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Probiotics application in aquaculture: improving nutrition and heath

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Abstract

Biotechnology has immense potential in enhancing animal nutrition, heath-status and productivity. One of such application of biotechnology in aquaculture is probiotics. Probiotics are micro-organisms that are beneficial to host in improving its health, digestive enzyme activity, immunity and nutrition. Probiotics may be administered to fish as a food supplement or as a water additive. These may prove as a boon to aquaculture as these not only improve heath of animal but are also helpful in bioremediation of water, and are eco-friendly. These also compete with the pathogenic bacteria; decrease their virulence, hence, limiting the use of antibiotics. The present review paper highlights the application of probiotics in aquaculture. It also summarizes the development and research highlights of the probiotic status and mode of action, which are of great significance from an eco-friendly and sustainable aquaculture point of view.

Introduction

Growing population of a developing country and to meet the requirements of food for such a large population is an issue of major concern. Aquaculture has gained much momentum in fulfilling demands of seafood for a considerable part of population. According to a recent data published by the Food and Agriculture Organization, Fisheries and Aquaculture Department, the world aquaculture production of food fish reached 62.7 million tonnes in 2011, up by 6.2% from 59 million tonnes in 2010 and contributing about 40.1% to the world's total fish production (FAO, 2011). Indian aquaculture production mainly consists (~ 87%) of 3 native major carps and 3 exotic carps. Besides this, fish and its products are trending much now-a-days due to its rich nutritive value and awareness among people. The use of vaccines, antimicrobial agents and disinfectants for increasing fish and shrimp production, has led to the expansion of antibiotic resistance among the microorganisms which have become a global concern (Esiobu et al., 2002).

Infectious diseases are considered to be of paramount importance to the development and sustainability of commercial aquaculture, in terms of direct losses of biomass and productivity as well as indirectly as trade restrictions and poor water quality (Verschuere et al., 2000; Sharifuzzaman et al., 2014). The pathogens, however, get congenial environment for multiplication causing disease manifestations, when the fishes constantly suffer from stress due to adverse conditions in the pond ecosystem like higher temperature, higher stocking densities, less oxygen and heavy organic load etc. Hence, semi-intensive and intensive systems are very much prone to disease outbreak. Bacterial infections are one of the important causes of disease problems in Indian aquaculture (Sahoo et al., 2011). *Aeromonas hydrophila* is the most common pathogen, and it can easily spread through accidental abrasions and causes haemorrhagic septicaemia, ulcers, exophthalmia, abdominal distension (Austin and Austin, 2012).

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Until now, prevention or controlling aquatic disease has mainly depended on antibiotics and disinfectants. However, the massive use of these chemicals has led to antibiotic resistance in some instances (Verschuere et al., 2000). Also the rapid expansion of intensive aquaculture industry, are often acccompanied by rotted uneaten feed, sedimentation of faeces and organic residue. The water quality rapidly deteriorates as a result. In particular, nitrogenous compounds such as ammonia and nitrite quickly built up, which are both harmful to fish even at low concentrations (Mohapatra et al., 2012; Xie et al., 2013). Water exchange can be applied to maintain good water quality, however frequent exchange is not only laborious and costly, but also may incur disease causing agents and pollute nearby water bodies (Mohapatra et al., 2012). Therefore, there is an urgent demand for cost-effective and environment-friendly approaches for remediation of aquaculture water.

In order to mitigate the antibiotic resistance, disease problem and match the demand of seafood, biotechnological interventions have been employed. One of the successful biotechnological applications in the field of aquaculture is the use of probiotics. Probiotics are harmless live microorganisms that help the wellbeing of the host animal by competitive exclusion of pathogenic bacteria through the production of inhibitory compounds, enhancing nutrition and immune response of host species and improving water quality (Thompson et al., 1999; Verschuere et al., 2000; Gupta and Dhawan, 2011, 2012).

