

Probiotics in fish and shellfish culture: immunomodulatory and ecophysiological responses

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Received: 26 April 2013 / Accepted: 6 December 2013
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Abstract Aquaculture is emerging as one of the most viable and promising enterprises for keeping pace with the surging need for animal protein, providing nutritional and food security to humans, particularly those residing in regions where livestock is relatively scarce. With every step toward intensification of aquaculture practices, there is an increase in the stress level in the animal as well as the environment. Hence, disease outbreak is being increasingly recognized as one of the most important constraints to aquaculture production in many countries, including India. Conventionally, the disease control in aquaculture has relied on the use of chemical compounds and antibiotics. The development of non-antibiotic and environmentally friendly agents is one of the key factors for health management in aquaculture.

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Consequently, with the emerging need for environmentally friendly aquaculture, the use of alternatives to antibiotic growth promoters in fish nutrition is now widely accepted. In recent years, probiotics have taken center stage and are being used as an unconventional approach that has numerous beneficial effects in fish and shellfish culture: improved activity of gastrointestinal microbiota and enhanced immune status, disease resistance, survival, feed utilization and growth performance. As natural products, probiotics have much potential to increase the efficiency and sustainability of aquaculture production. Therefore, comprehensive research to fully characterize the intestinal microbiota of prominent fish species, mechanisms of action of probiotics and their effects on the intestinal ecosystem, immunity, fish health and performance is reasonable. This review highlights the classifications and applications of probiotics in aquaculture. The review also summarizes the advancement and research highlights of the probiotic status and mode of action, which are of great significance from an ecofriendly, sustainable, intensive aquaculture point of view.

Keywords Aquaculture · Ecofriendly · Immunomodulation · Microbiota · Probiotics

Abbreviations

ACP	Alternative complement pathway
ALT	Alanine aminotransferase
ASS	Acid sulfate soils

AST	Aspartate aminotransferase
BFM	Bifidobacteria-fermented milk
BLIS	Bacteriocin-like inhibitory substances
CANON	Completely autotrophic nitrogen removal over nitrite
CER	Conversion efficiency ratio
CF	Condition factor
DGGE	Denaturing gradient gel electrophoresis
DO	Dissolved oxygen
FCR	Feed conversion ratio
FER	Feed efficiency ratio
FISH	Fluorescent in situ hybridization
FOS	Fructo-oligosaccharide
GIT	Gastrointestinal track
HUFA	Highly unsaturated fatty acid
LAB	Lactic acid bacteria
LCA	Life assessment cycle methodology
LDL-C	Low density lipoprotein cholesterol
LPO	Lipid peroxidation
LPS	Lipopolysaccharide
MOS	Mannose oligosaccharide
ODC	Ornithine-decarboxylase
PCR	Polymerase chain reaction
PER	Protein efficiency ratio
PET	Probiotic encapsulation technology
PHB	Poly-hydroxyl butyrate acid
QS	Quorum sensing
ROS	Reactive oxygen species
RPS	Relative percent survival
SGR	Specific growth rate
SOD	Sodium oxidase dismutase
SOD	Superoxide dismutase
TAN	Total ammonia nitrogen
TG	Triglycerides
WGR	Weight gain rate

Introduction

The concept of biological disease control, particularly using microbiological modulators for disease prevention, has received widespread attention. A bacterial supplement of a single or mixed culture of selected non-pathogenic bacterial strains is termed probiotics. The word “probiotic” was pioneered by Parker (1974), who described probiotics as organisms and substances that contribute to the intestinal microbial balance. Fuller (1989) revised the definition of

probiotics as ‘a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.’ This revised definition has emphasized the importance of live cells as the essential component of a potential probiotic, and it clears up the confusion created by the use of the term ‘substances.’ Probiotics, according to the currently adopted definition of the Food and Agricultural Organization and World Health Organization, are live microorganisms that when administered in adequate amounts confer a health benefit on the host (FAO and WHO 2001). Over the years, various strategies to modulate the composition of the gut microbiota for better growth, digestion, immunity and disease resistance of the host have been investigated in various kinds of livestock as well as in humans (Burr et al. 2007). The massive use of antimicrobials for disease control and growth promotion in animals increases the selective pressure exerted on the microbial communities and encourages the natural emergence of bacterial resistance (<http://www.who.int/inf-fs/en/fact194.html>). Probiotics thus are opening a new era in the health management strategy from human to aquatic species including fish and shellfish. Probiotics are gaining increasing scientific and commercial interest and now have a quite common place in health-promoting functional foods as well as therapeutic, prophylactic and growth supplements (Ouweland et al. 2002; O’Sullivan 2001). The bacteria present in the aquatic environment influence the composition of the gut biota as the host and microorganisms share the ecosystem (Verschuere et al. 2000). Therefore, it is preferable to give probiotics to fish in the larval stage, because the larval forms of most fish and shellfish are released in the external environment at an early ontogenetic stage (Lim et al. 2011; Kapareiko et al. 2011; Arig et al. 2013). Miniature larvae are highly vulnerable to gastrointestinal-associated disorders, because they start feeding even though the digestive tract has not yet been fully developed and the immune system is still incomplete. It has also been observed that temperature changes, intensity of stress management and density may have suppressive effects on olfactory and physiological responses, where selective food additives and immune stimulants can enhance the organism efficiency toward immediate responses (Magnadottir 2006, 2010). Probiotics may act as a microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as

