintaining of water quality parameters are important for disease free fish production (Bader et al., 2006; Sihag and Sharma, 2012). In general, high stocking densities and over feeding cause water pollution (Chambel et al., 2015), which enhances the susceptibility of several types of bacterial diseases (Ulcer, tail rot, fin rot, dropsy), viral diseases (Iridovirus, Lymphocystis), worm diseases (Gyrodactylosis, Dactylogrosis, Diplostomiasis, Ligulosis), protozoan diseases (Ichthyophyhiriasis, Trichodinosis, Myxosporidipsis), fungal diseases (Saprolegnia attack, Gill rot) and crustacean diseases (Argulosis, Anchor worm). Parasitic diseases in fish are very common, which cause a huge mortality in fresh water fish (Amare et al., 2014). In a detail study, Hossain et al. (2011) have investigated the impact of environmental factors on parasitic diseases in fish. Results of their investigation have concluded that the fluctuation in water salinity hampers the pond ecosystem and increases stress to aquatic animals, which enhances susceptibility of different diseases like ulcer, Argulosis, Epizootic Ulcerative Syndrome (EUS), Trichodiniasis, Myxoboliasis, Chilodoneliasis, Dactylogyrosis, Gyrodactylosis, Ichthyophthiriasis and Pernicious anaemia. Till date, the application of probiotics candidate against parasitic diseases is not so popular, as the probiotics are not so effective against ecto and endo parasites (Travers et al., 2011; Sihag and Sharma, 2012). Thus, an effective alternative method has to be developed to combat with this situation.

Unlike parasitic diseases, the use of probiotic isolates against both bacterial and viral diseases in fish is a common practise in aquaculture industries. In a study conducted by Harikrishnan et al. (2010) have stated that *Paralychthys olivaceus* fed with 2.4×10^8 CFU (colony forming unit) g^{-1} of Lactobacil and/or Sporolac (commercial lactic acid bacteria) significantly increases the survival rate in fish infected by lymphocystis disease virus (LCDV). Furthermore, Liu et al. (2012) also have demonstrated that *Bacillus subtilis* E20 (10^8 CFU g^{-1} feed) as a feed supplement can effectively reduces the mortality rate in grouper infected by *Iridovirus*. Similarly, Maeda (1999) has compared the survival rate in larvae of *Shima aji* infected by Irido virus and has reported that fish fed with probiotic strain *Pseudoalteromonas Undina* VKM-124 supplemented feed showed better survival compared to control. However, the efficacy of probiotic strain is not satisfactory against most of the viral diseases in fish. Viral diseases (such as Novirhabdoviruses, pancreatic necrosis virus, lymphocystis disease etc.) are the major problems in both cultured and marine water fishes, and till date there are no effective probiotic against these diseases. Most of the viral agents are opportunistic and cause disease during stressed condition (Matsuzaki and Chin, 2000; Panigrahi et al., 2004; Sihag and Sharma, 2012). It is already established that upon binding on mucosal surface, probiotic candidate directly modulate fish immunity (both innate and adaptive), which activates the defence system and helps fish to fight against various viral diseases. The advance researches have uncovered the role of probiotic strain as immune modulator in fish (Salinas, 2015), however the actual mechanism/working pathway is still unclear.

Like viral diseases, bacterial infections are also common in fish farm industries. In last few years, several investigations have been published regarding the beneficial effect of probiotic candidates against various bacterial infections (RingØ and Birkbeck, 1999; Irianto and Austin, 2002, Trachoo and Boudreaux, 2006; Anukam, 2007; Deeseenthum et al., 2007; Bansal et al., 2011; Yesillik et al., 2011). However, little is focused in aquaculture industries (Table 1). In a review, Balcázar et al. (2006) have listed the popular probiotic bacterial candidates such as, *Bacillus cereus* var. *toyoi*, *Enterococcus faecium*, *Lactobacillus plantarum*, *Strepinfantarius tococcus*, *Bacillus licheniformis*, *Pediococcus acidilactici*, *Bacillus subtilis*, *Lactobacillus rhamnosus*, *Lactobacillus farciminis* and *Lactobacillus casei* for aquaculture industries. Similarly, Sorroza et al. (2012) also have reported the beneficiary effects of probiotics strains, *Vagococcus fluvialis* on *Dicentrarchus labrax* infected with *Streptococcus* sp. and *Vibrio anguillarum*. Though, several studies have reported the probiotics effect of different bacterial strains, however most of these selected probiotic candidates belong to lactic acid bacteria group such as *Lactobacillus plantarum* (Son et al., 2009), *Lactobacillus plantarum* VSG-3 (Giri et al., 2013), *Lactobacillus rhamnosus* ATCC 53103 (Nikoskelainen et al., 2001) and *Lactococcus lactis* (Balcázar et al., 2007). Researchers also have reported the probiotic potential of *Streptococcus iniae* Dan-1, *Pseudomonas fluorescens* AH2, *Enterococcus casseliflavus* and *Bacillus licheniformis* in rainbow trout (*Oncorhynchus mykiss*) infected by pathogenic strain of *Streptococcus iniae*, *Vibrio anguillarum* and *Yersinia ruckeri*, respectively (Eldar et al., 1997; Gram et al., 1999; Raida et al., 2003; Safari et al., 2016). Similarly, Cha et al. (2013) have conducted a challenge test on *Paralichthys Olivaceus* exposed to a pathogenic strain of *Stretoccocus iniae* and have reported the lower mortality rate in fish fed with probiotic (*Bacillus subtilis*) supplemented diet. However, Kumar et al. (2008) did not detect any significant difference using *Bacillus subtilis* as a probiotic candidate in *Labeo rohita* challenged with *A. hydrophila*. Thus, the degree of beneficiary effects of probiotic candidate varies from one host to another and highly depends on genetic makeup of both the pathogens and the host. So, the selection of probiotic bacteria for aquaculture industries is a crucial step.