According to the definition of probiotic, effective probiotic treatments might provide broader-spectrum and greater disease protection as a result of immunity enhancement (Kesarcodi-Watson et al., 2008). Many micro-organisms like *Bacillus, Lactobacillus, Enterococcus, Bifidobacterium* are regarded as safe probiotics and have been commercialised for use. As *Bacillus* bacteria secrete many exoenzymes (Moriarty, 1996, 1998), these have been used widely as putative probiotics. Studies have shown that administration of probiotics in the freshwater prawn *M. rosenbergii* led to improvement in growth and survival as well as enhancement in immunity (Suralikar and Sahu, 2001; Venkat et al., 2004; Keysami et al., 2007; Shinde et al., 2008; Saad et al., 2009; Seenivasan et al., 2012). In aquaculture, probiotics can be administered either as a food supplement or as an additive to the water (Moriarty, 1998). The efficacy of a probiotic application depends on many factors such as species composition and viability, administration level, application method, frequency of application, diet, fish age, overall culture management and environmental stress factors (Lara-Flores, 2011).

Beneficial effects of probiotics on host

Probiotics may act in several ways as shown in Fig.1 and exerts beneficial effects on the host by:

a. Enhancing growth performance through establishment of healthy gut micro-environment (Merrifield et al., 2011; Gupta et al., 2014).

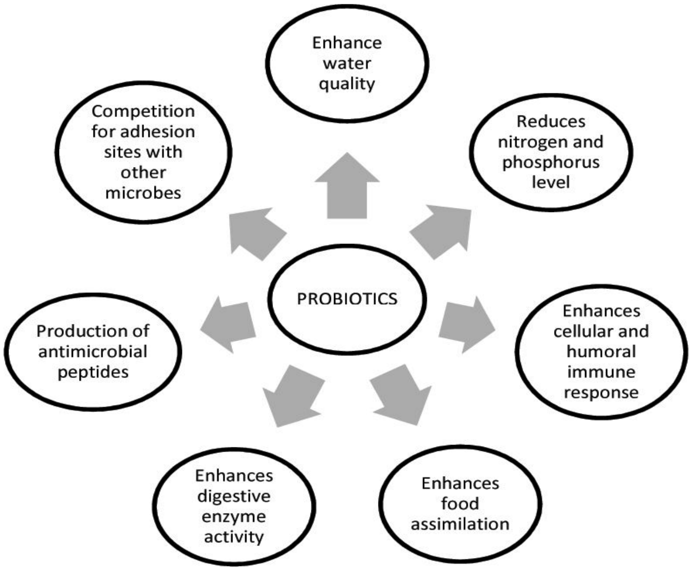
b. Providing nutrients to digestion through production of exogenous digestive enzymes and vitamins (Tinh et al., 2008; Gupta et al., 2015b).

c. Inhibiting pathogenic microorganisms through competition (Verschuere et al., 2000; Irianto and Austin., 2002; Gupta et al., 2014).

d. Enhancing immune response through elevating specific and innate immunity (Irianto and Austin., 2002; Gupta et al., 2014, 2015a); and

e. Improving water quality through water nitrogen remediation (Verschuere et al., 2000; Zhou et al., 2009; Gupta et al., 2015a; 2015b).