well as improving the nutritional and microbial balance in the intestinal tract (Villamil et al. 2002).

There are some common mechanisms of action that have been reported for the majority of probiotic strains. Probiotics were found to stimulate the feed conversion efficiency, augment live weight gain in fish and shrimp culture (Al-Dohail et al. 2009; Saenz de Rodriguez et al. 2009) and confer protection against pathogens by competitive exclusion for adhesion sites (Chabrillon et al. 2005; Vine et al. 2004), production of organic acids, hydrogen peroxide, antibiotics, bacteriocins, siderophores and lysozyme (El-Dakar 2007; Yan et al. 2002) and also modulate physiological and immunological responses in fish (Balcazar et al. 2006; Khattab et al. 2004). Moreover, probiotics are also being used as biological control agents in highly stocked intensive aquaculture ponds (Lim et al. 2011; Wu et al. 2012; Firouzbaksh et al. 2011; Rahiman et al. 2010; Nimrat et al. 2012; Morya et al. 2013). A wide range of microalgae (*Tetraselmis*), yeast (*Debaryomyces*, *Phaffia* and *Saccharomyces*), gram-positive (*Bacillus*, *Lactococcus*, *Micrococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Streptococcus*, *Weissella*) and gram-negative bacteria (*Aeromonas*, *Alteromonas*, *Photobacterium*, *Pseudomonas* and *Vibrio*) has been evaluated as probiotics (Gatesoupe 1999; He et al. 2011). Several microalgae, yeasts and gram-positive and -negative bacteria have been isolated from the aquatic medium (Cahill 1990; Liu et al. 2000; Alcaide 2003; Austin 2006; Dahiya and Sihag 2009; Dahiya et al. 2009). Likewise, probiotics have been characterized as new ecofriendly alternative measures of disease control in aquaculture (Irianto and Austin 2002a, b; Dahiya et al. 2012a, b; Sihag and Sharma 2012). This review touches upon the relevant recent findings from studies on a wide range of fish and shellfish.

Feed probiotics

The concept behind the composition of feed probiotics is to apply the beneficial bacterial strains in feed using binders such as eggs and cod liver oil to obtain the beneficial microbial effects with more efficacy and at less environmental cost. The majority of commercial preparations contain either *Lactobacillus* or *Saccharomyces cerevisiae* (Abidi 2003), nitrifying bacteria, *Streptococci*, *Roseobacter* (Wang et al. 2008; He et al.

2009) and *Bacillus* sp. (Yun-Zhang et al. 2010; He et al. 2011; Ran et al. 2012; Arig et al. 2013). Beneficial effects of regular use of probiotics in fish feed in the UK and other European countries have been reported (Cerrato 2000).

In aquaculture, probiotics can also be encapsulated in feed (Ramos et al. 2005) or in live food such as rotifers and *Artemia* (Mahdhi et al. 2011). Another efficient application of probiotics to aquatic animals is through bioencapsulation or infusions in diets. Preparation of probiotic diets has been demonstrated by Yassir et al. (2002). According to the FAO and WHO guidelines, probiotic organisms used in food must be capable of surviving passage through the gut. They must have the ability to resist gastric juices and exposure to bile (Senok et al. 2005). In addition, probiotics must be able to proliferate and colonize the digestive tract to be safe, effective and maintain their effectiveness and potency for the duration of the shelf life of the product (Senok et al. 2005). The benefits of inclusion of bacterial strains into feed ingredients include improvements in feed values, contributions to enzymatic digestion, inhibition of pathogenic microorganisms, anti-mutagenic and -carcinogenic activity, growth-promoting factors and enhanced immune response (Wang and Xu 2006; Wang 2007; Kuhlwein et al. 2013; Ambas et al. 2013). Purwandari and Chen (2013) studied the effects of probiotic *Bacillus subtilis* on intestinal microbial diversity and immunity of the Orange-spotted grouper *Epinephelus coioides*. The innate cellular response and respiratory burst activity of the supplemental groups were significantly higher compared to the control at 10 and 20 days after feeding and even more significant at 30 days. Probiotic *B. subtilis* increased the intestinal microbial diversity by stimulating the bacterial populations of *Paenibacillus* sp., *Lactobacillus oeni* strain 59 b and *Methyacidiphilum infernorum* strain V4, which are beneficial for *E. coioides*. The best dose of the probiotic *B. subtilis* based on the growth performance, innate cellular responses and microbial profile of fish intestines is 0.1 %, which showed equal efficacy as the 1 % diet. In this way, the use of feed probiotics in aquaculture has opened the window onto the possibility of sustainable commercial aquaculture (Gatesoupe 1999). Feed probiotics were found to provide better growth results, an improved feed conversion ratio and enhanced appetite than had been experienced in aquaculture previously (Panigrahi et al. 2005; Salah