In general, fungal diseases are called mycoses and considered to be a major problem in temperate fish. In a review, Verma (2016) has described several types of fungal infections in fish like saprolegniasis. Furthermore, Howe et al. (1998) have stated that saprolegniasis is a secondary infection seen to the wound area of fish integument. On the other side, *Ichthyosporidium* is a fungal pathogen that damages liver and kidney in fish. Robert (1989) also has reported that *Exophiala salmonis* and *Exophiala Psychrophila* are potent fish fungal pathogens that increase the mortality rate in fish farm. During last two decades, EUS is recognised as the most serious problem in brackish water fish (Sihag and Sharma, 2012), which is caused by *Aphanomyces invadans* or *Aphanomyces piscicida*. The outbreak of this disease was first reported in Japan in 1971 and later on reported from other several countries such as Sri Lanka, Burma, Australia, Indonesia, Malaysia and Thailand (Sihag and Sharma, 2012). Till date, information on probiotic candidate against fungal pathogens are scanty. Future investigation should be conducted to discover the promising probiotic strain against such fungal infections.

4. Mode of action of probiotics

The intensive researches have clearly demonstrated the mode of action of probiotics in animal sectors, including fish (Ibrahem, 2015). Until

Table 1
Effect of probiotics on several fish diseases.

Fish species	Probiotic strains	Bacterial pathogens	References
Salmo salar	*Tetraselmis suecica*	Four pathogens[a]	Austin et al. 1992
Oncorhynchus mykiss	*Clostridium botyricum*	*Vibrio anguillarum*	Sakai et al. 1995
Anguilla anguilla	*Enterococcus faecium*	*Edwardsiella tarda 981210L1*	Chang and Liu, 2002
Labeo rohita	*Bacillus subtilis*	*A. hydrophila*	Kumar et al. 2006
Catla catla	*L. plantarum* and *Bacillus megaterium*	*A. hydrophila*	Parthasarathy and Ravi 2011
Clarias batrachus	Several Probiotic mixture[e]	*Micrococcus* sp.	Dahiya et al. 2012
Ictalurus punctatus	*Bacillus* sp.	Four pathogens[c]	Ran et al. 2012
Labeo rohita	Probiotic mixture[b]	*A. hydrophila*	Saini et al. 2014
Heteropnuestes fossilis	*Bacillus subtilis*	*A. hydrophila*	Mohideen and Haniffa 2015
Carassius auratus	Three strains[d]	*V. parahaemolyticus*	Subharanjani et al. 2015

[a] *Aeromonas hydrophila*, *Aeromonas salmonicida* (strains LL and NG), *Serratia liquefaciens*, *Vibrio anguillarum*, *Vibrio salmonicida* and *Yersinia ruckeri* type I.
[b] *Bacillus* sp., *Lactobacillus* sp. and *Arthrobacter* sp.
[c] *Edwardsiella tarda*, *Streptococcus iniae*, *Yersinia ruckeri*, *Flavobacterium columnare*
[d] *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus sporogenes*.
[e] *D. sulphuricum*, *Rhodococcus*, *Bacillus megaterium*, *L. formis*, *G. acetobactor*, *D. sulphuricum*, *Chromatium*, *Chlorobium*, *Thioxidants*, *Psuedomonas*, *M. methyanica*, *Azospirillum*, *Trichoderma*, *S. commune* and *S. gluconicum*, *Thiobacillus*, *T. ferroxidant*, *Nitromonas*.

now, a wide range of probiotics candidates have been studied deeply regarding their beneficial effects to host, including their antagonistic activities against different bacterial, fungal and viral diseases in fish, but few strains have been selected for commercial use. Among several properties, antagonistic activity, extracellular enzymes production, competition for colonization site, immune modulation and competition for iron uptake are considered to be the most important properties of a successful probiotic candidate. In this present review, we have summarized all of these facts in details from fisheries point of view.