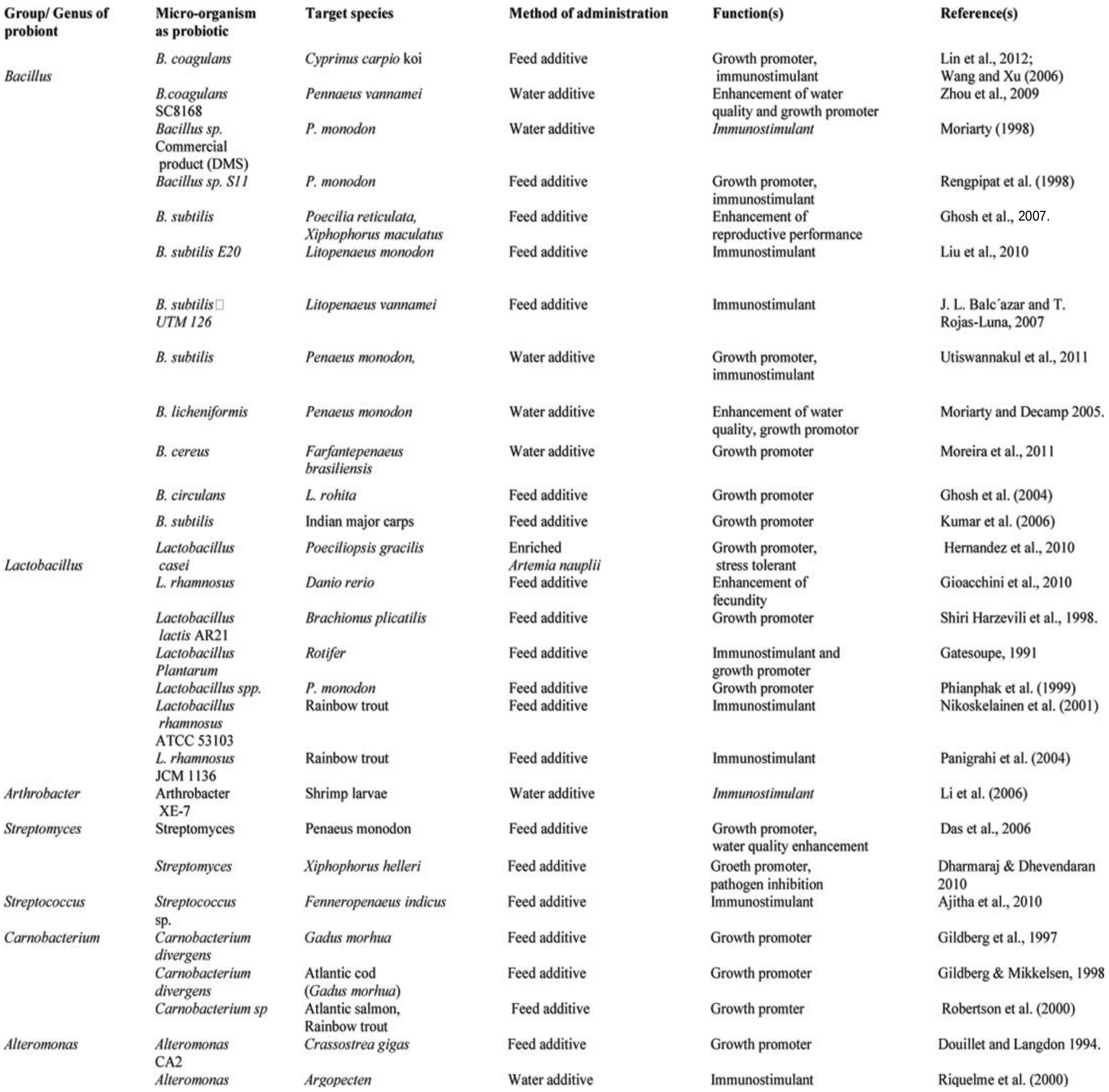
Fig. 1: Beneficial effects of probiotics



Mechanism of probiotic action

Probiotics modulate the growth of intestinal microbiota, suppress potentially harmful bacteria and reinforce the body's natural defense mechanisms (Giorgio et al. 2010; Bidhan et al. 2014), thus improving resistance against infectious diseases (Gildberg et al. 1997). Bacterial probiotics do not have specific mode of action but act on species specific or even strain-specific and immune responses of the animal, and their interaction with intestinal bacterial communities plays a key role (Simon 2010). Probiotics produce inhibitory substances that may be antagonistic to the growth of pathogens in the intestine. The ability of some probiotics to adhere to the intestinal mucus may block the intestinal infection route common to many pathogens (Ringo et al. 2010; Gatesoupe 1999). They can also stimulate the appetite and improve nutrition by the production of vitamins, detoxification of compounds in the diet and breakdown of indigestible components (Abdelhamid et al. 2009; Bidhan et al. 2014). Due to multiple advantages, they have gained a lot of importance in area of research in aquaculture. Some of micro-organisms used as a probiotic are listed in Table 1. However, yeast, algae and mixed strains has also been used. Mixed cultures of micro-organisms used as probiotic are listed in Table 2.

Table 1: List of micro-organisms used as probiotics in aquaculture.