Mesalhy et al. 2008; Chae-Woo et al. 2009). El-Dakar et al. (2007) demonstrated a marked (73–78 %) reduction in the feed cost of rabbit fish (*Siganus rivulatus*) culture only through Biogen^R (a mixture of probiotics and prebiotics) supplementation. It will soon be necessary to conduct studies relating to probiotic resistance to antibiotics and the possibility of transmission of genetic elements to other microorganisms in the fish GIT, and thus to humans when consuming the aquaculture product (Munoz-Atienza et al. 2013; Touraki et al. 2012).

General 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), thus improving resistance against infectious diseases (Gildberg et al. 1997). Bacterial probiotics do not have a 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).

Symbiosis

Combined application of probiotics and prebiotics, known as synbiotics, is based on the principle of providing a probiont with a competitive advantage over endogenous populations, improving the survival and implantation of the live microbial dietary supplement in the gastrointestinal tract of the host (Gibson and Roberfroid 1995). The use of synbiotics may make possible the production of greater benefits than the application of individual probionts (Merrifield et al. 2010b). The main rationale for using a synbiotic is that

a true probiotic, without its prebiotic food, does not survive well in digestive systems; it will have a greater intolerance for oxygen, low pH and temperature. As prebiotics provide enormous support for probiotics to thrive, the population of these good bacteria is preserved (Sekhon and Jairath 2010).

Synbiotic effects to improve the feed value

Symbiosis affects the host beneficially by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth and activating the metabolism of a limited number of health-promoting bacteria studied in rainbow trout (*Oncorhynchus mykiss*) (Alak and Hisar 2012). Partida-Arangure et al. (2013) found that inulin and bacteria improved immunity in the cultured shrimp *Litopenaeus vannamei*. In humans, Gibson and Roberfroid (1995) could also find the same synergistic effect of probiotics and prebiotics. Rodriguez-Estrada et al. (2009) assessed the potential of a commercial preparation of *E. faecalis* and two kinds of prebiotic ingredients, MOS and PHB, respectively, to enhance survival, improve the feed conversion and ensure better growth in rainbow trout (*Oncorhynchus mykiss*).

Ye et al. (2011) supplemented FOS with MOS and *B. clausii* alone or in combination with a *B. clausii* strain that had previously been isolated from grouper (*Epinephelus coioides*) intestine. Ye et al. (2011) found that protease activity was significantly higher in fish fed diets of BM and BFM than in fish fed the control diet, obtaining a similar value in both diets. These results indicated that administration of FOS in combination with MOS and *B. clausii* did not lead to a major increase in protease activity compared to that observed with the BM diet. Moreover, fish fed the BFM diet exhibited the highest amylase activity, which was significantly higher than in fish fed BFM and control diets. The increase in digestive enzyme activities would allow more efficient nutrient digestion, improving assimilation and promoting a possible increase in the WGR and feed efficiency. In another study, Mehrabi et al. (2011) assessed the dietary inclusion of a commercial symbiotic, Biomin IMBO (Biomin, Herzogenburg, Austria), comprised of a probiotic (*E. faecium* 5×10^{11} cfu/kg) and FOS as a prebiotic, in rainbow trout fed for 60 days with diets containing different levels of synbiotics (0.5, 1.0 and

1.5), which gave rise to a body weight gain of about 50, 59 and 53 %, respectively, in comparison to the control group. Even though the highest average of final weight and weight gain was observed in the T₂ group, there was no significant difference between T₂ and T₃. The addition of a synbiotic to the feed also produced better SGR, FCR and CF with values significantly higher than in the control, more specifically in groups treated with 0.1 % synbiotic. In this study, significantly better growth performance was observed in *O. mykiss* fingerlings maintained on the diet supplemented with a synbiotic.