4.1. Antagonistic activity

The competition for nutrients uptake is a common phenomenon in natural habitats. Several bacterial species use the antagonistic activity or inhibition property as a weapon against its competitors (El-Kholy et al., 2014). In general, bacteria produce several types of anti-microbial compounds or bacteriocins to inhibit/kill other competitor bacterial species (Desriac et al., 2010; Pisano et al., 2014). Dobson et al. (2012) have stated that bacteriocins are protein (>10 kDa) or small peptide (<10 kDa) in nature and highly differ from antiobiotics (secondary metabolites) regarding their mode of action and chemical structure. Furthermore, Klaenhammer (1988) and Cascales et al. (2007) also have stated that a vast majority of bacteria and archaea produce at least one bacteriocin for their own defence. *In vitro* antagonistic activity of probiotic bacteria against different fish pathogens is a very popular area of research, and several investigations have been conducted in this particular direction (Desriac et al., 2010; Bibiana and Nithyanand, 2014; Pannu et al., 2014). Chavan and Riley (2007) have demonstrated the typical structure of a bacterocin that contains three parts; a toxin gene (encodes a toxic protein), an immunity/protection gene (protect from own toxin) and a lysis gene (destroy bacterial cell wall). The nature, structure and mode of action of bacteriocin differ greatly from one bacterial species to another (Lee and Kim, 2011). For example, bacteriocins produced by *Pseudomonas aeruginosa* and *Escherichia coli* are known as pyocins (Govan and Harris, 1985) and colicin (Riley and Gordon, 1992), respectively. Most of the aquatic researchers have focused their investigations on the evaluation of crude culture supernatant of probiotic candidates against fish pathogens. The details information regarding the characterization of bacteriocin produced by probiotic candidates are scanty; however, in recent years few reports have been published (Table 2). Furthermore, the diversity of bacteriocins produced by Gram negative bacteria is quite larger compared to Gram positive bacteria. Among several bacterial species, Lactobacilli are the well characterized group for their antagonistic activities, bacteriocins diversity and wide distribution (Perez et al., 2014; Hernández et al., 2005). Several researchers have isolated different strains of *Lactobacillus* from aquatic organisms, including water and soil sediments (Karthikeya and Santosh, 2009; Ghosh et al., 2014; Ibrahem, 2015). In the year 2014, Sahnouni et al. have isolated and screened 38 strains of *Lactococcus lactis* from the gastrointestinal tract of marine fish (*Sardina pilchardus* and *Boops boops*) and evaluated their antagonistic activities against various deadly pathogens, including *A. hydrophila*. It is already established that the anti-microbial protein produced by different *Lactobacillus* sp. differ in size from <5 kDa to >30 kDa (Ghosh et al., 2014). Until now, the researches on bacteriocin are mostly focused on the pathogens related to human and other veterinary animals such as, cow, pig, goat and sheep. To enhance the momentum of probiotic research in future, detail investigations of anti-microbial activity of probiotic strains against potent fish pathogens should be conducted.

4.2. Enzymes production

Probiotic bacteria produce several types of extracellular enzymes to support the host metabolism process. Fish produce a wide range of endogenous enzymes such as, protease, amylase, lipase, cellulase, phytase etc (Fagbenro et al., 2000, Krogdahl et al., 2005; Sriket, 2014; Liu et al., 2016). Among these, proteases are very important digestive enzymes and reported in several species of freshwater and marine water fish (Sriket et al., 2012). Similarly, endogenous and exogenous (produced by gut endosymbionts) amylase is very important in herbivorous and omnivorous fish species, which degrades the complex carbohydrate into simple monosaccharide (Alarcón et al., 2001; German et al., 2004; Ray et al., 2012). Enzymes activities in alimentary tract are highly depend on dietary supplements (carbohydrate, protein and lipid), physiological factors (age, body temperature, gut pH, structure of digestive tract) and other environmental conditions (Cahu and Zambonino Infante, 2001; Skea et al., 2007; Miegel et al., 2010; Allameh et al., 2015). Furthermore, Liu et al. (2016) have stated that enzyme activity (both endogenous and exogenous) in fish is highly influenced by tropic levels also. Cellulase and chitinase are also important enzymes and reported in several aquatic organisms, including fish (Ray et al., 2012). In an investigation, Saha and Ray (1998) have reported that endogenous cellulase activity was very poor in *Labeo rohita* and gut microbiota plays a leading role in glucose metabolism in this fish species. Similar findings were also reported in *Cyprinus carpio* and other fish species (Ray et al., 2012). The quantity and activity of endogenous enzymes is not sufficient for complete metabolism of the ingested food materials in fish. Thus, enzymes secreted by permanent gut endosymbionts and probiotics are crucial from nutritional point of view (Banerjee et al., 2013a,b). In recent year, extracellular enzyme production by several fish probiotic strains have been investigated extensively (Table 3). Most of these studies in this direction evaluated the enzyme production capacity through *In vitro* studies. However, the actual contribution of these enzymes in fish metabolism is still not well understood.

