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REVIEW ARTICLE

Aquaculture and stress management: a review of probiotic intervention

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Summary

To meet the ever-increasing demand for animal protein, aquaculture continuously requires new techniques to increase the production yield. However, with every step towards intensification of aquaculture practices, there is an increase in stress level on the animal as well as on the environment. Feeding practices in aqua farming usually plays an important role, and the addition of various additives to a balanced feed formula to achieve better growth is a common practice among the fish and shrimp culturists. Probiotics, also known as 'bio-friendly agents', such as LAB (Lactobacillus), yeasts and *Bacillus* sp., can be introduced into the culture environment to control and compete with pathogenic bacteria as well as to promote the growth of the cultured organisms. In addition, probiotics are non-pathogenic and non-toxic micro-organisms, having no undesirable side effects when administered to aquatic organisms. Probiotics are also known to play an important role in developing innate immunity among the fishes, and hence help them to fight against any pathogenic bacterias as well as against environmental stressors. The present review is a brief but informative compilation of the different essential and desirable traits of probiotics, their mode of action and their useful effects on fishes. The review also highlights the role of probiotics in helping the fishes to combat against the different physical, chemical and biological stress.

Introduction

Aquaculture is one of the fastest growing industries in the food sector. According to Food and Agricultural Organization (FAO) report, the average consumption of aquaculture products relative to total *per capita* fish for human consumption rose from 14% in 1986 to 47% in 2006 and it can be expected to reach 50% in the next few years (Desriac et al., 2010). However, this rapid increase in growth has been marred by the outbreak of numerous fish diseases, mainly the bacterial disease, leading to very high stock mortality (Kurath, 2008). Prevention and control of diseases have led to a substantial increase in the use of veterinary medicines in the recent years. However, the utility of antimicrobial agents and antibiotics as a remedial measure has been questioned. These huge amounts of antibiotics have exerted a very strong selection pressure on the resistance among bacteria which have adapted to this situation, mainly by a horizontal and philandering flow of resistance genes (SCAN 2003; Cabello, 2006; Yousefian and Amiri, 2009).

Therefore, to combat diseases and prevent the dependency mainly on antibiotics, microbial

intervention in terms of bioremediation, vaccine, immunostimulants and probiotics are the need of the day (Panigrahi and Azad, 2007). The beneficial microbes that manipulate the gut microbiota through dietary supplementation are a novel approach from both the nutritional as well as immunological aspect. The term ''probiotics'' was originated from the Greek words ''pro'' and ''bios'' which mean life (Parker, 1974). According to FAO, probiotics can be defined as live micro-organisms, which administered in adequate amounts confer a health benefit on the host (Food and Agriculture Organization of the United Nations (FAO), 2001). After the administration of these useful microbes into the host, they are able to colonize and multiply in the gut of the host and show numerous beneficial effects by modulating various biological systems in the host (Cross, 2002). The application of probiotics has gained special interest because of their help in promoting the indigenous microbe(s) in the intestines, and thus helps to restore the microbial balance (Cross, 2002; Morelli et al., 2003). Probiotics can be implemented at larval and early fry stages, where vaccines cannot be administered.

The purpose of this review is to summarize the probiotics (microbial strains) that have convalesced from different aquaculture species, mode of action of probiotics and their role on different fish and shellfish with respect to immune responses, metabolism, stress and ameliorating effect on oxidative stress.

Mode of action of probiotics

One of the most important properties of probiotics is to adhere and proliferate at the specific location for the maximum usefulness to the host species. Therefore, to have maximum benefit, the probiotics should reach the specified location where it is most required. Modes of actions of probiotics are listed below.

Production of inhibitory compounds

Probiotics play a major role in preventing the occurrence of diseases by producing certain inhibitory compounds that act antagonistically against the pathogenic microbes and hence, preventing their proliferation in the host bodies (Tinh et al., 2007). The anti-pathogenic activity may be due to singular or combination of production of antibiotics (Williams and Vickers, 1986), bacteriocins (Vandenbergh, 1993; Pybus et al., 1994; Panigrahi and Azad, 2007; Tinh et al., 2007), siderophores, lysozymes, proteases, hydrogen peroxide and the alteration of pH values (Sugita et al., 2009). LAB inhibit the multiplication of microbes by the production of inhibitory compounds, called bacteriocins (Vandenbergh, 1993). Some bacterias also produce compounds other than bacteriocins, which are also useful in restricting the activity of the pathogens. An Aeromonas media strain A199 exhibited antagonistic activity against a wide range of fish/shellfish pathogens *in vitro* (Gibson et al., 1998) which was later identified as indole (s,3-benzopyrrole) (Lategan et al., 2006), having anti-bacterial and anti-fungal activity (Desriac et al., 2010).

Competitions for adhesion sites

Competition for space for adhesion and colonization on the gut and other tissue surface is another mode of action of the probiotics to fight against harmful pathogens (Ringø et al., 2007), as proper adhesion to the enteric mucus and intestinal wall surface is most important for any pathogen to cause damage to the host animal (Olsson et al., 1992; Vine et al., 2004). Adhesion of the probiotics is either non-specific, based on the physico-chemical factors or specific, based on the adhesion of the probiotics on the surface of the adherent bacteria and receptor molecules on the epithelial cells (Salminen et al., 1996). Different strategies have been put forth regarding the attachment of micro-organisms to the intestinal tract, such as, passive forces, electrostatic interactions, hydrophobic, steric forces, lipoteichoic acids, adhesins and specific structures of adhesion (Lara-Flores and Aguirre-Guzman, 2009). It has been reported that the intestinal isolates compete more effectively with *Vibrio anguillarum* for adhesion sites on the mucosal intestinal surface (Joborn et al., 1997). In this regard, Kankainen et al. (2009) suggests that intact pilus fibers with mucus-binding capacity on the cell surface of a probiotic strain of lactic acid bacteria are helpful for competing with *Escherichia coli* in the human intestine.

Modulation of host immune responses

Probiotics render protection against pathogens by overcoming the adverse consequences of antibiotics and chemotherapeutic agents. Probiotics help in achieving natural resistance and high survivability of larvae and post larvae of fishes, due to the elevated immunity of the fish. Different modes of probiotics have a different stimulatory effect on the immune system of fish, namely, effect on the immune cell,

anti-bodies, acid phosphatase, lysozyme and anti-microbial peptides (Nayak, 2010). An increase in the acid phosphatase activity was observed in *Miichtys miiuy*, when fed with *Clostridium butyricum*, hence indicating an enhancement in the immune response of the animal (Lara-Flores and Aguirre-Guzman, 2009). Rengpipat et al. (2000) showed that *Bacillus* sp. (strain S11) can be provided for disease protection by activating the *Penaeus monodon* immune defenses. Stimulation of immune response is increased by anti-body activity and macrophage activity as reported by Marteua and Ramboud (1993). The addition of probiotic bacteria in the diet of cod and herring larvae has led to an increased stimulation of the immune system and hence better immunity against pathogens (Olafsen, 1998).

Competition for chemicals or available energy

The basis for the existence of any microbial population depends on its ability to compete for chemicals and available energy with the other microbes residing the same ecosysytem (Verschuere et al., 2000a). Heterotrophs, which dominate the aquatic environment, compete for organic substrates, such as carbon and other energy sources. Rico-Mora et al. (1998) inoculated a bacterial strain, which had a capacity to grow actively in an organic-poor substrate, into a diatom culture and reported that it prevented the establishment of a pathogenic strain of *V. alginolyticus*. As the inoculated strain lacked any *in vitro* inhibitory effect on *V. alginolyticus*, so this prevention might be due to the bacterial strains ability to utilize the exudates of the diatoms and fight against the pathogen. In another study, omission of iron (important for many *Vibrio* pathogens, Rorvik et al., 1991) from the diet of early weaned sea bass larvae helped in reducing the microbial load of the larvae without affecting its growth rate and survivability (Gatesoupe, 1997).

Competition for nutrients

Probiotics make up a part of the resident micro-flora by adhering to the mucus, gastrointestinal tract, epithelial cells and other tissues, further contributing to the health or well-being of the host (Gatesoupe, 1999; Farzanfar, 2006). The attachment ability of some bacteria has been tested *in vitro* and *in vivo* and their results suggest that the pathogen gets displaced by the potential probiotic based on the competition for essential nutrients, space, etc. (Verschuere et al., 2000b). Probiotics utilize the nutrients otherwise consumed by pathogenic microbes. The useful microbiota sometimes serves as a supplementary source of food and microbial activity in the digestive tract and also is a source of vitamins or essential amino acids. It has been seen that *Bacteroides* and *Clostridium* species contribute to the host's nutrition, especially by supplying fatty acids and vitamins (Sakata, 1990).