Ai et al. (2011) evaluated the effects of different concentrations of *B. subtilis* and FOS, combined or separated, on yellow croaker (*Larimichthys crocea*). In this study, dietary supplementation of FOS did not exert beneficial effects on the growth performance, survival and feed utilization, and no significant interactions were observed between *B. subtilis* and FOS. However, the significantly enhanced SGR and FER using *B. subtilis* in this study clearly showed its growth-improving property. Geng et al. (2011) reported the better growth performance of cobia, *Rachycentron canadum*, fed with various levels of dietary *B. subtilis* and chitosan.

Synbiotic effects on immunity and disease resistant

Major contigs of the fish immune system activities are lysozyme, the alternative complement pathway, phagocytosis, respiratory burst, superoxide dismutase and mucus production (Cerezuela et al. 2011).

Lysozyme activity constitutes an essential defense mechanism against pathogens in fish, and the bacteriolytic activity of lysozyme in fish skin mucus and other tissues contributes to the host defense mechanism against bacterial infection (Subramanian et al. 2007; Saurabh and Sahoo 2008; Sanchooli et al. 2012). Ye et al. (2011) studied dietary effects of lysozyme activity on Japanese flounder. The lysozyme activity was significantly higher in fish fed a symbiotic diet than in those whose diets were supplemented with individual prebiotics, and the activity tended to be enhanced compared to those fed the *B. clausii* diet, which suggested that the dietary administration of FOS or MOS combined with *B. clausii* synergistically modulates lysozyme activity. In contrast to this study, Ai et al. (2011) revealed that an increase in lysozyme

activity could be independent of FOS, being a response to *B. subtilis*. Although no significant differences were observed between the two dietary *B. subtilis* levels, a tendency was observed toward increased activity with increased *B. subtilis* concentration. Nekoubin and Sudagar (2012) also found the same results with a synbiotic (*Bioimin IMBO*) supplemented with artificial diets of varying protein levels on the growth performance and survival rate in Grass carp (*Ctenopharyngodon idella*). Geng et al. (2011) determined that chitosan alone or in combination with *B. subtilis* in the diet of cobia (*Rachycentron canadum*) increased the lysozyme activity.

Magda et al. (2011) investigated the effect of dietary supplementation of the probiotics *Bacillus subtilis*, *Lactobacillus plantarum*, a mixture of bacterial isolates (*B. subtilis* and *L. plantarum*) and the yeast *Saccharomyces cerevisiae* on the immune response of the Nile tilapia, *Oreochromis niloticus*. Experimental results showed significantly increased phagocytic, acid phosphatase, lysozyme and total immunoglobulin activities in blood samples of the fish compared to those on the control diet.

Alternative complement pathway activity has only been evaluated in yellow croaker and cobia following the administration of *B. subtilis* combined with FOS or chitosan, respectively (Ai et al. 2011; Ye et al. 2011). In yellow croaker, ACP activity was not significantly affected by the pro- and prebiotic supplementation. In cobia, this activity was significantly enhanced with diets supplemented with a low chitosan level (3.0 g/kg) at any *B. subtilis* level, whereas the high chitosan level (6.0 g/kg) only produced an increase in ACP when administered with *B. subtilis* at 2.0 g/kg. Biller-Takahashi et al. (2012) determined that the hemolytic activity of the proteins of the complement system increased after the pathogen challenge, but was not influenced by β -glucan treatment. Alternatively, Zhao et al. (2011) found that *Bacillus* TC22 and prebiotic fructo-oligosaccharide, when fed to sea cucumbers as TC22 at 10⁹ cfu/g feed and 0.5 % FOS alone or in combination, resulted in enhanced phagocytosis, respiratory burst and phenoloxidase activity of sea cucumber coelomocytes and disease resistance against *V. splendidus* infection.

Stimulatory effects to enzymatic digestion

Digestive tract and enzyme physiologies seem to be affected by ingested feed ingredients, which are finally