4.3. Competition for colonization on mucosal surface

To exert the harmful effect, pathogens need to be attached on the mucosal surface of the gut. Probiotic bacteria not only directly inhibit pathogens, but also block the attachment sites for pathogenic bacteria

Table 2
Bacteriocin produced by probiotics bacteria against common fish pathogens.

Probiotic bacteria	Size/name	Pathogens	References
Vibrio mediterranei 1	63–65 kDa	*V. parahaemolyticus*	Carraturo et al. 2006
Enterococcus faecium MC13	2.148 kDa	*V. parahaemolyticus*	Satish Kumar et al. 2011
Bacillus subtilis NCIMB 3610	16 kDa	*V. anguillarum* 408 O1, *Photobacterium damselae*, *Vibrio* sp., *Aeromonas* sp.	Maria et al. 2012
Pseudoalteromonas flavipulchra JG1	77.0 kDa	Five pathogens[a]	Yu et al. 2012
Lactobacillus brevis FPTLB3	54 kDa	Five potent pathogens[c]	Banerjee et al. 2013a,b
Lactobacillus sp. MSU3IR	39.26, 6.38 kDa	*Carnobacterium*	
Enterococcus feacium	4.5 kDa	*maltaromaticum*	Sarra et al. 2013
Lactobacillus murinus AU06	21 kDa	*Vibrio* sp., *Micrococcus*	Elayaraja et al. 2014
Bacillus sp. SW1-1	38 kDa	Five pathogens[b]	Kim et al. 2014
Pediococcus acidilactici L-14	pediocin PA-1	–	Araújo et al. 2016

[a] *Escherichia coli* MTCC 1563, *Enterococcus faecalis* MTCC 2729, *Lactobacillus casei* MTCC 1423, *Lactobacillus sakei* ATCC 15521 and *Staphylococcus aureus* ATCC 25923.
[b] *Edwardsiella tarda*, *Streptococcus iniae*, *S. parauberis*, *Vibrio anguillarum* and *V. harveyi*.
[c] *Aeromonas hydrophila*, *Vibrio harveyi*, *V. parahaemolyticus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

Table 3
Enzyme producing fish probiotics bacteria.

Fiah	Probiotic bacteria	Enzymes produced	References
Oreochromis leucostictus, *Oreochromis mossambicus*, *Etroplus suratenis*	*Acinetobacter*, *Vibrio*, *Bacillus*, *Alcaligens*, *Photobacterium*, *Flavobacterium*	Amylase, protease, lipase	Sivasubramanian and Kavitha, 2012
Sparus aurata	*Bacillus* sp.	Alkaline and acid protease	Arığ et al. 2013
Cirrhinus mrigala	*Bacillus cereus*	Protease, amylase, cellulase	Bhatnagar and Lamba 2014
Oreochromis niloticus	*B. subtilis* strain VITNJ1	Protease	Efendi and Yusra, 2014
Catla catla	*B. methylotrophicus*, *B. subtilis*, *Enterobacter hormaechei*	Protease, amylase cellulase	Mukherjee and Ghosh 2014
Barbus grypus	*Lactobacillus casei*	Lipase, amylase, alkaline phosphatase	Vand et al. 2014
Cirrhinus mrigala	*B. amyloliquefaciens*, *B. sonorensis*	Protease, amylase cellulase, lipsase, phytase, xylanase	Dutta and Ghosh, 2015
Catla catla	*B. aerius*, *B. sonorensis*	Protease, amylase cellulase, lipsase, phytase, xylanase	Dutta et al., 2015