Improvement of water quality

Gram-positive bacteria, such as *Bacillus* spp. enhance the immune system of the animal and also act beneficially in improving the quality of the water system. *Bacillus* spp. acts more efficiently in converting organic matter into carbon dioxide in comparison to the Gram-negative bacteria, which converts a greater proportion of organic matter into bacterial biomass or slime. The introduction of *Bacillus* spp in proximity to pond aerators reduced chemical oxygen demand (Porubcan, 1991). Certain strains of probiotics have significant algicidal effect on many species of microalgae, particularly the red tide plankton (Fukami et al., 1997). The major water quality problems are due to ammonia and nitrite toxicity, which can be rectified by the application of nitrifying cultures into the fish ponds (Lewis and Morris, 1986). Probiotics are supplied as beneficial bacterial strains to rearing water that helps to increase microbial species composition in the environment and hence improve the quality of water. The temperature of water, pH, dissolved oxygen, NH_3 and H_2S were found better in trials with probiotics and a daily administration of probiotics showed a possibility of maintaining healthy environment for shrimp and prawn larval in the improved green water system (Banerjee et al., 2010).

Interference with quorum sensing

The term ''quorum sensing'' can be defined as a process of bacterial cell-to-cell communication. The disruption of quorum sensing (QS) is known to be a new anti-infective strategy in aquaculture (Defoirdt et al., 2004). Defoirdt et al. (2005) reported that AI-2 (autoinducer-2) mediated system is responsible for the virulence of *V. harveyi* toward gnotobiotic *Artemia franciscana* (Defoirdt et al., 2005), while both HAI-1 (Harveyi autoinducer-1) and AI-2–mediated systems are involved in the growth-retarding effect of this bacterium toward gnotobiotic *Brachionus plicatilis* (Tinh et al., 2007). This might suggest the existence of host dependent QS in *V. harveyi*. It has

been found that the marine red algae, *Delisea pulchra,* act as a very good QS antagonist (Manefield et al., 1999; Tinh et al., 2007). These compounds, protected *Brachionus, Artemia*, and rainbow trout (*Oncorhynchus mykiss)* from the negative effects of pathogenic *Vibrio* sp., when added at adequate concentrations (Rasch et al., 2004; Defoirdt et al., 2006; Tinh et al., 2007). However, usage of the probiotic bacteria, which can act as QS-disrupting agents in aquaculture systems, needs to be ascertained. Therefore, the determination of the concentration of QS molecules *in vivo* would provide a better knowledge about the importance of QS *in vivo*, and also help to clarify the mechanism of action of the QS-disrupting bacteria.

Antibacterial activity

Bacterial antagonism is a common phenomenon in nature; therefore, microbial interactions play a major role in the equilibrium between competing beneficial and potentially pathogenic micro-organisms (Balcázar et al., 2004). It was demonstrated that feeding of rotifers with food additives containing live lactic acid bacteria or *Bacillus* spores decreased the amount of pathogenic *Vibrio* species in the rotifers. The administration of *C. butyricum* bacteria to rainbow trout enhanced the resistance of the fish to Vibriosis (Sakai et al., 1995). It has been reported that *Lactobacillus* displays a higher antagonistic effect against *E, coli* and *P. aeruginosa* (Oyetayo, 2004). *B. subtilis* was also seen to have significantly lowered the count of motile Aeromonads, presumptive Pseudomonads and total Coliforms in live bearing ornamental fishes (Ghosh et al., 2008). *B. pumilus, Bacillus firmus* and *C. freundii* showed inhibitory effect against *A. hydrophila* in *O. niloticus* (Aly et al., 2008a).

Antiviral activity

Though vaccination being an age old practice to control viral diseases, its success rate is highly variable and the duration of immunity against the virus is questionable (McLoughlin and Graham, 2007). Some probiotics have antiviral effects, although the exact mechanism is not yet known. Strains of *Pseudomonas* sp., *Vibrio* sp., *Aeromonas* sp. and groups of *Coryneforms* showed antiviral activity against infectious haematopoietic necrosis virus (IHNV) (Kamei et al., 1988). Moreover, feed supplementation with a *B. megeterium* strain has resulted in increased resistance to white spot syndrome virus (WSSV) in shrimp *Litopenaeus vannamei* (Li et al., 2009). Probiotics, like *Bacillus* and *Vibrio* sp., positively influenced the protective effect against WSSV (Balcázar, 2003).

Antifungal activity

Aeromonas media (strain A199) isolated from fresh water, in culture of eels (*Anguilla australis* Richardson), presented antagonistic activity against *Saprolegnia* sp. It has also been reported that *Aeromonas* media strain A199 protects the fish against Saprolegniosis (Lategan et al., 2004b).

Role of probiotics on different aquaculture organisms

In many countries the aquaculture has become an important source of income. Therefore, on large-scale production facilities, where fish and shellfish are open to stressful conditions, problems related to diseases and worsening of environmental circumstances often occur and result in staid economic damages. Since decade, the prevention and control of disease in aquatic environment led to the use of different kind of drugs. Nevertheless, the efficacy of antimicrobial agents as a preventive measure has been examined, given the extensive documentation of the advancement of antimicrobial resistance among pathogenic bacteria. The use of probiotics or beneficial bacteria, which control pathogens through a variety of mechanisms, is increasingly viewed as an alternative to antibiotic treatment and recently the demand of probiotics has increased heavily in aqua industry for eco-friendly and sustainable aquaculture.

However, careful attention should be given while dealing with larval culture because of their lableness and underdeveloped immunity. Larval endogenous microbial population depends on both egg and environmental microbial composition (Ringo et al., 1997). The larval digestive tract is immature during hatching, the gall bladder has yet to develop and subsequently bile is not secreted until later on during development (Ringo et al., 1997). Hence, the probiotics are not required to move through an acidic environment en route to the gut and, unlike a probiont designed for adults, it does not need to be resistant to acid and bile. To maximize the competitive advantage of probiotics, early delivery especially before first feeding increases the chances of producing persistant population (Ringo et al., 1997).

Impact on fish

Fish is one of the richest sources of animal protein and is the fastest food-producing sector in the world. The fish production is maximized through intensification with addition of commercial diets, growth promoters, antibiotics, and several other additives. Therefore, to overcome these problems in a healthy way, probiotics are used. The beneficial effects of probiotics in larval rearing have been demonstrated in fishes by several investigators (Alami-Durante et al., 1991). A relatively dense non-pathogenic and diverse adherent microbiota is reported to be present on the fish egg, which probably acts as an effective barrier against colony formation by pathogen on the egg (Olafsen, 1998). Survivability and resistance to pathogens were also increased significantly after probiotic supplementation during larval life (Gatesoupe, 1997). Swain et al. (1994) reported significantly higher growth and nutrient utilization in catla juveniles through probiotic supplemented diet. However, no improvement in growth was noticed in flounder and Tilapia, when they were fed with *Lactobacillus,* isolated from salmon intestine, as a feed additive (Gildberg et al., 1995). Many probiotics (micro-organisms) are recovered from different aquaculture species are summarized in Table 1.

Impact on crustaceans

Worldwide, people obtain approximately 25% of their animal protein from fish and shellfish (Naylor et al., 2001). Hence, crustaceans have a huge role to play in the international food market. Probiotics have also found a way into the crustaceans' aquaculture system. Nogami and Maeda (1992) isolated a bacterial strain that was found to improve the growth of crab (*Portunus trituberculatus* M) and suppress the growth of pathogenic *Vibrio* sp. They also suggested that the bacterium might improve the physiological rate of crab larvae by serving as nutrient source during its growth. Moriarty (1998) also reported that several *Bacillus* strains used as probiotics in the culture ponds of penaeid shrimps completed the growth period in 160 days without any problem, compared to almost failure of culture ponds where no *Bacillus* was used. Gatesoupe (1989) reported that LAB showed an improvement in the dietary value of rotifers. Uma (1995) also reported enhanced growth rate and survival of *Penaeus indicus* through the addition of *L. plantarum* in the water. *Bacillus* S11 when incorporated into the diet of *Penaeus monodon*, showed mean weight increase and survival of larvae and post larvae, decreased mortality after being challenged with the pathogen *V. harveyi*