reflected in fish health and growth (Bolasina et al. 2006; Shan et al. 2008). Fish may adapt their metabolic functions to the dietary substrates through regulation of enzyme secretion in order to improve the utilization of feed ingredients. The enzyme profiles found in axillary sea bream fed two different diets confirmed this for proteases and amylases (Reimer 1982). A study has suggested that probiotics have a beneficial effect on the digestive processes of aquatic animals because probiotic strains synthesize extracellular enzymes such as proteases, amylases and lipases as well as provide growth factors such as vitamins, fatty acids and amino acids (Balcazar et al. 2006). Therefore, nutrients are absorbed more efficiently when the feed is supplemented with probiotics (El-Haroun et al. 2006). However, only a few studies have investigated the changes in enzyme patterns that are altered because of intake of feed ingredients or their digestion in some fish species: *Hypophthalmichthys molitrix*, *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala* (Ismat et al. 2013) and *Anguilla anguilla* (Caruso et al. 2008). Elevated secretion of the enzymes amylase, trypsin, protease and lipase was reported in the sea bass *Labeo rohita* after being fed with live yeast and a mixture of *Bacillus subtilis*, *Lactococcus lactis* and *Saccharomyces cerevisiae* (Tovar-Ramirez et al. 2002; Mohapatra et al. 2012). Amylase and lipase, being the major enzymes associated with carbohydrate and fat digestion, were found to increase nutrient digestibility and improve health conditions, which might be because of better availability of exoenzymes produced by probiotics (Bairagi et al. 2002; Vine et al. 2006; Lara-Flores and Aguirre-Guzman 2009; Mohapatra et al. 2012). Studies in juveniles of the common dentex (*Dentex dentex*) showed that when the diet was supplemented with 0.5 g of *Bacillus cereus* strain per kg of food, increased fish growth occurred because of more efficient use of the food (Hidalgo et al. 2006). In the case of rainbow trout, similar results were obtained using *B. subtilis*, *B. licheniformis* and *Enterococcus faecium* when these probiotics were provided for 10 weeks along with the fish diet (Merrifield et al. 2010b). In some trials, the diet of European sea bass larvae (*Dicentrarchus labrax*) was supplemented with probiotic yeast (*Saccharomyces cerevisiae* strain X2180); fish growth, activity and expression of antioxidant key enzymes were assessed, with differences found in enzyme activity and gene expression patterns between probiotic-supplemented and non-

supplemented treatments, which was attributed to the presence of the yeast (Tovar-Ramirez et al. 2010). In white shrimp *Litopenaeus vannamei* and *Fenneropenaeus indicus*, various strains of *Bacillus* have been used as probiotics to increase the apparent digestibility of dry matter, crude protein and phosphorus. Results showed larger sizes when the diet was supplemented with 50 g of probiotic per kg of food (Heizhao et al. 2004). Other researchers had suggested the importance of managing the probiotic in all ontogenetic stages of the shrimp to generate a constant effect on the production of digestive enzymes (Zhou et al. 2009). A study by Bertotto et al. (2011) on the early stages of sea bass illustrated that probiotic treatment was found to modulate the heat-resistant properties because of the different level of heat shock protein available. In ornamental fish such as guppies (*Poecilia reticulata*, *P. sphenops*) and swordtail (*Xiphophorus helleri*, *X. maculatus*), the effect of incorporating *Bacillus subtilis* isolated from the intestine of *Cirrhinus mrigala* into their diet has also been evaluated. Some of the studies highlighted the specific activity of proteases and amylases in the digestive tract of ornamental fish, which results in better follicle competence and temporal development (Kamei et al. 2011; Avella et al. 2012; Gioacchini et al. 2012). It has been reported that *Bacillus* could exhibit a wide range of exoenzymes that complemented the activities of the fish and increased enzymatic digestion in earlier studies (Arig et al. 2013; Purwandari and Chen 2013). In fact, the bacteria isolated from the digestive tract of aquatic animals have been shown to produce chitinases, proteases, cellulases, lipases and trypsin, which may contribute to fish nutrition (Vine et al. 2006; Ray et al. 2012). Moreover, probiotics also have a very positive effect on the digestive processes as well as the assimilation of food components (Irianto and Austin 2002a, b).

Competitive exclusion of pathogenic bacteria

Competitive exclusion is a phenomenon whereby an established microflora prevents or reduces the colonization of a competing bacterial challenge for the same location on the intestine (Pandiyani et al. 2013). The aim of probiotic products designed under competitive exclusion is to obtain stable, agreeable and controlled microbiota in cultures based on the following: competition for attachment sites on the mucosa, and

competition for nutrients and production of inhibitory substances by the microflora that prevent replication and destroy the challenging bacteria, hence reducing colonization (Moriarty 1998).