and reduce the harmful effect of the pathogens (Yirga, 2015). Mucosal surface of aquatic animal is more important than terrestrial counterparts, as they are continuously interacting with the microbiota in their water environment. The microbial population in gut of fish is very dense and consists of several types of bacteria, fungi and yeast (Ray et al., 2012; Banerjee et al., 2013a,b). Fish are always susceptible to a wide range of pathogenic bacteria, which enter in the gut, attach to gut mucus surface, colonize and finally cause disease (Cruz et al., 2012). The binding ability of bacteria on mucosal surface depends on the presence of mucus binding protein in cell wall (Jensen et al., 2014). In an investigation, MacKenzie et al. (2010) have reported the expression pettern of mub, or mub-like gene in several *Lactobacillus* strains, which differ greatly from each other in term of binding affinity. Furthermore, bacterial lipopolyscharide (LPS) and extracellular polymeric substance (EPS) were also reported to be a vital substance in attachment process (Walker et al., 2004; Santander et al., 2014). In a study, Horne and Baxendale (1983) have compared the adhesion capability of *Vibrio anguillarum* at different regions of rainbow trout (*Oncorhynchus mykiss*) intestine and have reported the higher adhesion in upper and mid gut regions compare to lower region of the gut. Similarly, Krovacek et al. (1987) also have demonstrated the adhesion efficiency of two potent fish pathogens (*Vibrio anguillarum* and *Aeromonas hydrophila*) and a probiotic candidate; *Carnobacterium* strain K1 in the gastrointestinal tract of fish. In a similar *in vivo* study, Divya et al. (2012) have confirmed the colonization ability of three pribiotic strains namely *Bifidobacterium infantis*, *B. coagulans* and *B. mesentericus* in the gut of *Puntius conchonius*. In last few years, several researches have been done on fish probiotic bacteria, but few investigations have reported the mode of association of probiotic bacteria on the gut mucosal layer (Vine et al., 2006; Balcazar et al., 2007; Lara-Flores and Aguirre-Guzmán, 2009; Rendueles et al., 2012; Ibrahem, 2015). Probiotic candidates must have possesses colonization ability and thus, before commercial use adhesion property should be checked properly through *in vitro* and *in vivo* experiments. Presence of mucus binding protein secreting gene can also be confirmed using polymerase chain reaction technique, which indirectly will support the colonization property of the probiotic candidate.

4.4. Immune modulation

Gut is the primary attachment site for the probiotic bacteria. Gut-associated lymphoid tissues (GALT) are considered to be key regulator for gut immunity. Immunomodulation is a prophylactic strategy in fish which comes from the beneficial effects of the probiotics (Lazado and Caipang, 2014). Probiotics or normal gut endosymbionts maintain a close relation with mucosal immunity of fish. Upon entering a pathogens inside the body, gut microbiota/probiotics induce the mucosal immunity (Magnadottir, 2010), which immediately activates the adaptive immune system (B cell and T cell responses) and the complement system (Lovoll et al., 2007; Salinas et al., 2011; Heinecke and Buchmann, 2013; Nuñez Ortiz et al., 2014). According to Cerezuela et al. (2013) "Major contigs of the fish immune system activities are lysozyme, the alternative complement pathway, phagocytosis, respiratory burst, superoxide dismutase and mucus production". Several articles have been published regarding the probiotics and its effects on fish immune system, but most of these researches just have measured the complement proteins concentration, immunity related gene expression, interlukins and cytokines concentration (Song et al., 2006; Biswas et al., 2013; Meena et al., 2013). The exact mechanism/working pathway of probiotics in fish are not clear till date.

It is well established that upon binding on mucosal surface, probiotic candidate modulates the mucosal immunity in fish (Lazado and Caipang, 2014), but the mechanistic pathway of immune modulation by probiotic strain is not well understood. Several internal and external factors such as water quality, gut pH, body temperature and food ingredients have been identified, which can effectively alter the efficiency of probiotic candidate in fish gut environment (Brown et al., 2012; Ray et al., 2012; Xia et al., 2014). In recent year, few reports have been published regarding the functions of probiotic bacteria in modulating gut commensal flora and mucosal immunity (Otte and Vordenbaumen, 2011; Gallo and Nakatsuji, 2011; Foey and Picchietti, 2014). Probiotic bacteria when ingested with food stuff, modulate the microbial composition (usually increase the number of beneficial microbe) in gut and activate the host defence system (Fig. 2). However, the direct relation between probiotic and host immune system is not well defined. Bandyopadhyay and Mohapatra (2009) have reported the role of probiotic strain *Bacillus circulans* PB7 in immune modulation in *Catla catla*. In a similar investigation Brunt and Austin (2005) also have recorded the immune modulation in *Oncorhynchus mykiss* fed with probiotic bacteria. Probiotic candidates usually influence the innate and adaptive immunity in term of several cytokines and chemokines production such as TNF-a, interlukins (IL-6, IL-10, IL-12) and IFN-c (Peddie et al., 2002; Montes et al., 2003; Nayak, 2010; Hemaiswarya et al., 2013; Foey and Picchietti, 2014). Similarly, Biswas et al. (2013) have reported the expression of a wide range of cytokines gene in *Takifugu rubripes* fed with heat killed probiotic strain *Lactobacillus paracasei* spp. paracasei 06TCa22 and *L. plantarum* 06CC2. Whereas, Kim et al. (2013) have investigated the effects of probiotic on immune system of *Paralichthys olivaceus* infected by *Lactococcus garvieae*. Results of their study have clearly indicated that the probiotic strain *Enterococcus faecium* can effectively stimulates the expression of complement system associated gene, lysozyme activity, protease activity and other cytokines like TNF-α and IL-1β in kidney, however no such expression was detected in gill and liver. On the other hand, He et al. (2013) have stated that probiotc bacterium *Bacillus subtilis* C-3102 enhances the adhesion of beneficial bacteria in gut mucosal surface of hybrid tilapia (*Oreochromis niloticus* ♀ × *Oreochromis aureus* ♂) and also enhances the expression of intestinal cytokines like IL-1b, TGF-β and TNF-α, but down regulates the expression of heat shock protein HSP70. During last few years, some other investigations have slao been conducted to explore the roles of