Group/ Genus of probiont	Micro-organism as probiotic	Target species	Method of administration	Function(s)	Reference(s)
Bacillus	*B. coagulans*	*Cyprinus carpio* koi	Feed additive	Growth promoter, immunostimulant	Lin et al., 2012; Wang and Xu (2006)
	B.coagulans SC8168	*Pennaeus vannamei*	Water additive	Enhancement of water quality and growth promoter	Zhou et al., 2009
	Bacillus sp. Commercial product (DMS)	*P. monodon*	Water additive	*Immunostimulant*	Moriarty (1998)
	Bacillus sp. S11	*P. monodon*	Feed additive	Growth promoter, immunostimulant	Rengpipat et al. (1998)
	B. subtilis	*Poecilia reticulata, Xiphophorus maculatus*	Feed additive	Enhancement of reproductive performance	Ghosh et al., 2007.
	B. subtilis E20	*Litopenaeus monodon*	Feed additive	Immunostimulant	Liu et al., 2010
	B. subtilis□ *UTM 126*	*Litopenaeus vannamei*	Feed additive	Immunostimulant	J. L. Balc´azar and T. Rojas-Luna, 2007
	B. subtilis	*Penaeus monodon,*	Water additive	Growth promoter, immunostimulant	Utiswannakul et al., 2011
	B. licheniformis	*Penaeus monodon*	Water additive	Enhancement of water quality, growth promotor	Moriarty and Decamp 2005.
	B. cereus	*Farfantepenaeus brasiliensis*	Water additive	Growth promoter	Moreira et al., 2011
	B. circulans	*L. rohita*	Feed additive	Growth promoter	Ghosh et al. (2004)
	B. subtilis	Indian major carps	Feed additive	Growth promoter	Kumar et al. (2006)
Lactobacillus	*Lactobacillus casei*	*Poeciliopsis gracilis*	Enriched *Artemia nauplii*	Growth promoter, stress tolerant	Hernandez et al., 2010
	L. rhamnosus	*Danio rerio*	Feed additive	Enhancement of fecundity	Gioacchini et al., 2010
	Lactobacillus lactis AR21	*Brachionus plicatilis*	Feed additive	Growth promoter	Shiri Harzevili et al., 1998.
	Lactobacillus Plantarum	*Rotifer*	Feed additive	Immunostimulant and growth promoter	Gatesoupe, 1991
	Lactobacillus spp.	*P. monodon*	Feed additive	Growth promoter	Phianphak et al. (1999)
	Lactobacillus rhamnosus ATCC 53103	Rainbow trout	Feed additive	Immunostimulant	Nikoskelainen et al. (2001)
	L. rhamnosus JCM 1136	Rainbow trout	Feed additive	Immunostimulant	Panigrahi et al. (2004)
Arthrobacter	Arthrobacter XE-7	Shrimp larvae	Water additive	*Immunostimulant*	Li et al. (2006)
Streptomyces	Streptomyces	Penaeus monodon	Feed additive	Growth promoter, water quality enhancement	Das et al., 2006
	Streptomyces	*Xiphophorus helleri*	Feed additive	Groeth promoter, pathogen inhibition	Dharmaraj & Dhevendaran 2010
Streptococcus	*Streptococcus* sp.	*Fenneropenaeus indicus*	Feed additive	Immunostimulant	Ajitha et al., 2010
Carnobacterium	*Carnobacterium divergens*	*Gadus morhua*	Feed additive	Growth promoter	Gildberg et al., 1997
	Carnobacterium divergens	Atlantic cod (*Gadus morhua*)	Feed additive	Growth promoter	Gildberg & Mikkelsen, 1998
	Carnobacterium sp	Atlantic salmon, Rainbow trout	Feed additive	Growth promter	Robertson et al. (2000)
Alteromonas	*Alteromonas* CA2	*Crassostrea gigas*	Feed additive	Growth promoter	Douillet and Langdon 1994.
	Alteromonas	*Argopecten*	Water additive	Immunostimulant	Riquelme et al. (2000)