Table 1 Probiotics recovered from different aquaculture species

Micro-organism	Isolated from	Reference
Vibrio alginolyticus	*O. mykiss*	Irianto and Austin, 2002b
Vibrio fluvialis	*O. mykiss*	Irianto and Austin, 2002b
Aeromonas hydrophila	*O. mykiss*	Irianto and Austin, 2002b
Bacillus subtilis	*O. mykiss, Litopenaeus vannamei, Catla catla, Labeo rohita, Cirrhinus mrigala*	Newaj-Fyzul et al., 2007; Liu et al., 2010; Nayak and Mukherjee, 2011;.
Bacillus licheniformis	*O. mykiss*	Merrifield et al., 2009
C. inhibens	*O. mykiss, S. salar*	Irianto and Austin, 2002b; Gildberg et al., 1995
Carnobacterium spp.	*O. mykiss*	Irianto and Austin, 2002b
Carnobacterium divergens	*O. mykiss, S. salar*	Kim and Austin, 2006b
Carnobacterium maltaromaticum	*O. mykiss*	Kim and Austin, 2006b
P. acidilactici	*O. mykiss*	Aubin et al., 2005
S. cerevisiae	*O. mykiss*	Wache' et al., 2006
Leu. Mesenteroides	*O. mykiss, S. trutta*	Balcázar et al., 2007b; Vendrell et al., 2008
La. Lactis	*O. mykiss, S. trutta*	Balcázar et al., 2007b
L. sakei	*O. mykiss, S. trutta*	Balcázar et al., 2007b
L. planatarum	*O. mykiss*	Vendrell et al., 2008
L. rhamnosus	*O. mykiss*	Nikoskelainen et al., 2003
E. facium	*O. mykiss*	Merrifield et al., 2009
Bacillus circulans PB7	*Catla catla*	Bandyopadhyay and Das Mohapatra, 2009
Pseudomonas spp	*Catla catla, L. rohita, C. mrigala*	Nayak and Mukherjee, 2011
Cornyebacterium spp.	*Catla catla, L. rohita, C. mrigala*	Nayak and Mukherjee, 2011
Micrococcus spp.	*Catla catla, L. rohita, C. mrigala*	Nayak and Mukherjee, 2011
Plesiomonas spp.	*Catla catla, L. rohita, C. mrigala*	Nayak and Mukherjee, 2011

D331 (Rengpipat et al., 1998). Harzevili et al. (1998) used *Lactococcus lactis* AR21, which stimulated the growth of rotifers and inhibited the growth of *V. anguillarum*. Hirata et al. (1998) used mixed cultures consisting mainly of *Bacillus* species to improve performance of rotifer *Brachionus plicatilis* in water.

Giant fresh water prawn, *Macrobrachium rosenbergii*, zoea larvae had a higher survival and a faster rate of metamorphosis when fed with *B. subtilis* enriched *Artemia* naupli compared to zoea larvae fed unenriched *Artemia* (Keysami et al., 2007). Similarly, Liu et al. (2010) reported that shrimp (*L. vannamei*) larval development, metamorphosis, immuno-stimulation and stress response was significantly accelerated after the addition of the probiotic (*B. subtilis* E20) to the larval rearing water at a level of 10^9 cfu/L. In addition, the use of *B. subtilis* E20 in shrimp larval culture may have delayed the primary proliferation of *Vibrio* spp. In crustaceans, the PPO system plays an important role in the defense reaction (Soderhall and Cerenius, 1998) through the conversion of prophenol oxidase (PPO) to PO by an serine Protease (SP) named ppAE. Increases of phenoloxidase (PO) activity and phagocytic activity of shrimp after being treated with *B. subtilis* E 20 were documented by Liu et al. (2010) which signifies the increase of phagocytic activity and further clearance activity (Liu et al., 2009; Tseng et al., 2009).

Impact on bivalves

Bivalve larvae, which are filter feeders, need a constant flow of seawater through their organisms, which makes it difficult for the establishment of a bacterial population in the digestive tract. Hence, it is advisable to control the microbiota present in the environment, to maintain a beneficial balance for the larval development and survival, and preventing the proliferation of opportunistic pathogens. The presence of a strain, sharing the same ecological niche with pathogens and with the ability to inhibit them, allows a control of the microbiological quality of seawater without any allochthonous species. It has been assayed in the effect of strain *Phaeobacter* PP-154, diffusible-pigment producer, in the settlement of *O. edulis* larvae and also observed similar effect. Therefore, the induction of these processes and the enhancement of survival and settlement mediated by bacteria may be considered as a mode of probiosis, with special relevance in bivalve larval cultures.

The nutritional aspect of molluscan larvae has gained a lot of importance in the recent years. Douillet and Langdon (1994) observed enhanced growth of *Crassostrea gigas* larvae when supplemented with bacterial strain CA2 as a feed supplement. A bacterial strain identified as *Aeromonas haloplanctis*, obtained from the gonads of Chilean scallop (*Argopecten purputatus*) showed *in vitro* inhibiting activity against many pathogenic bacteria like *Vibrio ordalii*, *V. parahaemolyticus, V. anguillarum* and *Aeromonas hydrophila* (Riquelme et al., 1996).

Effect of probiotics on fish metabolism

Effect of probiotics mainly depends on several factors i.e. the probiont, supplementation form, vector of administration, dosage level and duration of application (Mohapatra et al., 2012). Most commonly, in aquaculture related studies, live-cultures are sprayed or top-dressed onto basal diets (Balcázar et al., 2007a; b; Vendrell et al., 2008; Merrifield et al., 2010a,b,c, 2011) but freeze-dried/lyophilised cells (Panigrahi et al., 2007; Merrifield et al., 2010c), dead cells (Irianto and Austin, 2003; Newaj-Fyzul et al., 2007), disrupted cells (Brunt and Austin, 2005; Newaj-Fyzul et al., 2007), cell-free supernatants (e.g. Brunt and Austin, 2005; Newaj-Fyzul et al., 2007) and spores (Raida et al., 2003; Bagheri et al., 2008) have all showed some degree of success (Table 2).

Effect on growth

The use of probiotics for enhancing bio-growth parameters and in improving disease resistance ability has been well documented in aquaculture for human consumption (Robertson et al., 2000). Bjornsdottir et al. (2010) demonstrated an improved survival and growth of halibut larvae as a result of live prey treatment using selected autochthonous bacteria. Indeed numerous studies have shown that the application of probiotics can improve feed conversion, growth rates and weight gain of fish including salmonids (Taoka et al., 2006; Bagheri et al., 2008; Wang et al., 2008b). The addition of probiotics in the diet of common carp lead to enhanced feed utilization and hence better feed conversion ratio (FCR). Bagheri et al. (2008) demonstrated that the application of *B. subtilis* and *B. licheniformis* could significantly improve the FCR, specific growth rate (SGR), weight gain and protein efficiency ratio (PER) after 2 months feeding on diets containing 3.8Å∼109 CFU/g in the rainbow trout fry.

Improvement in growth of *Penaeus vannamei* larvae and reduction in the incidence and severity of diseases was observed on the widespread use of the probiotic, *Vibrio alginolyticus* when applied to the lar-

Table 2 Different parameters investigated using probiotics in aquacultural species

Probiont	Parameters studied	Species tested	Reference
L. lactis	DR, IR, GM	*Oncorhynchus mykiss, Salmo trutta*	Balcázar et al., 2007b
Leu. mesenteroids	DR, IR, GM, GP	*O. mykiss, S. trutta*	Balcázar et al., 2007b; Vendrell et al., 2008
L. sakei	DR, IR, GM	*O. mykiss, S. trutta*	Balcázar et al., 2007b
L. plantaram	DR, GM, GP	*O. mykiss*	Vendrell et al., 2008
L. rhamnosus	DR, GP, GM, IR	*O. mykiss*	Nikoskelainen et al., 2001, 2003; Panigrahi et al., 2007
E. faecalis	BC, GP, IR, DR	*O. mykiss, Lithobates catesbeianus*	Newaj-Fyzul et al., 2007
B. subtilis + B. licheniformis	BC, DR, GM, GH, GP, FU, IR	*O. mykiss*	Bagheri et al., 2008
B. subtilis	DR, IR	*O. mykiss, L. catesbeianus*	Merrifield et al., 2009
E. faecium	BC, FU, GM, GP, GH, IR	*O. mykiss*	Merrifield et al., 2009
P. acidilactici	BC, FU, GM, GP, SM, IR	*O. mykiss*	Merrifield et al., 2009; Quentel et al., 2004
A. sobria	DR, IR	*O. mykiss*	Brunt and Austin, 2005; Pieters et al., 2008
S. cerevisiae	BC, BE, FU, GM, GP, SM, IR	*O. mykiss*	Wache' et al., 2006
C. divergens	DR, IR, GH, GM, GP	*O. mykiss, Salmo salar*	Kim and Austin, 2006a; b; Gildberg et al., 1995
C. maltaromaticum	IR	*O. mykiss*	Kim and Austin, 2006a; b
C. inhibens	DR, IR, GM	*O. mykiss, S. salar*	Robertson et al., 2000
Brachothix thermosphacta	DR, IR	*O. mykiss*	Pieters et al., 2008
A. hydrophila	DR, IR	*O. mykiss*	Irianto and Austin, 2002b, 2003
V. fulvialis	DR, IR	*O. mykiss*	Irianto and Austin, 2002b, 2003
Carnobacterium spp	DR, IR, GM	*O. mykiss*	Irianto and Austin, 2002b, 2003
V. alginolyticus	DR, IR, GM	*O. mykiss, S. salar*	Austin et al., 1995; Irianto and Austin, 2002b
Pseudomonus fluorescens	DR	*O. mykiss, S. salar*	Spanggaard et al., 2001; Gram et al., 2001
D. hansenii	GM	*O. mykiss*	Andlid et al., 1995
R. glutinis	GM	*O. mykiss*	Andlid et al., 1995
Kocuria SM1	DR, IR	*O. mykiss*	Sharifuzzaman and Austin, 2010b
Enterobactor	DR, IR	*O. mykiss*	Capkin and Altinok, 2009
B. mojavensis	DR, IR	*O. mykiss*	Capkin and Altinok, 2009
L. delbrueckii	GH, DR, GM	*S. salar*	Salinas et al., 2008
Bifidobacterium bifidium	IR	*L. catesbeianus*	De Carla Dias et al., 2010
Zooshikella sp.	IR	*Paralichthys olivaceus*	Kim et al., 2010
V. anguillarum	DR, IR	*O. mykiss*	Sharifuzzaman and Austin, 2010a
Pdp11	GP, IR	*Sparus auratus*	Varela et al., 2010