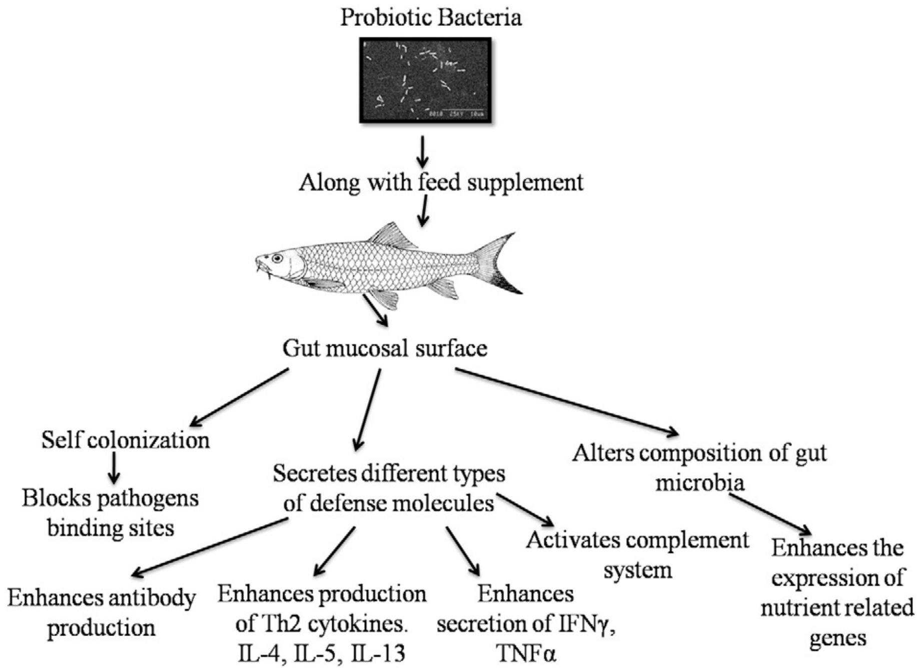

Fig. 2. Demonstrates the role of probiotic in immune modulation in fish after colonization on the gut mucosal surface.

probiotic in immune stimulation in fish (Villamil et al., 2002; Balcazar, 2003; Liu et al., 2012). Culture dependent studies are not sufficient to evaluate the role of probiotic in gut microbial alteration, as a high percentage of gut commensals are un-culturable (>90%). Recent advancements in molecular biology field have introduced several types of sophisticated techniques such as denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), RNA-seq and next generation sequencing (NGS) to explore the total microbial compositions in gut (He et al., 2009; Ye et al., 2014; Hiergeist et al., 2015).

4.5. Competition for iron uptake

All organisms, including bacteria require a continuous source of nutrients for survival, growth and proliferation. Among several nutrients, iron is recognised to be the most important one (Sihag and Sharma, 2012), as it is required for DNA replication, oxygen transport, protection against oxidative stress, enzyme activity and energy generation (Skaar, 2010). Siderophores are low molecular weight iron chelating compound (<1500) produced by several bacterial and fungal candidates (Stephan 2005; Miethke and Marahiel, 2007; Ahmed and Holmström, 2014). The battle for iron between pathogenic bacteria and it's vertebrate host is an interesting phenomenon and has been reported by several researchers (Gao et al., 2012; Kortman et al., 2012). The siderophore produced by probiotic candidate or beneficial gut endosymbiont reduce the availability of iron for pathogenic bacteria, as it has high affinity for ferric ion (Tan et al., 2016). Several reports have been published regarding the siderophores production by different fish probiotics strains (Gatesoupe et al., 1997; Gram et al., 1999; Verschuere et al., 2000; Cruz et al., 2012). In a detail study, Sugita et al. (2012) have isolated 2637 bacterial specimens from gut of thirteen different costal fish species and tested their siderophores producing ability. Results of their investigation have demonstrated that siderophores producing bacterial strains belong mostly to *Vibrio splendidus* (32.7%), *Vibrio ichthyoenteri* (19.5%) and *Vibrio crassostreae* (11.3%). Furthermore, they also have suggested that siderophores producing bacterial species such as *Vibrio crassostrea*, *Enterovibri norvegicus*, *Photobacterium phosphoreum*, *Photobacterium leiognathi*, *Vibrio scophthalmi* and *Photobacterium rosenbergii*, might be a possible probiotics in fish farming industries. Available sources of nutrients in fish gastrointestinal tract influence the survival of pathogenic bacteria and use of siderophore producing probiotic strain is an important strategy to reduce the energy availability for pathogens (Verschuere et al., 2000; Pacheco and Sperandio, 2015). The siderophore mediated competition between pathogenic and beneficial bacteria is more common in marine environment (Verschuere et al., 2000; Ibrahem, 2015). Iron removal by siderophores producing probiotic strains definitely enhances the nutritional immunity in fish. Until now, the siderophore research is not so explored in probiotic bacteria of fish. Intensive research on the mechanism of siderophore production by probiotic bacteria should be conducted.