	haloplanktis	*purpuratus*			
Aeromonas	A. hydrophila	Goldfish	Feed additive	Immunostimulant	Irianto et al., 2003
	Aeromonas media A 199	Pacific oyster larvae *(Crassostreagigas)*	Water additive	Growth promoter	Gibson et al., 1998
Enterococcus	*Enterococcus faecium* SF68	*Anguilla anguilla*	Feed additive	Immunostimulant	Chang and Liu 2002
Pseudomonas	Fluorescent *pseudomonad* F19/3	Atlantic salmon presmolts	Bathing in bacterial suspension	Growth promoter	Smith and Davey 1993
	Pseudomonas spp.	Rainbow trout	Water additive	Immunostimulant	Spanggaard et al., 2001
	Pseudomonas fluorescens AH2	Rainbow trout juveniles	Water additive	Growth promoter	Gram et al., 1999
	Pseudomonas fluorescens	Rainbow trout *(O. mykiss)*	Water additive	Growth promoter	Gram et al., 1999
Lactococcus	*Lactococcus lactis* AR21	Rotifers	Water additive	Growth promoter	Harzevili et al., 1998
Pediococcus	*Pediococcus acidilactici*	*Artemia*	Water additive	Growth promoter	Gatesoupe 2002
	Pediococcus acidilactici	*Artemia*	Feed additive	Immunostimulant	Villami et al., 2003
Roseobacter	*Roseobacter sp.* BS107	*Pecten maximus*	Water additive	Growth promoter	Ruiz-Ponte et al. (1999)
	Roseobacter sp. strain 27-4	Turbot larvae	Water additive	Growth promoter	Hjelm et al., 2004
Yeast (Saccharomyces)	*Saccharomyces cerevisiae*	*Litopenaeus vannamei*	Feed additive	Immunostimulant	Scholz et al., 1999
Unicellular alga (*Tetraselmis*)	*Tetraselmis suecica*	Atlantic Salmon juveniles	Feed additive	Growth promoter	Austin et al.,1992
Vibrio	*Vibrio alginolyticus*	*L. vannamei*	Water additive	Growth promoter	Garriques and Arevalo 1995
	Vibrio alginolyticus	Atlantic Salmon juveniles	Bathing in bacterial suspension	Growth promoter	Austin et al., 1995
	Vibrio pelagius	Turbot	Water additive	Growth promoter	Ringø and Vadstein 1998
	Vibrio alginolyticus C14	*Artemia* nauplii	Water additive	Growth promoter	Gomez-Gil et al., 1998

Table 2: List of mixed cultures (various strains of micro-organisms) used as probiotics in aquaculture.

Mixed cultures	**Target species**	**Mode of administration**	**Function(s)**	**Reference**
Bacillus coagulans, Paenibacillus polymyxa and *B. licheniformes*	*Cyprinus carpio*	Feed additive	Growth promoter, immunostimulant	Gupta et al., 2014
Bacillus megaterium, B. polymyxa, B. licheniformis, B. subtilis (Biostart)	*Channel catfish*	Water additive	*Growth promotor*	Queiroz and Boyd 1998
Cytophaga sp., Roseobacter sp., Ruergeria sp., Paracoccus sp., Aeromonas sp., Shewanella sp.	Gilthead sea bream larvae *(Sparus aurata*)	Water additive	*Growth promotor*	Makridis et al. (2005)
Vibrio spp., Micrococcus sp.	Gilthead sea bream	Feed additive	*Immunostimulant*	Chabrillón et al. (2006)
Vibrio P62, Vibrio P63, Bacillus P64	*P. vannamei*	Water additive	Immunostimulant	Gullian et al. (2004)
Vibrio hepatarius, Vibrio sp., Bacillus sp.	*P. vannamei*	Feed additive	Growth promotor	Balca´zar (2003)
Streptococcus lactis and Lactobacillus bulgaricus	Turbot larvae (*Scophthalmus maximus)*	Enrichment of live food	Growth promotor	Garcı´a de la Banda et al. (1992)
Lactobacillus sp. and Carnobacterium sp.	Turbot larvae	Enrichment of rotifers	Growth promotor	Gatesoupe (1994)
Aeromonas hydrophila, Vibrio fluvialis,Carnobacterium sp., Micrococcus luteus	Rainbow trout	Feed addititve	Disease resistance	Irianto and Austin (2002)
Saccharomyces cerevisiae, S. exiguus, Phaffia rhodozyma	*Penaeus vannamei*	Feed additive	Disease resistance	Scholz et al. (1999)
Pseudomonas sp., Vibrio fluvialis	*P. monodon*	Water additive	Immunostimulant	Alavandi et al. (2004)
Lactobacillus casei, L. brevis,L. helveticus, Lactococcus lactic spp. lactis, Leuconostoc, Mesenteroides spp. mesenteroides, Pediococcus acidilactici	*Artemia nauplii*	Water additive	Immunostimulant	Villamil et al. (2003)
Streptococcus faecium, L. acidophilus, S. cerevisiae	*Nile tilapia*	Feed additive	Growth promotor	Lara-Flores et al. (2003)
Commercial product: Bactocell (*Pediococcus acidilactici*), Levucell (*Saccaromyces cerevisiae*)	*Pollack*	Enriched Artemia	Growth promotor	Gatesoupe (2002)
Improval (*L. sporogenes* and *Saccharomyces cerevisiae*)	*Macrobrachium rosenbergii*	Feed additive	Growth promotor	Gupta and Dhawan 2012
Bacillus NL 110, *Vibrio* NE 17	*M. rosenbergii*	Feed additive	Enhancement of water quality, growth promotor	Rahiman et al., 2010