Genera abbreviations: A., Aeromonas; B., Bacillus; C., Carnobacterium; D., Debaryomyces; E., Enterococcus; L., Lactobacillus; La., Lactococcus; Leu., Leuconostoc; P., Pediococcus; Ps., Pseudomonas; R., Rhodotorula; S., Saccharomyces; V.,Vibrio.
Parameters investigated: BC, body composition; BE, brush border enzymes; DR, disease resistance; FU, feed olonizatio; GH, gut histology; GM, gut microbiota (inclusive of probiont olonization) ; GP, growth performance; IR, immunological/haematological response; SM, skeletal malformation.

val rearing tanks (Garriques and Arevalo, 1995). Uma et al. (1999) observed a significant improvement in FCR, FER (Food Efficiency Ratio) and PER of shrimp larvae when fed with *L. plantarum* bioencapsulated *Artemia*. Similar observations were made by Suralikar and Sahu (2001) when probiotic *L. cremoris* at 8.5×10^{11} cfu/g diet was fed to post larvae of *M. rosenbergii*.

Effect on gut micro-flora

The gut is the major organ, where probiotics establish and execute their functions. Therefore, the discussion between probiotics and gut environment warrants high consideration. The continual application of bacterial cells (LAB, *Bacillus* spp. and certain Gram-negative spp.) to salmonids may lead to high levels of colonization and modulated gastrointestinal (GI) microbial populations (Irianto and Austin, 2002b; Kim and Austin, 2006a; Balcázar et al., 2007a,; b; Bagheri et al., 2008; Merrifield et al., 2010a,b,c, 2011). Several reports suggest that most of probiotics exert their effect through colonization in host and excretion of several growth-enhancing nutrients (Bagheri et al., 2008). Mohapatra et al. (2012) observed significant reduction in the total hetrotrophic bacteria and higher colonization of the useful microbes in the digestive tract in the multispecies probiotic supplemented fed *Labeo rohita*, resulting in better growth and immunity.

Effect on digestive enzymes

It is reported that the digestive organs are very sensitive to food composition and cause immediate changes in activities of the digestive enzymes (Bolasina et al., 2006; Shan et al., 2008), which is finally reflected in fish health and growth. The enzymes liberated by probionts helps in increasing the digestive utilization of feed or detoxifying injurious metabolites liberated by the harmful micro-flora. The alteration of microbial metabolism is however affected either by increased or decreased enzymatic activity. Amylase and lipase are the major enzymes related to carbohydrate and fat digestion, respectively. Tovar et al. (2002) reported an increase in amylase and trypsin secretion in sea bass *(Dicentrarchus labrax)* larvae after being fed with live yeast *Debaryomyces hansenii.* Moreover, Mohapatra et al. (2012) noted elevated level of digestive enzyme (protease, amylase and lipase) activities in *Labeo rohita* when fed with a mixture of *Bacillus subtilis*, *Lactococcus lactis* and *Saccharomyces cerevisiae*. Bacteria also secrete proteases to digest the peptide bonds in proteins and therefore break down the proteins into their constituent monomers and free amino acids, which can benefit the nutritional status of the animal. Higher alkaline phosphatase activity was observed in probiotic fed Nile Tilapia (*Oreochromis niloticus*), thereby reflecting a possible development of brush border membrane of enterocytes, and hence, indicating that the carbohydrate and lipid absorption has been enhanced due to probiotic supplementation (Lara-Flores and Aguirre-Guzman, 2009). The *Bacillus* sp. isolated from *Cyprinus carpio* has considerable extracellular amylolytic, cellulolytic, proteolytic and lipolytic activities (Bairagi et al., 2002). Probiotics also play a very positive effect on the digestive processes as well as the assimilation of food components (Irianto and Austin, 2002b). This increase in the nutrient digestibility maybe because of better availability of exoenzymes produced by probiotics (Vine et al., 2006) or better health condition (Mohapatra et al., 2012).

Effect on metabolic enzymes

Bacteria, particularly members of the genus *Bacillus,* secrete a wide range of exo-enzymes (Moriarty, 1998). The exogenous enzymes produced by the probiotics represent only a small contribution to the total enzyme activity of the gut (Ziaei-Nejad et al., 2006), and the presence of the probiotics might stimulate the production of endogenous enzymes by the shrimp. The dietary yeast (*Saccharomyces cerevisiae* var. *boulardii*) in reared trout showed higher activity of three enzymes in the brush border membrane of the enterocytes: alkaline phosphatase (ALP), g-glut-amyl-transpeptidase (GGT) and leucine-amino-peptidase N (LAP) (Wache' et al., 2006). Decreased activity of the enzymes, like aspartate amino aransferase (AST), alanine amino transferase (ALT) and lactate dehydrogenase (LDH) was observed in *Oreochromis niloticus* after being fed with a diet containing *Pseudomonas* spp. and a mixture of *Micrococcus luteus* and *Pseudomonas* spp. Similar results were also observed in *Cyprinus carpio* which was fed with the extract of *Cyanobacteria* (Palikova et al., 2004). Yeasts are well known in animal nutrition because they can act as a producer of polyamines, which enhance intestinal maturation (Peulen et al., 2000).

Effect on haematological parameters

Haematological parameters of fish are used as indicators of their physiological state and their study has become widespread in the control of pathologies and manipulation of stress in fish farming. It is reported that the percentage volume of erythrocytes and the total and differential leucocyte count in the blood provides a clue about the health status of the fish (Sampath et al., 1998). Irianto and Austin (2002a) revealed that the feeding of Gram-positive and Gram-negative probiotic bacteria at 10^7 cells/g of feed led to a notably increase in the number of erythrocyte within two weeks of feeding trial. Apart from this, the increased white blood cell (WBC) count helps in the non-specific immunity via neutrophils and macrophages. According to Oboh and Akindahunsi (2005), the WBC content of the *S. cerevisae* fermented cassava flour diet was significantly lower than that of the control. This low WBC count might be attributed as an added advantage to the use of micro-fungi fermentation, since some of these fungi are capable of secreting antimicrobial substances that would restrict the growth of any contaminated organism. Higher counts (%) of phagocytic cells (neutrophils and monocytes) and lymphocytes are also indicative of infection in fish. Probiotics interact with the immune cells such as mononuclear phagocytic cells (monocytes and macrophages) and polymorphonuclear leucocytes (neutrophils) and NK cells to enhance innate immune responses (Irianto and Austin, 2002a; Nikoskelainen et al., 2003; Kumar et al., 2008). Probiotics also actively stimulate the proliferation of lymphocytes (both B and T cells) and further immunoglobulin production in fish (Al-Dohail et al., 2009; Picchietti et al., 2009).