The direct effect, such as inhibiting the growth of other organisms, might be the main action that can occur in cultured systems (Kesarcodi-Watson et al. 2008; Jiang et al. 2013; Giri et al. 2013), and studies have illustrated that indigenous microorganisms have great potential because of the higher probability of competitive exclusion due to adaptation to the same ecological niche (Lalloo et al. 2010). Adhesion and colonization of the mucosal surfaces are possible protective mechanisms against pathogens through competition for binding sites and nutrients (Westerdahl et al. 1991; Salyers and White 2002). The study showed that lactobacilli reduced the adhesion of *A. salmonicida*, *C. piscicola* and *Yersinia ruckeri* to intestinal mucus of rainbow trout (Balcazar et al. 2006). *Aeromonas hydrophila* inhabits as a normal constituent of the gut microflora of fish, which exhibit hemotoxic responses to cause mass mortalities in several species including carp, snakehead, gouramies and catfish; it is considered an etiological agent in more than a few diseases including emaciation, hemorrhagic septicemia, asymptomatic septicemia, ulcerative infection tail rot and fin rot (Rahman et al. 2001; Kumar et al. 2006). Korkea-aho et al. (2012) used the *Pseudomonas* M162 strain against *Flavobacterium psychrophilum* to reduce the mass mortality in salmonid culture during the early life stages of rainbow trout and found a significant result in vivo. Another approach is to apply probiotics as a feed additive: formalin-inactivated cells of *Aeromonas hydrophila* A3-51 against *A. salmonicida* in goldfish (Irianto et al. 2003); a combination of *Bacillus subtilis* and *B. licheniformis* (Bio-Plus2B) infection against *Yersinia ruckeri* provided better growth (Raida et al. 2003). Kim et al. (2010) showed that the administration of normal trout feed supplemented with *Zooshikella* strain JE-34, a bacterium from marine sediments, may help to control *Streptococcus iniae* infections and improve the innate immune system in olive flounder. These findings are in accordance with the findings of Kumar et al. (2006) (Table 1), who also obtained higher survivability in probiotics fed (*B. subtilis*) to rohu after *A. hydrophila* infection as the survivability of the infected fishes also increased after probiotic supplementation (Kumar et al. 2008; Kesarcodi-Watson et al. 2008). Recent studies using Biomin (2009) have

demonstrated that various probiotic strains exhibited antibacterial activities against several common fish pathogens, including *Enterococcus durans*, *Escherichia coli*, *Micrococcus luteus* and *Pseudomonas aeruginosa* (Biomin 2009).

Production of inhibitory compounds

Probiotic bacteria produce a variety of wide-spectrum chemical compounds including bacteriocins, siderophores, lysozymes, proteases and hydrogen peroxides, which are in the intestine of the host, thus constituting a barrier against the proliferation of opportunistic pathogens as well as alteration of the intestinal pH due to the production of organic acids (Strom-Bestor and Wiklund 2011; Korkea-aho et al. 2011, 2012; Perez-Sanchez et al. 2011; Oppegard et al. 2007; Martin-Visscher et al. 2009; Zai et al. 2009). Several researchers worked on various species of *Vibrio* (*V. mediterranei* 1, *V. mediterranei* 4 and *V. fluvialis* V. harveyi-VIB 571), which exhibit either antagonistic activity on solid agar medium against pathogenic *V. parahaemolyticus* and *V. mediterranei* or produce bacteriocin-like inhibitory substances (Carraturo et al. 2006; Prasad et al. 2005a, b). Other probiotic bacterial strains such as *Pseudomonas* M174, *Pseudomonas* M162 and *Pseudomonas* MSB1 also executed siderophore production and antagonistic activity against *Flavobacterium psychrophilum*, which causes hemorrhagic septicemia and meningoencephalitis particularly in rainbow trout (Korkea-aho et al. 2011, 2012; Strom-Bestor and Wiklund 2011). Zai et al. (2009) have isolated and identified 50 strains of the genus *Vibrio* to produce BLIS (bacteriocin-like inhibitory substances), called vibriocin AVP10, from the gills and gut region of healthy and infected catfishes (*Arius thalassinus*). Taoka et al. (2006a, b) showed that viable probiotics administered to tilapia *Oreochromis niloticus* increased the nonspecific immune response determined by parameters such as lysozyme activity, neutrophil migration and bactericidal activity, which improved the resistance of fish to infection by *Edwardsiella tarda*. Consecutively, Robertson et al. (2000) isolated a strain of *Carnobacterium* sp. from salmon bowel and administered it alive to rainbow trout and Atlantic salmon, demonstrating in vitro antagonism against known fish pathogens: *Aeromonas hydrophila*, *A. salmonicida*, *Flavobacterium psychrophilum*, *Photobacterium damsela* and *Vibrio* species.