Improvement in water quality

Remediation of aquaculture water using microorganisms is a burgeoning trend for the sustainable development of aquaculture industries (Verschuere et al., 2000). It is very important to provide fish with a healthy environment and probiotics has great deal of potential (Zhou et al., 2009). *Bacillus* species are widely used for water remediation because they are stable for long period due to spore formation, easily prepared by fermentation and possess antagonistic effects on pathogens (Xie et al., 2013; Zhou et al., 2009; Hong et al., 2005). Screening strains with good remediation characteristic in conjunction with their influence on survival, immune response and disease resistance still remains a fundamental step towards developing commercial microbial agents. Very few reports are available on spore forming bacteria to describe the remediation effect in freshwater aquaculture. Wang and He (2009) observed that the application of probiotics would mitigate the nitrogen and phosphate pollution in ponds sediments. Xie et al. (2013) demonstrated the use of *Bacillus amyloliquefaciens* for remediation of aquaculture water. Wang et al. (2005) investigated the effect of commercial probiotics on water quality of *P. vannamei* ponds and the results showed that probiotics could significantly reduce the concentrations of nitrogen in pond water. Similarly, Ma et al. (2009) evaluated the feasibility of *Lactobacillus* strains for the removal of nitrogen and observed simultaneous removal of ammonia, nitrite and nitrate. However, Zhou et al. (2009) observed inconsistence results by using different concentrations of *B. coagulans* as water additive in the culture of shrimp *P. vannamei*. Gupta et al. (2015a) used varied concentrations of *P. polymyxa* in common carp as water additive and found no effect of probiotics on the water quality improvement.

Enhancement of growth performance

Probiotics administration also has been shown to increase animal survival by enhancing resistance to pathogens by activating both cellular and humoral immune defences. The enhanced growth performance of animal might be induced by the probiotics via synthesis of vitamins and cofactors or enzymatic improvement (Gatesoupe, 1999). *Bacillus* strains are widely used in aquaculture industry through dietary supplementations for the improvement of growth (Ghosh et al., 2008) and feed utilization through digestive enzymes enhancement (Zhou et al., 2009). Gupta et al. (2015b) studied that inoculation of varied concentrations of *P. polymyxa* in water resulted in significant improvements in growth performance in terms of weight gain, specific growth rate, survival, relative per cent survival and feed utilization in terms of food conversion ratio and protein efficiency ratio of *C. carpio*. Previous study showed that *Bacillus* sp when applied as probiotic was able to colonize both in the culture water and the fish digestive tract, thereby increasing the fish survival (Rengpipat et al., 1998; Zhou et al., 2009; Gupta et al. 2014). Similar experiments had previously been documented in preliminary trials on Nile tilapia *Oreochromis niloticus* (Lara-Flores et al., 2003; Haroun et al., 2006), Indian carp *Cirrhinus mrigala* (Swain et al., 1996), Persian sturgeon *Acipenser persicus* (Faramarzi et al. 2011), Chinese carp *Cyprinus carpio* (Ramkrishnan et al., 2008) and giant freshwater prawn *Macrobrachium rosenbergii* (Gupta and Dhawan, 2011, 2012). However, Shariff et al. (2001) found that treatment of *P. monodon* with a commercial *Bacillus* probiotic did not significantly increase growth and survival. It was difficult to directly assess different studies using probiotics, because the efficacy of probiotic application depended on many factors (Gomez-Gil et al., 2000; Gupta et al., 2014) such as species composition, application level, frequency of application and environmental conditions.