Effect on immunological parameters

Among the numerous beneficial effects of probiotics, modulation of immune system is one of the most common benefits of the probiotics (Table 3). The immune system of teleost fish appears to be an efficient means by which the host protects itself upon pathogenic challenge. The inter-relationship between gut mucosal epithelial cells, mucus, antimicrobial products, commensal organisms resident in the gut and immune cells in the mucosa/submucosa are vital for the health and well-being of the fish. Endogenous commensal microbiota plays an important role in tolerance induction vs. immune activation decisions. It has been reported that bacterial compounds act as immunostimulants in fish and shrimp (Sakai, 1999). He also reported that the presence of probiotics, *Bacillus coagulans* B16 and *R. palustris* G06, were able to increase immune responses such as myeloperoxidase (MPO) activity, respiratory burst activities, superoxide dismutase (SOD) activity and Catalase activity of tilapia. It has been shown that injection of β -glucan induced significantly elevated lysozyme activity (Misra et al., 2006). Salinas et al. (2006) reported that respiratory burst activity of teleost fish (*Sparus aurat*a L.) increased *in vitro* by the addition of heat-inactivated *Lactobacillus delbrüeckii* ssp. *lactis*. Similar result was also observed by Nikoskelainen et al. (2003), who reported that rainbow trout fed *L. rhamnosus* (8×10^4 cfu/g) for 2 weeks showed a significant increase in respiratory burst activity compared with the control group. In another study, *A. salmonicida* and *Y. ruckeri* was seen to increase the phagocytic activity, respiratory burst as well as serum and gut mucosal lysozyme activity (Kim and Austin, 2006a). In a separate study by Panigrahi et al. (2007), Rainbow trout fed with three freeze-dried probionts (*Lactobacillus rhamnosus, Enterococcus faecium* and *Bacillus subtilis*) displayed enhanced superoxide anion production, serum alternative complement activity and elevated IL-1 β, TNF and TGFβ expression in spleen and head kidney. Such results are again suggestive of augmentation of innate immunity and possibly regulatory mechanisms behind mucosal tolerance (Kim and Austin, 2006b; Newaj-Fyzul et al., 2007; Merrifield et al., 2010a,b). Probiotics are responsible for the enhancement of the natural complement activity of the fish (Panigrahi et al., 2007; Salinas et al., 2008). An increased complement activity was recorded on *O. mykiss* from fourth week of feeding heat-inactivated probiotics (Pdp 11 or 51M6) (Choi and Yoon, 2008).

With respect to probiotic effects on the adaptive immune system, Arijo et al. (2008) demonstrated that the administration of live probiotic strains resulted in the expression of cross-reactive antibodies which were specific for outer membrane proteins and extracellular products of bacterial pathogens, conferring a protective effect upon challenge with *Vibrio harveyi*. Fermentation products such as the short-chain fatty acid, butyrate, both modulate barrier function and regulate inflammatory processes, by decreasing epithelial permeability through upregulation of tight junction proteins and suppression of pro-inflammatory cytokines by induction of expression of anti-inflammatory, regulatory cytokines, respectively (Van Nuenen et al., 2005). These short-chain fatty acids (SCFAs) are effectively acting as adopted regulators of both innate and adaptive immune mechanisms.

Effect on immunoglobulin production

Probiotics have very profound impact on the specific and innate immune system of fish (Nikoskelainen et al., 2003). Staykov (2004) found higher levels of bactericidal activity, lysozyme, antibody levels and alternative complement pathway activity in rainbow trout and common carp fed mannan oligosaccharides (MOS). Therefore, MOS could activate and facilitate antigen processing and serve to stimulate the initial stages of the immune response (Moran, 2004). The oral administration of heat- inactivated *L. delbrueckii* ssp. *lactis* and *B. subtilis*, individually or combined, increased the total serum IgM and numbers of gut IgM super (+) cells and acidophilic granulocytes on gilthead sea bream (Salinas et al., 2008).

Histological changes

Histological methods remain the primary tools for the evaluation of pathological changes in tissues in toxicological studies and are getting considerable attention while conducting sub-lethal exposure of different toxicant in aquatic organisms. The histological analysis of cells and tissues provides essential information on the pathological changes occurring in a variety of organelles, which can be related to both biochemical changes at cellular level and to tissue pathology.

Light and electron microscopy demonstrated that pathogen-induced damage to the Atlantic salmon foregut could not be prevented or reversed, but could be marginally reduced in some cases. Merrifield and colleagues demonstrated in a preliminary

Table 3 Effect of different probiotics supplement on various immune responses in fish

Probiotics	Forms of probiotics	Mode of supplementation	Immunological effect	References
Bacillus subtilis, *Lactobacillus acidophilus*	Viable	Individual and combination	Increased RB, SBA, NA, lysozyme	Aly et al., 2008b
Lactobacillus sakei	Viable	Individual	Increased RB, IG, CA, PA, and decreased lysozyme	Balcázar et al., 2006, 2007a; b
Lactococcus lectis	Viable	Individual	Increased RB, IG, PA, lysozyme, CA	Balcázar et al., 2006, 2007a; b
Lenuconostoc mesenteroides	Viable	Individual	Increased RB, IG, PA, lysozyme, CA	Balcázar et al., 2006, 2007a; b Balcázar et al., 2009
Aeromonas sorbia	Viable	individual	Increased RB, PA, leucocytes and decreased serum lysozyme and AP activity.	Brunt and Austin, 2005; Brunt et al., 2007
Pdp11,51M6	Heat killed	Individual and combination	Incrased PA, RB	Choi and Yoon, 2008
Shewanella putrefaciens, *S. baltica*	Inactivated	Individual and combination	Increased PA, CA, AP, RB	Diaz-Rosales et al., 2006a
Shewanella putrefaciens, *S. baltica*	Viable	Individual	Increased RB	Diaz-Rosales et al., 2006b, 2009
Vibrio fluvialis, Micrococcus luteus, Aeromonas hydrophilla	Viable	Indidividual and combination	Increased bloodlets and lysozyme activity	Diaz-Rosales et al., 2009
Gram-positive coccus	Viable	Individual	Increased bloodlets, lysozyme activity and PB	Irianto and Austin, 2002b
Vibrio fulvialis	Viable	Individual	Increased bloodlets, lysozyme activity and PB	Irianto and Austin, 2002b
A. hydrophilla	Viable	Individual	Increased bloodlets, lysozyme activity and PB	Irianto and Austin, 2003
Carnobacteium maltaromaticum	Viable	Individual	Increased RB, lysozyme, serum mucus, PB	Kim and Austin, 2006a; b
Carnobacterium divergens	Viable	Individual	Increased RB, lysozyme, serum mucus, PB	Kim and Austin, 2006a; b
Bacillus subtilis	Viable	Individual	Increased RB, SBA, IG, PB, AP, CA, lysozyme, gut mucus	Kumar et al., 2006; Newaj-Fyzul et al., 2007
Lactobacillus rhamnosus	Viable	Individual	IG, RB, CA, PA	Nikoskelainen et al., 2003
Saccharomyces cerevisiae	Viable	Individual	PA, RB, CA, Myeloperoxidase	Ortuño et al., 2002
Clostridium butyricum	Viable and inactivated	Individual	Increased Lysozyme, PA, CA, IG, RB	Pan et al., 2008
Lactobacillus Rhamnosus, Bacillus subtilis, Enterococcus facium	Viable (freeze dired)	Individual	Tissue and strain dependent modulation	Panigrahi et al., 2007
Lactobacillus delbrueckii	Viable	Individual	Increased IG and related genes	Picchietti et al., 2009
Aeromonas sorbia,	Viable	Individual	Increased RB, CA, PA, lysozyme, bloodlets, IG	Pieters et al., 2008
Brochothrix thermosphacta	Viable	Individual	Increased PA and decreased CA, RB, Lysozyme, IG	Pirarat et al., 2006
Lactobacillus delbrueckii ssp. *lactis, Bacillus subtilis*	Viable	Individual and combination	Increased PA, Cytotoxic activity	Salinas et al., 2005
Lactobacillus delbrueckii, Bacillus subtilis, Pdp11, 51M6	Inactivated	individual	Increased RB, cytotoxic activity	Salinas et al., 2006
Lactobacillus delbrueckii, Bacillus subtilis	Heat killed	Individual/ Combination	Increased RB, AP, PA, CA, IG depending on mixing of probiont	Salinas et al., 2008
Kocuria spp.	Viable	Individual	Increased PA, AP, RB, Lysozyme	Sharifuzzaman and Austin, 2010b
Lactobacillus plantarum	Viable	Individual	Lysozyme, PA, AP, CA	Son et al., 2009
Bacillus subtilis, Lactobacillus acidophilus, Clostridium butyrium, Saccharomyces cerevisiae	Viable	Combination	Increased neutrophil migration, Lysoyme, RB, bacteriocidal activity	Song et al., 2006