Table 1 Probiotics used for biological control in aquaculture

Sl. no.	Probiotic strain	Source	Used on	Method of application	Observation	References
1.	Strain E (<i>Vibrio alginolyticus</i> -like)	Healthy turbot larvae	Turbot larvae	Enrichment of rotifer	Increased survival rates of turbot larvae when challenged with <i>Vibrio</i> strain P	Gatesoupe (1997)
2.	<i>Streptococcus lactis</i> and <i>Lactobacillus bulgaricus</i>	Unknown	Turbot larvae	Enrichment of live food	Enrichment of rotifers and Artemia	Garcia et al. (1997)
3.	<i>Vibrio pelagius</i>	Copepod-fed turbot larvae	Turbot larvae	Addition to culture water	Decreased mortality of turbot larvae challenged with <i>A. caviae</i>	Ringo and Vadstein (1998)
4.	Microbially matured water	Unknown	Turbot larvae	As culture water	Growth rate improved	Skjermo et al. (1997)
5.	<i>Carnobacterium divergens</i> (lyophilized)	Atlantic salmon intestine	–	Addition to diet	–	Gildberg et al. (1995)
6.	<i>Carnobacterium divergens</i> (lyophilized)	Atlantic salmon intestine	Atlantic cod	Addition to diet	Decrease of mortality of fry challenged with <i>V. anguillarum</i>	Gildberg et al. (1997)
7.	<i>Carnobacterium</i> bacterium strain K1	Atlantic salmon intestine	–	Unknown	Growth inhibition of <i>V. anguillarum</i> and <i>A. salmonicida</i> in fish intestinal mucus and fecal extract (both in vivo and vitro)	Joborn et al. (1997)
8.	<i>Vibrio alginolyticus</i>	Commercial shrimp hatchery	Atlantic salmon (<i>Salmo salar</i> L.)	Bathing in bacterial suspension	Decrease of mortality of juveniles challenged with <i>A. salmonicida</i> , <i>V. anguillarum</i> and <i>V. ordalii</i>	Austin et al. (1995)
9.	<i>Bacillus megaterium</i> , <i>B. subtilis</i> , <i>B. polymyxa</i> , <i>B. licheniformis</i>	Commercial product (Biostart)	Channel catfish	Addition to pond water	Improved growth rate and survival of catfish	Queiroz and Boyd (1998)
10.	<i>Vibrio pelagius</i>	Turbot larvae	Turbot	Addition to culture water	Improve larval survival when fish exposed to <i>A. Caviae</i>	Ringo and Vadstein (1998)
11.	<i>G-probiotic</i>	Commercial product	Oreochromis niloticus	Addition to diet	–	Naik et al. (1999)
12.	<i>Pseudomonas fluorescens</i>	Iced freshwater fish (<i>Lates niloticus</i>)	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Addition to culture water	Growth rate improved and 46 % reduction in mortality	Gram et al. (1999)
13.	<i>Carnobacterium</i> sp.	Intestines of Atlantic salmon	Atlantic salmon	Addition to diet	Antagonism against <i>Aeromonas hydrophila</i> , <i>A. salmonicida</i> , <i>Flavobacterium psychrophilum</i>	Robertson et al. (2000)
14.	<i>Lactobacillus rhamnosus</i> ATCC 53103	Culture collection	Rainbow trout	Addition to diet	<i>Rhannosus</i> ATCC 53103; <i>L. bulgaricus</i> can be used as a novel and safe treatment in aquaculture	Nikoskelainen et al. (2001)