Enhancement of digestive enzyme activity

Studies indicate that some probiotics increase the content of digestive enzymes in the gastro-intestinal tract (GIT) and thereby facilitate nutrient utilization and digestion (Haroun et al., 2006; Abdelhamid et al., 2009). The increased activities of digestive enzymes in fish fed probiotics are due to the improvement in the digestion of protein, starch, fat and cellulose and increase in absorption of food, which in turn contributed to the improved growth and survival. Effects have been reported for fish and shrimp, in which digestion was shown to increase considerably in response to probiotics in the diet (Lara-Flores et al., 2003; Ziaei-Nejad et al., 2006; Wang, 2007). Bacteria, particularly members of the genus *Bacillus* secreted a wide range of exoenzymes (Moriarty, 1998). The exogenous enzymes produced by the probiotic would represent, at most, only a small contribution to the total enzyme activity of the gut (Ding et al., 2004; Zhou et al., 2009), and the presence of the probiotic might stimulate the production of endogenous enzymes by the fish. The increase in specific activities of digestive enzymes in probiotic inoculated fish led to enhanced digestion and increased absorption of food, which in turn contributed to the improved growth and survival in fish (Gupta et al. 2015b).

Enhancement of immune responses

The innate immune system, comprising physical barriers, and cellular and humoral components, serves as a defense weapon in aquatic organisms (Magnadóttir, 2006). The beneficial effects of probiotics as immunostimulants have already been studied in several freshwater fish for example *Labeo rohita* (Nayak et al., 2007), *Oreochromis niloticus* (Aly et al., 2008), *O. mykiss* (Sharifuzzaman and Austin, 2010) and *C. carpio* (Gupta et al., 2014). Lysozyme is a cationic enzyme that attack the â-1, 4 glycosidic bond between N-acetlymuramic acid and N-acetylglucosamine in the peptidoglycan of bacterial cell walls. This enables lysozyme to lyse certain Gram-positive bacteria, and in conjunction with complement, even some Gram-negative bacteria (Alexander and Ingram, 1992). Administration of *Bacillus* strains could significantly enhance serum lysozyme activity of rainbow trout, *O. mykiss* (Merrifield et al., 2009) and common carp, *C. carpio* fry (Gupta et al., 2014). In contrast, the serum lysozyme content of tilapia (*O. niloticus*) was not affected by treatment with *B. subtilis* B10 and *B. coagulans* B16 as water additive for 40 days (Zhou et al., 2009). The differences in the effects of lysozyme activity can be due to the inclusion levels as well as the fish species under study. Respiratory bursts are produced by phagocytes to attack invasive pathogens during phagocytosis and have been widely used to evaluate host defense capabilities against pathogens; however, excessive accumulation of reactive oxygen intermediates (ROIs) is extremely toxic to host cells (Dalmo et al., 1997). The stimulation of respiratory burst activity after dietary probiotic supplementation involving feeding regimes and feeding durations have been previously reported in various fish (Aly et al., 2008; Kumar et al., 2008; Giri et al., 2012; Geng et al., 2011; Sun et al., 2010; Gupta et al., 2014). Studies have also demonstrated that dietary administration of high levels of probiotics for longer periods affects respiratory burst activity in *L. rohita* (Kumar et al., 2008; Giri et al., 2012); *O. niloticus* (Aly et al., 2008) and *R. canadum* (Geng et al., 2012). The myeloperoxidase is an important enzyme that utilizes oxidative radicals to produce hypochlorous acid to kill pathogens. During oxidative respiratory burst, it is mostly released by the azurophilic granules of neutrophils (Dalmo et al., 1997). Result of elevated myeloperoxidase level in serum was observed for *B. amyloliquifaciens* in carp, *C. catla* (Das et al., 2013); *B. subtilis* in rainbow trout, *O. mykiss* (Newaj-Fyzul et al., 2007); *L. plantarum* in grouper, *E. coioides* (Son et al., 2009) and *P. polymyxa* in common carp, *C. carpio* (Gupta et al., 2014).