Table 3 Continued

Probiotics	Forms of probiotics	Mode of supplementation	Immunological effect	References
Lactococcus lactis, Leuconostoc mesenteroides	Viable, inactibated	Individual	Increased PA, nitric oxide	Taoka et al., 2006
Enterococcus faecium	Viable	Individual	Increase CA, RB, MPO and lysozyme	Wang et al., 2008a
Bacillus coagulans, B. subtilis, Rhodopseudomonas palustris	Viable	Individual	Increased RB, SOD, Catalase, MPO	Zhou et al., 2009
Zooshikella spp.	Viable	Individual	IG, disease resistance	Kim et al., 2010

RB, respiratory brust activity; IG, immunoglobulin; SBA, serum bacteriocidal activity; NA, neutrophill dherence; CA, complement activity; PA, phagocytic activity; AP, antiperoxidase; MPO, myeloperoxidase.

study that dietary applications of *P. acidilactici* could significantly improve microvilli length of the rainbow trout proximal intestine as compared to the control group (Merrifield et al., 2011). Rodriguez-Estrada et al. (2009) in their experiment showed that the enterocytes of fish receiving the probiotic supplemented diets showed a normal appearance, with a reduced number of lipid vacuoles. It is presumed that functionality of such cells should be better than that of the control group and this could contribute to the higher growth observed in such fish. The result of the histo-pathological examination of the kidney, heart, spleen and liver of the rats fed diet containing *S. cerevisae* fermented cassava flour revealed that the diet did not cause any damage to the kidney and heart. However, it caused darkish red colouration on the spleen while the liver had some necrotic lesion, which is an indication of possible damage to the spleen and liver (Oboh and Akindahunsi, 2005).

Probiotic response to stress

Pathogenic infection

Aeromonas hydrophila is the normal constituent of the gut micro-flora of fish (Kumar et al., 2006) that is present in freshwater, aquatic plants and fish, which exhibit haemotoxic responses to the mucus of freshwater fish. *Aeromonas hydrophila* has also been reported to cause mass mortalities in several species including carps, snake head, gouramies and catfishes and is considered as an etiological agent of more than a few diseases including emaciation, haemorrhagic septicaemia, asymptomatic septicaemia, ulcerative infection tail rot and fin rot (Rahman et al., 2001). In a study conducted by Irianto et al. (2003), it was shown that formalin-inactivated cells of *Aeromonas hydrophila* A3-51 when applied as a feed additive, shows beneficial effect in controlling infection by atypical *A. salmonicida* in gold fish. The administration of normal trout feed supplemented with spores of *Bacillus subtilis* and *B. licheniformis* (Bio-Plus2B) is one of the several methods to improve resistance in fish against infection with *Yersinia ruckeri* (Raida et al., 2003). Recently, Kim et al. (2010) showed that adding *Zooshikella* strain JE-34, a bacterium from marine sediment, may help to control streptococcus inane infections and improve the innate immune system in olive flounder. These studies indicate that the indigenous/natural micro-organisms have much potential because of higher probability of competitive exclusion due to adaptation to same ecological niche (Lalloo et al., 2010). Survivability of the infected fishes also increased after probiotic supplementation. This is in accordance with the findings of Kumar et al. (2006), who also obtained higher survivability, in probiotic fed (*B. subtilis*) rohu after *A. hydrophila* infection. A summary of different probiotics tested against several fish specific pathogens are given in Table 4.

Oxidative stress

Apart from pathogen pressure, aquatic animals are also subjected to temperature and other environmental perturbations that can severely affect their physiological state (Wabete et al., 2008). Studies include elucidation of the presence of a wide range of contaminants (xenobiotics) (Ferreira et al., 2005), UV-radiation, hypoxia and hyperoxia (Zenteno-Savín et al., 2006), and other environmental physicochemical parameters (Lesser, 2006) being linked to changes to physiological states of shrimps. All these factors are responsible for the oxidative stress in the animal. Vijayavel and Balasubramanian (2009) reported significant inhibition of antioxidant enzymes like SOD and catalase upon fenvalerate exposure to brackish water prawn, *Penaeus monodon*. Induction of stress by environmental temperature variation determines whether an organism adapts to

Table 4 Summary of fish specific probiotic-pathogen interaction in aquaculture

Animal tested	Probiotic used	Pathogen tested	References
Gadus morhua (Atlantic cod)	*Carnobacterium divergens*	*V. anguillarum*	Gildberg et al., 1997
Salmo salar (Atlantic salmon)	*Lactobacillus plantarum*	*A. salmonicida*	Gildberg et al., 1995
Salmo salar (Atlantic salmon)	*Carnobacterium* spp.	*V. anguillarum, A. salmonicida*	Robertson et al., 2000
Salmo salar (Atlantic salmon)	*P. fluorescens*	*A. salmonicida*	Gram et al., 2001
Oncorhynchus mykiss (Rainbow trout)	*Carnobacterium* spp.	*V. anguillarum, A. salmonicida, V. ordalii, Y. ruckeri*	Robertson et al., 2000
Anguilla anguilla (Eel)	*E. facium*	*E. tarda*	Chang and Liu, 2002
Anguilla anguilla (Eel)	Aeromonas media	*Saprolegnia* spp.	Lategan et al., 2004b
Sparus auratus (Gilthhead sea bream)	*Vibrio* spp., *Micrococcus* spp.	*L. anguillarum*	Chabrillon et al., 2006
Carassius auratus (Gold fish)	*A. hydrophila*	*A. salmonicida*	Irianto et al., 2003b
Labeo rohita (Indian major carp)	*B. subtilis*	*A. hydrophila*	Kumar et al., 2006
Oncorhynchus mykiss (Rainbow trout)	*P. fluorescens*	*V. anguillarum*	Gram et al., 1999
Oncorhynchus mykiss (Rainbow trout)	*Lactobacillus rhamnosus*	*A. salmonicida*	Nikoskelainen et al., 2003
Oncorhynchus mykiss (Rainbow trout)	*Pseudomonus* spp.	*V. anguilarum*	Spanggaard et al., 2001
Oncorhynchus mykiss (Rainbow trout)	*A. hydrophilla, V. fluvialis,* Carnobacterium spp.	*A. salmonicida*	Irianto and Austin, 2003a
Oncorhynchus mykiss (Rainbow trout)	BioPlus 2B	*Y. ruckeri*	Raida et al., 2003
Oncorhynchus mykiss (Rainbow trout)	*A. sobria*	*L.garvieae, S., iniae*	Brunt and Austin, 2005
Solea senegalensis (Senegalese sole)	*Vibrio* spp., Pseudomonas spp., Micrococcus spp.	*V. harveyi*	Chabrillon et al., 2005
Bidyanus bidyanus (Silver perch)	A. media	*Saprolegnia* spp.	Lategan et al., 2004a
Paralichthys olivaceus (Japanese flounder)	Commercial product	*E. tarda*	Taoka et al., 2006
Scophthalmus maximus (Turbot)	*Roseobactor* spp., *Vibrio* spp.	*V. anguillarum, V. splendidus*	Hjelm et al., 2004
Salmo salar (Atlantic salmon)	*Carnobacterium* spp.	*A. salmonicida, Vibrio* spp.	Robertson et al., 2000
Gadus morhua (Atlantic cod)	*C. divergens*	*V. anguillarum*	Gildberg et al., 1997
Salmo trutta fario (Brown trout)	*L. lactis*	*A. salmonicida*	Balcázar et al., 2009
Solea senegalensis (Senegalese Sole)	*S. putirficance, S. baltica*	*Photobacterium*	Diaz-Rosales et al., 2009
Oncorhynchus mykiss (Rainbow trout)	*C. maltaromaticum, C. divergens*	*A. salmonicida, Y. ruckeri*	Kim and Austin, 2006a; b
Labeo rohita (Indian major carp)	*B. subtilis*	*E. tarda*	Nayak, 2010
Miichthys miiuy (Brown Croaker)	*C. butyricum*	*V. anguillarum*	Sakai et al., 1995
Oncorhynchus mykiss (Rainbow trout)	*Kocuria* sp.	*V. anguillarum*	Sharifuzzaman and Austin, 2010b
Epinephelus coioides (Orange-spotted grouper)	*L. plantarum*	*Streptococcus* spp., *iridovirus*	Son et al., 2009
Carassius auratus (Gold fish)	Commercial probiotics	*P. fluorescens*	Abraham et al., 2008
Oncorhynchus mykiss (Rainbow trout)	*L. plantarum*	*L. garvieae*	Vendrell et al., 2008
Salmo trutta fario (Brown trout)	*S. putrefaciens*	*L. anguillarum*	Chabrillon et al., 2006
Scophthalmus maximus (turbot)	*Roseobacter* spp.	*V. anguillarum, V. splendidus*	Hjelm et al., 2004
Oreochromis mossambicus (Mossambic tilapia)	Lactic acid bacteria	*A. hydrophila*	Vijayabaskar and Somasundaram, 2008
Shrimp	Commercial product, *B. subtilis*, *Vibrio* spp.	Gram-negative bacteria, pathogenic vibrios,	Reviewed in Kesarcodi-Watson et al., 2008
Haliotis midae (Abalone)	Unidentified yeast and bacterium	*V. anguillarum*	Macey and Coyne, 2005
Argopecten purpuratus (Scallop larvae)	Marine bacteria	Pathogenic *Vibrio* and *Aeromonas* spp.	Riquelme et al., 2000