5. Probiotics and reproduction

The role of probiotics in maintaining fish health is well established, but some recent investigations also have demonstrated the relation between probiotics and fish reproduction (Gioacchini et al., 2011a,b; Lombardo et al., 2011a,b; Ibrahem, 2015). In general, during reproductive period female fish requires more energy (in term of protein, lipid and carbohydrate metabolism) to enhances the fecundity (egg producing capacity) rate. Probiotics are good producers of different types of extracellular enzymes like protease, amylase, cellulose etc (Mukherjee and Ghosh, 2014; Dutta and Ghosh, 2015`), which enhance the host metabolism and increase the availability of nutrients. Avella et al. (2012) have conducted an experiment to evaluate the effect of probiotic strain *L. rhamnosus* on the development and reproduction of zebrafish (*Danio rerio*). Results of their study have indicated the over expression of several genes such as insulin like growth factors I and II, vitamin D receptor-α, gonadotropin releasing hormone 3 and retinoic acid receptor γ in fish fed with probiotic supplemented feed. On the other hand, Abasali and Mohamad (2010) have reported the enhancement of gonadosomatic index (GSI) and fecundity rate in *Xiphophorus hellerii* fed with commercial probiotic strain *Lactobacillus* sp. Similarly, Ghosh et al. (2007) also have evaluated the probiotic potential of *Bacillus subtilis* on reproduction of four ornamental fish species (*Poecilia sphenops*, *Xiphophorus maculates*, *Poecilia reticulate* and *Xiphophorus helleri*) in response to GSI, fry production and fecundity rate. While, Chitra and Krishnaveni (2013) have stated that commercial probiotic, Vibact significantly increased GSI, fry survival rate and fecundity in black molly (*Poecilia*

Table 4
Role of probiotics on fish reproduction.

Fish species	Probiotic strains	Effects on reproduction	References
Danio rerio	*Lactobacillus rhamnosus* IMC 501	Estradiol receptor, vtg, cyclin B, activinβA1, smad2 gene expression increase	Gioacchini et al. 2011a,b
Xiphophorus maculates	Primalac	GSI and fecundity rate increase	Abasali and Mohamad 2011
Fundulus heteroclitus	*Lactobacillus rhamnosus* IMC 501	Enhance gonadal growth, fecundity and embryo survival	Lombardo et al. 2011a,b
Danio rerio	*Lactobacillus rhamnosus* IMC 501	Induce follicles maturation process	Gioacchini et al. 2012
Danio rerio	*Lactobacillus rhamnosus* IMC 501	Enhance follicles development and embryo quality	Carnevali et al. 2013
Danio rerio	*Lactobacillus rhamnosus* CICC 6141 and *Lactobacillus casei* BL23	Up-regulate the expression of leptin, kiss2, gnrh3, fsh, lh, hcgr, and paqr8 genes responsible for fecundity and follicle maturation	Qin et al. 2014
Anguilla anguilla	*rhamnosus* IMC 501	Enhance sperm count	Vílchez et al., 2015

spenops). The reports regarding the probiotic potential on fish reproduction are scanty; however, few reports have been published in this direction (Table 4). Most of the studies have just reported the change of GSI and fecundity rate, but have not uncover the under laying mechanism. GSI is directly related to reproductive hormones like follicle stimulating hormones (FSH) and luteinizing hormones (LH), and thus the evaluation of the expression of these hormonal genes in presence of probiotic candidate might be an important clue.