In vertebrates, phagocytic process is followed by the production of reactive oxygen molecules, such as superoxide anion (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH), all of which are highly microbicidal (Sun et al., 2010). The main enzymes which detoxify reactive oxygen molecules are superoxide dismutase (SOD), catalase and glutathione peroxidase, all of them abundant in fish tissues (Di Giulio et al., 1993). SOD catalyses the dismutation of the highly reactive O_2 to the less reactive H_2O_2 and belongs to the main antioxidant defence pathways in response to oxidative stress (Fridovich, 1995). Catalase is the primary cellular enzymatic defense against H_2O_2, converting it into H_2O and O_2, and is critical for the process of scavenging free radicals (Dorval et al., 2003). Zhou et al. (2009) and Sun et al. (2010) demonstrated that the SOD activities of tilapia (*O. niloticus*) and grouper (*E. coioides*), respectively increased significantly after treated with *Bacillus* spp. However, Son et al. (2009) found that dietary administration of different levels of *L. plantarum* significantly decreased the SOD activity. The authors hypothesized that the decreased SOD in those fish fed *L. plantarum*-supplemented diets may occur in order to retain the superoxide anion level or to convert it into the singlet oxygen ($^1O_2$) and / or hydroxyl radicals (OH) via a metal-catalyzed interaction to enhance the microbial-killing capacity of phagocytes. Therefore, further study is needed to illustrate the effects of probiotics on the antioxidant enzymes and their related immune function in fish.

Protection against infectious diseases

The activation of non-specific immunity by immunostimulants is associated with increased protection against infectious disease (Sakai, 1999). Probiotics help in achieving natural resistance and controlling disease-related loss among farmed fish (Abraham et al., 2007). Gupta et al. (2015a) examined application of probiotic *P. polymyxa* as water additive and its effect on fish injected with *A. hydrophilla in vivo*. Bacterium *A. hydrophilla* is the causative agent of haemorrhagic septicaemia in a wide range of commercially important fish species including carps (Zhang et al., 2012). Occurrence of antibiotic resistant strains of *A. hydrophilla* in fish was reported (Giri et al., 2012). Therefore, prophylactic against this bacterium by the inclusion of immunostimulants in water becomes more practical to implement in a fish farm. In a previous study protection was achieved in eel (*A. anguilla*) and Indian carp (*C. catla*) against *A. hydrophila* infection after the fish were fed with diet supplemented with *B. amyloliquefaciens* (Cao et al., 2011; Das et al., 2013). Protection against edwardsiellosis (Nayak et al., 2007); enteric red mouth disease (Kim and Austin, 2006); furunculosis (Irianto and Austin, 2002); lactococossi and streptococcosis (Brunt and Austin, 2005) and aeromoniasis (Newaj-Fyzul et al., 2007) have successfully been accomplished through probiotics feeding. Increased non-specific immune responses such as lysozyme, RBA, MPO, SOD by the application of probiotics resulted in enhancement of fish disease resistance.

Conclusions

The present review highlighted the application of probiotics in aquaculture. Probiotics improve nutrition as well as the health of fish, prawn and molluscs. The supplementation of probiotics through feed is a better method to ensure the efficiency of the probiotic bacteria in the gastro-intestine of fish without interacting with the surrounding medium. The negative environment effects on fish could be mitigated through the use of probiotics as water additive. The use of a probiotic in culture water is a special focus of environmental research.

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