changed conditions and survives or suffers from physiological disturbances (Peuranen et al., 2003). Temperature change fundamentally influences the concentrations of enzymes via differential effect on protein synthesis and protein degradation (Willmer et al., 2000). Molecular chaperons like heat shock proteins (HSPs) are also expressed differently with varying temperature (Hartl, 1996). Change in HSP

lead to change in plasma cortisol level and further the immunity of fish (Langston et al., 2002). Even though there are controversies over the effects of temperature on the innate immune responses, it is well established that the effect of temperature also affects the microbial or probiotic strain. Hagi et al. (2004), observed a shift from *Lactococcus raffinolactis* to *Lactococcus lactis* in fish intestine in summer, when temperature rose above 20 °C.

Ameliorating effect on oxidative stress

Apart from pathogen pressure, aquatic animals are also subjected to temperature and other environmental perturbations that can severely affect their physiological state (Wabete et al., 2008). Studies include elucidation of the presence of a wide range of contaminants (xenobiotics) (Ferreira et al., 2005), UV-radiation, hypoxia and hyperoxia (Zenteno-Savín et al., 2006), and other environmental physico-chemical parameters (Lesser, 2006) being linked to changes to physiological states of shrimps. All these factors are responsible for the oxidative stress in the animal. Hence, to ameliorate the effect of these oxidative stress factors, probiotics are fed to the experimental animals. The feeding of probiotics acts in such a way as to increase the antioxidant status of the experimental shrimps (Castex et al., 2009). It can therefore be assumed that the probiotic diet may: (i) improve the diet utilization (Castex et al., 2009) and contribute to increasing the assimilation of dietary antioxidants from the feed and/or (ii) plays a role in antioxidant activity. Castex et al. (2009) hypothesized that the anti-oxidative properties of a *Lactobacillus fermentum* strain may serve as a defensive mechanism in the intestinal microbial ecosystem and therefore help overcome exo- and endogenous oxidative stress (ess). *Lactobacillus fructivorans* and *L. plantarum*, when administered to sea bream, *Sparus auratus*, fry using the rotifer, *Brachionus plicatilis*, and/or *Artemia salina* as carrier, caused a significantly lower cortisol level and higher HSP 70 gene expression, and also resulted in significantly lower cumulative mortality in probiotic-treated sea bream, subjected to an acute pH stress (from 8.6 to 6.3) (Liu et al., 2010). Recent studies by different research workers have revealed that the protective mechanism of the animal are influenced by normal intestinal micro-flora commonly taken as probiotics, by increasing the immunity especially the putative protective HSP under stress conditions (Koninkx and Malago, 2008). It has been reported by Taoka et al. (2006), that a commercial probiotic, Alchem Poseidon (a mixture of *Bacillus subtilis*, *Lactobacillus acidophilus*, *Clostridium butyricum* and *Saccharomyces cerevisiae*), is helpful in increasing the stress tolerance in *P. Olivaceus*, under closed recirculatory system. *Sparus auratus*, when fed with *Lactobacillus fructivorans* and *Lactobacillus plantarum*, resulted in checking the increase in cortisol level when subjected to acute stress condition (Varela et al., 2010). Some of the Amazonian ornamental fishes (marbled hatchetfish *Carnegiella strigata*, Gomes et al., 2008; cardinal tetra *Paracheirodon axelrodi*, Gomes et al., 2009), also showed enhanced survival and decreased cortisol level when subjected to transportation stress. Varela et al. (2010) also reported that the administration of the probiotic strain, Pdp11, in the diet of *Sparus auratus*, helps in promoting the growth as well as improves the stress tolerance to high stocking density, thus suggesting its beneficial role in aquaculture industry. Mohapatra et al. (unpublished) reported the useful effect of feeding a multi-species probiotic diet to the *Labeo rohita* fingerlings in tolerating the stress caused by Fenvalerate, a widely used synthetic pyrethroid.

Synbiotics

Along with probiotics, prebiotics (Table 5), Bacteriocins (Table 6) and immunostimulants can also be used to enhance immunity in the host organism. Prebiotic also play a role in increasing growth rate, improving immune system as well as changing the community of bacterial in gastrointestinal track (Yousefian and Amiri, 2009). Prebiotics also offer a rational approach to the probiotic concept, e.g. reduction of gut pH through SCFA formation; secretion of antimicrobial substances; blocking of adhesion sites; attenuation of virulence; blocking of toxin receptor sites; immune stimulation; competition for nutrients, and suppression of toxin production (Fooks et al., 1999; Gibson, 1999) (Table 5). Therefore, the term, synbiotics, can be very well applied in aquaculture to better the nutritional supplements, in a form of synergism. As the probiotic are mainly active in the small intestine and the probiotics play a role in large intestine in humans, a combination of both would render a much effective effect (Gibson and Roberfroid, 1995).

Molecular approach in probiotic use

Majority of generalized methodologies for assessment of probiotic effect on microbial ecology of the gut is based on anaerobic-cultivation approach, which gives incomplete idea about the microbial population,

Table 5 Prebiotics candidate for aquaculture

Preobiotics	Parameters investigated	Species tested	References
GroBiotac A	↑ feed efficiency, ↑ respiratory burst, ↑ resistance against *Streptococcus iniae*, ↑ growth performance	Hybrid striped bass (*Morone chrysops* X *Morone saxatilis*)	Li and Gatlin, 2004; Ringø et al., 2010
GOS	↑ growth performance, better feed utilization, body composition	Atlantic salmon (*Salmo salar*)	Sealey et al., 2007
	↑ Protein and organic ADC values↓ Lipid ADC	Red drum (*Sciaenops ocellatus*)	Burr et al., 2008; Ringø et al., 2010
MOS	↑ growth performance, feed utilization, body composition, gut histology, Resistance to parasitic infection	Atlantic salmon (*Salmo salar*)	Grisdale-Helland et al., 2008
FOS	Growth performance, haematology or immune function	Channel catfish (*Ictalurus punctatus*)	Grisdale-Helland et al., 2008
	Larval survival, Early survival and morphological development of early juvenile stages	European lobster (*Homarus gammarus*)	Ringø et al., 2010
	Gut microbiota, gut histology, growth performance, feed utilization, immune response, body composition, disease resistance	Rainbow trout (*Oncorhynchus mykiss*)	Daniels et al., 2006, 2007; Ringø et al., 2010
	Growth performance, feed utilization, body composition	Atlantic salmon (*Salmo salar*)	Dimitroglou et al., 2009; Rodriguez-Estrada et al., 2009
scFOS	↑ growth rate	Soft-shell turtle (*Triortyx sinensis*)	Grisdale-Helland et al., 2008
	↑ growth rate, effect on gut microbiota	Turbot larvae (*Scophthalmus maximus*)	Ringø et al., 2010
	↑ growth rate, feed intake, feed conversion, survival and condition factor	Hybrid Tilapia (*Oreochromis niloticus* ♀ X *Oreochromis aureus* ♂)	Ringø et al., 2010; Mahious et al., 2006
XOS	Weight gain, feed conversion, gut microbiota	white shrimp (*Litopenaeus vannamei*)	Ringø et al., 2010; Hui-Yuan et al., 2007
	↑ growth, survival, ↑ enzymatic activity	Crucian carp (*Carassius auratus gibelio*)	Li et al., 2007; Ringø et al., 2010
AXOS	Growth, butyrate production	African catfish (*Clarius gariepinus*)	Ringø et al., 2010; Rurangwa et al., 2008
	Microbial community in the hindgut, butyrate production, growth	Siberian sturgeon (*Acipenser baeri*)	Rurangwa et al., 2008; Ringø et al., 2010
IMO	Microbial population, immune response, resistance to WSSV (White Spot Syndrome Virus)	Pacific white shrimp (*Litopenaeus vannamei*)	Li et al., 2009; Ringø et al., 2010
Inulin	Susceptibility against *A. hydrophila* and *E. tarda*	Tilapia (*Tilapia aureus*)	Ringø et al., 2010
	↓Intestinal cell damage, ↓TVC, microbiota control	Arctic charr (*Salvelinus alpinus*)	Ringø et al., 2006, 2010
	↓Intestinal cell damage, ↑ Intestinal growth	Atlantic salmon (*Salmo salar*)	Bakke-McKellep et al., 2007; Refstie et al., 2006
	↓Intestinal cell damage, inhibition of phagocytosis and respiratory burst in leucocytes	Gilthead seabream (*Sparus auratus*)	Ringø et al., 2006