6. Probiotics and water quality

Water quality parameters such as acidity, alkalinity, dissolved oxygen (DO), carbon-di oxide, nitrate, phosphorus, ammonia and hardness play vital role in fish production in aquaculture industries. Low water quality (low DO, high ammonia, high nitrate etc.) enhances the percentage of diseases susceptibility in fish farming industries. Probiotic bacteria not only trigger the defense system in fish, but also improve the water quality (Table 5). Several researchers have stated that probiotics can be used as an eco-friendly bio-control or bioremediation agents for sustainable development in aquaculture (Shariff et al., 2001; Dimitroglou et al., 2011; Iribarren et al., 2012). Padmavathi et al. (2012) have investigated the effects of two probiotic bacterial candidates namely *Nitrosomonas* sp. and *Nitrobacter* sp., and reported that the uses of such beneficial bacteria decrease the pathogenic load in culture pond. However, ammonia and nitrite concentration in probiotics treated ponds have decreased dramatically. Furthermore, Melgar Valdes et al. (2013) have proved the potentiality of several probiotic candidates (*Rhodopseudomonas palustris*, *Lactobacillus plantarum*, *Lactobacillus casei* and *Saccharomyces cerevisiae*) in maintaining water quality. Phototropic bacteria are very important in marine ecosystem, which enhance the water quality in term of phosphorous availability, nitrate reduction and salt enhancement. In an experiment, Li et al. (1997) have stated that addition of probiotic bacteria in shrimp culture pond enhance the water quality and reduce the toxic material concentration. Several industries directly release their waste into water bodies without any prior processing, which cause pollution, kill the natural habitats and unbalance the ecosystem. Addition of beneficial or probiotic bacteria reduce the pollutant load (heavy metal like Pb, Cd, Hg, Ni, *etc.*) and maintain a healthy condition for aquatic animals (Banerjee et al., 2016b). Nimrat et al. (2012) have indicated that the addition of mixed probiotic accelerated the survival rate of white shrimp (*Litopenaeus vannamei*) and enhance the water quality significantly (maintain pH level, increase ammonia and nitrite concentration). In a study conducted by Sharma and Bhukhar (2000) have applied different concentrations of commercial probiotic, Aquazyn-TM-1000 in *Cyprinus carpio* cultured pond. Results of their study have indicated the better growth of zooplankton, whereas no such alteration was detected in pH, temperature, nitrate-nitrogen, orthophosphate and dissolved oxygen concentration. In a review, Ibrahem (2015) has Stated that probiotic bacteria serve as a positive agent in maintaining water quality such as, decrease algal growth and organic load, increase nutrients concentration, increase beneficial bacterial population, inhibit potential pathogens and increase dissolved oxygen concentration. Until now, the use of probiotic candidates in fish culture ponds are not so popular, but in near future it would be randomly used in aquaculture industries and local fish farming sectors.

7. Conclusion and future perspectives

The aquaculture is the fastest growing food producing sectors in different countries like China, India, Brazil, Norway, Malaysia, Sri Lanka, Japan and USA, which mainly produce fish, molluses and crabs. Bacterial disease in aquaculture is a very common problem which causes a huge mortality in both wild and farmed fish. To reduce the risk of pathogenic bacteria and to get the rapid result, farmers usually use antibiotics, which are not environment friendly indeed. Furthermore, antibiotics create a selective pressure for emerging drug resistant bacteria, which can be transmitted to human through food chain. At present, different types of probiotic candidates or probiotics mixtures are discovered, but their applications aquaculture sectors are still limited. Commercialization of these candidates is important for sustainable aquaculture practise. Furthermore, farmers are unaware about the importance of

Table 5
The role of probiotics in maintaining water quality for sustainable aquaculture.

Probiotic strains/commercial product	Water quality parameters and production		References
	Increase	Decrease	
Bacillus sp.	Dissolved oxygen, survival rate	Total ammonia	Zink et al. 2011
Super biotic, Pro-w, mutagen, zymatin and Pro-2	Dissolved oxygen	Ammonia	Hossain et al. 2013
Nitrosomonas, sp. *Nitrobacter* sp.	Dissolved oxygen, phosphorous, zooplankton	Pathogenic bacterial load, ammonia	Sunitha and Padmavathi 2013
Bacillus subtilis FY99-01	Increase production	pH, reactive phosphorous, nitrate	Wu et al. 2014
Staphylococcus sp.	Dissolved oxygen	Nitrogen, nitrites, nitrates, phosphates	Lakshmi et al. 2015
Ecotech	–	Ammonia, nitrate, nitrite	Rahul gaddipati et al. 2015
Zymetin	Conductivity, production	Nitrate, ammonia	Ghosh et al. 2016
PRO-W	Dissolved oxygen	Total ammonia nitrogen	Nitya Jeevan Kumar et al. 2016

probiotics and their application process in ponds. Thus, regular government campaigning is the only way to train the fish farmers which would be helpful in replacing antibiotics with probiotics.

The role of probiotics in fish reproduction is a new area of research. It was reported that probiotics enhance fish reproduction (enhance vitellogenin synthesis, induce follicle maturation and increase GSI index), but the underlying mechanisms are not clear. Furthermore, researches on human being have established that probiotic bacteria modulate gut bacteria which play an important role in brain development (gut-brain axis) and behaviour control. However, such information are not available in fish. Further investigations in this particular field should be conducted for better health management practise in aquaculture.

Conflict of interest

We declare that none of the authors have any types of conflict of interest.

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