FOS, fructooligosaccharides; scFOS, short-chain fructooligosaccharides; MOS, mannanoligosaccharides; GOS, galactooligosaccharides; XOS, xylooligo-saccharides; AXOS, arabinoxylooligosaccharides; IMO, isomaltooligosaccharides; TVC, total viable count; ADC, apparent digestability coefficient.

their interation with other inhibitant and host. However, the rapid advancement in molecular techniques, metagenomics and high-resolution post genomics has opened avenue for better understanding of probiotic interection in gut. The development of gnobiotic technology and specific germ free animal models opens new avenue for greater understanding of host- pathogen interaction and their effect on global development of a particular organism (Kleerebezem and Vaughan, 2009). Moreover, the availability of genome sequence data for numerous microbes generates a alternative method, i.e. 16s ribosomal RNA analysis-apporach, for detailed analysis of gut population (Ravi et al., 2007).

Table 6 Bacteriocin candidates for aquaculture

Organism	Class	Molecular weight (kDa)	Mode of action	References
E. coli	Colicin	40–80	Nuclease/pore-forming	Riley and Wertz, 2002
	Microcin	3.1	Intracellular enzymes	Duquesne et al., 2007
Prochloron didemni	Microcin like	0.7	Not yet defined	Schmidt et al., 2005
P. aeruginosa	Pyocins	270 (AA)/75–94	Pore-forming/Phage tail like	Duport et al., 1995
Hafnia alvei	Alveicins	358–408 (AA)	Pore-forming	Wertz and Riley, 2004
K. pneumonia	Klebcin	96	Nuclease	Riley et al., 2001
Serratia plymithicum	Serracin	66	Phage tail like	Jabrane et al., 2002
Xanthomonas campestris	Glynericin	50	Phage tail like	Pham et al., 2004
Yersinia enterocolitica	Enterocoliticin	669	Phage tail like	Strauch et al., 2001
Erwinia carotovora	Carotocoricin	68/76	Phage tail like	Nguyen et al., 2002
Other lactic acid bacteria	Class I/II/Lantibiotic	0.7–5.8	Pore-forming	Oppegård et al., 2007; Martin-Visscher et al., 2009
Streotococcus milleri	Millericin	30	Peptidoglycan hydrolysis	Beukes et al., 2000
Enterococcus faecalis	Enterolysin	345	Peptidoglycan hydrolysis	Nilsen et al., 2003
Staphylococcus aureus	Lysostaphin	25	Peptidoglycan hydrolysis	Kumar et al., 2008
Listyonella anguillarum	Vibriocin Avp10		Inhibit *E. coli*	Zai et al., 2009
Vibrio mediterranei	Bacteriocin Like Inhibitory Substances (BLIS)	63–65	Inhibit pathogenic strains of *Vibrio*	Carraturo et al., 2006
Aeromonas hydrophila	BLIS		Pore-forming	Messi et al., 2003
Pseudoalteromonas spp.	Antibiotic protein	280	Peptidoglycan hydrolysis	Longeon et al., 2004
Cornibacterium divergens V41	Divercin V41	4509	Inhibit *Listeria monocytogenes*	Richard et al., 2004
Cornibacterium piscicola V1	Piscicocin V1a/b	4416/4526	Inhibits *L. monocytogens*	Richard et al., 2004

Recently, in this regard, Avella et al. (2010) have found that clown fish larvae fed with lactic acid bacteria has both faster growth and development as well as higher survivility due to increase in several related gene i.e. insulin like growth factors, retinoic acid receptor, vitamin D receptor, Myostatin, and reduction in glucocorticoid receptor and 70-KD heat shock protein respectively. This improvement is due to the presence of two different kinds of pilus operon (spaCBA, spaFED) of *Lactobacillus* spp. which improves the mucus interaction and further adherance and colonization (Kankainen et al., 2009).

Safety and evaluation of probiotics

The known benefits of probiotics in aquaculture farming include direct effects like, immunity enhancement, disease control; feed conversion improvement effects on the organism through microbial colonization of the digestive tract and indirect/environmental effects i.e. water quality improvement, reduction of water exchange, and reduction of sludge accumulation. All of these effects combined have a synergist impact on the financial performance of the farm. It is expected that probiotics will be used in aquaculture to replace the use of antibiotics and will lead the aquaculture industry to future organic farming. Probiotic also reduces the cost of fish farming by decreasing the feed cost per unit growth of fish (El-Haroun et al., 2006). El-Dakar et al. (2007) demonstrated a marked 73%–78% reduction in feed cost of rabbit fish (*Siganus rivulatus*) culture only through Biogen® (mixture of probiotic and prebiotic) supplimentation. The dose-effect ratio should be carefully monitored for different stages of culture to get a higher benefit (Verschuere et al., 2000a). Some bacteria especially the gram-negative bacteria may loose their beneficial properties in artificial culture condition (Ringo et al., 2004). A simple and rapid means of culturing, storing and administering would be preferred by aquaculturists. However, with many candidate probiotics being Gram-negative, sterile laboratory techniques, equipment and skills are required to ensure product quality, all at extra expense. Therefore, endospore forming gram-positive bacteria and non-bacterial microbes (Yeast and microalgae) are economically viable competetor (Eddy and Jones, 2002; Tovar-Ramirez et al., 2004).

As the search for probiotic bacteria continues, novel species and species-specific strains of probiotic bacteria are constantly identified. Treatment with these new probiotics is relatively safe, but not entirely risk-free. Probiotics are originally pathogenic in nature (Ishibashi and Yamazaki, 2001) with a

potential of bacterial translocation. Considering this situation, safety of probiotics become utmost important and therefore safety considerations of putative probiotic should be a pre-requesite of the process of development and marketing. Evaluation should include consideration for the both end-product formulation and mechanism of action, since these can induce adverse effects in some subjects or negate the positive effects altogether. However, conventional methods relying on phenotypic characterization, growth requirements and characteristics, fermentation profiles, and serology studies have been proven useful but carry inherent deficiencies (Qi et al., 2009). The modern molecular techniques may be used to over the conventional lacunies and help to identify the correct strain of probiotics. Presently, various molecular finger printing techniques i.e. polymerase chain reaction – denaturing gradient gel electrophoresis/temperature gradient gel electrophoresis (PCR-DGGE/TGGE), terminal restriction fragment length polymorphism (T-RFLP), multi-locus sequence typing (MLST) and fluorescence amplified fragment length polymorphism (F-AFLP) have been proven useful in strain differentiation in case of higher organism (Huys et al., 2006). These techniques can be customized for probiotics assessment in aquculture.

Conclusion and future prospective

Probiotics or the useful microbes help in playing a vital role in sustainable aquaculture production. As the ban on antibiotics is being implemented all over the world, probiotics are gaining much importance (Panigrahi and Azad, 2007). Therefore, a wide knowledge of the mode of action of the probiotics is highly essential. Despite the potential benefits to health and performance as noted in various terrestrial species, less information is available about the effect of probiotics in fish. Although in fish, the effect of probiotics on growth, feed efficiency, gut microbiota, disease susceptibility, on innate immune parameters, mucosal barriers, cell damage/morphology have been investigated quite thoroughly but their detailed mechanism of action is largely unknown. Moreover, species/strain/stage specificity of probiotics are a great concern which need to be addressed carefully and promptly.

There are several initiative on multiple combination of probiotics to get a pronounced effect but more detailed and focused studies are necessary for betterment of future aquaculture. Furthermore, scarce studies are reported on role of probiotics in different environmental stress management. In-depth analysis of the surrounding environment-probiotic correlation might have greater influences on future fisheries and aquaculture management.

Following the numerous genome-sequencing tools that are currently used, future research on probiotic effects should involve transcriptome and proteome analysis using high throughput assays. In addition, transcriptome and proteome profiling of gut microbiota should be thoroughly documented in order to know the varying mode of action of different probiotic organism. Studies on probiotics should, therefore, be given high priority in the future, and molecular analysis should be included as standard criteria to assess their effects on fish health and nutrition.

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