Table 1 continued

Sl. no.	Probiotic strain	Source	Used on	Method of application	Observation	References
15.	<i>Aeromonas hydrophila</i> , <i>Vibrio fluvialis</i> , <i>Carnobacterium</i> sp., <i>Micrococcus luteus</i>	Digestive tract of rainbow trout	Rainbow trout	Addition to diet	Enhanced pathogen-resistant and lysozyme activity in the fish	Irianto and Austin (2002a, b)
16.	<i>Enterococcus faecium</i> SF68	Commercial product (Cernivet)	Eel (<i>Anguilla anguilla</i>)	Addition to diet	Increased the survival rates of eels	Chang and Liu (2002)
17.	<i>L. rhamnosus</i> JCM 1136	Culture collection	Rainbow trout	Addition to diet	Potential immune-regulatory role of probiotic organisms in rainbow trout	Panigrahi et al. (2004)
18.	<i>Roseobacter</i> sp. strain 27-4	Turbot larvae, Tetraselmis copepod-fed larvae	Turbot larvae	Addition to culture water	Mortality of larvae decreased	Hjelm et al. (2004)
19.	<i>Bacillus circulans</i>	Intestines of <i>Labeo rohita</i>	<i>L. rohita</i>	Addition to diet	Better growth and survival of rohu spawn	Ghosh et al. (2004)
20.	<i>Bacillus subtilis</i>	Chicken intestine	<i>Macrobrachium rosenbergii</i>	Added to feed	Increased the survival rate of prawns against <i>Aeromonas hydrophila</i>	Mehran and Masoumeh (2012)
21.	<i>Enterococcus faecium</i> MC13	<i>Mugil cephalus</i>	<i>Cyprinus carpio</i>	Injection and oral administration	Higher protection against <i>Aeromonas hydrophila</i>	Ayyaru and Venkatesan (2011)
22.	<i>B. subtilis</i> strains L10 and G1	–	White shrimp (<i>Litopenaeus vannamei</i>)	Added to commercial feed	Upregulation of the genes related to secondary defense mechanism and immune-related genes	Zokaeifar et al. (2012)
23.	<i>Shewanella putrefaciens</i> (Pd11 strain)	Skin mucus of gilthead seabream	<i>Solea senegalensis</i>	Added to commercial feed	Conferred protection against <i>P. damsela</i> sub Piscicida	Ines et al. (2012)
24.	<i>Bacillus subtilis</i> and <i>Lactobacillus acidophilus</i>	–	Nile tilapia (<i>Oreochromis niloticus</i>)	Added to feed	Increase in bactericidal activity, hematocrit values and lysozyme activity	Salah Mesalhy et al. (2008)
25.	<i>Lactobacillus</i> spp. strain 3RM	Dairy products	<i>Cyprinus carpio</i>	Added to feed	Enhanced growth and survival rate of fish	Vignesh et al. (2011)
26.	<i>B. subtilis</i> , <i>L. lactis</i> and <i>S. cerevisiae</i>	–	<i>Labeo rohita</i>	Added with basal feed	Higher growth, protein efficiency ratio, digestibility and lower FCR	Mohapatra et al. (2012)
27.	<i>Bacillus subtilis</i> and vitamin C in the form of ascorbyl polyphosphate	–	<i>Labeo rohita</i>	Added with basal feed	Respiratory burst activity of blood neutrophils and high antibody level	Nayak et al. (2007)
28.	<i>Bacillus subtilis</i> C-3102	Commercial product (Calsorin®)	Koi carp (<i>Cyprinus carpio</i>)	Added with basal feed	Upregulation of HSP70 and cytokines gene	He et al. (2011)

Table 1 continued

Sl. no.	Probiotic strain	Source	Used on	Method of application	Observation	References
29.	<i>Lactobacillus lactis</i> h ₂ L, <i>raffinolactis</i> h47, Ent, <i>pseudovarium</i> h50	Adult common carp intestine	–	–	Probiotics show cholic acid resistance and antimicrobial activity	Hagi and Hoshino (2009)
30.	<i>Pediococcus pentosaceus</i> and <i>Staphylococcus hemolyticus</i>	Gut of wild brown shrimp (<i>Farfantepenaeus californiensis</i>)	Whiteleg shrimp (<i>Litopenaeus vannamei</i>)	Sprayed on commercial feed	Total blood count and survival against WSSV and IHNV diseases	Karla et al. (2011)
31.	<i>Lactobacillus sporogenes</i>	–	<i>C. batrachus</i>	Study against <i>Aeromonas hydrophila</i>	Antagonism effects against pathogenic bacterium, <i>A. hydrophila</i>	Dahiya et al. (2012a, b)
32.	<i>Anoxybacillus</i> , <i>Leuconostoc</i> , <i>Clostridium</i> , <i>Actinomyces</i> and <i>Citrobacter</i>	From the gut of grass carp (<i>Ctenopharyngodon idellus</i>)	–	–	Manage and improve the microbial community in fish intestine	Wu et al. (2012)
33.	<i>Bacillus</i> spp. strain NM 12	Intestine of the dragonets (<i>Callionymus</i> spp.)	–	–	Antibacterial ability against <i>Vibrio vulnificus</i> RIMD 2219009	Sugita et al. (1998)
34.	<i>Bacillus</i> NL110 and <i>Vibrio</i> NE17	Larvae and egg samples of <i>Macrobrachium rosenbergii</i>	Juveniles of <i>M. rosenbergii</i> (0.080–0.001 g)	Feed, water and both	Improvements in water quality, growth, survival, SGR, FCR and other immune parameters	Rahman et al. (2010)
35.	<i>Vagococcus fluvialis</i>	Gilthead sea bream (<i>S. aurata</i>), sea bass (<i>D. labrax</i>)	–	–	Probiotics showed good protection against <i>Vibrio anguillarum</i> 975-1 (in vivo)	Sorrosa et al. (2012)
36.	<i>Bacillus subtilis</i> , <i>Bacillus egaterium</i>	–	<i>Litopenaeus vannamei</i>	Basal diet plus probiotics	Stress tolerance	Olmos et al. (2011)
37.	<i>Pseudomonas</i> M174	Rainbow trout eggs at 0, 1, 10 and 20 days postfertilization	Rainbow trout	Fish bathing	Shows potential probiotic against <i>Flavobacterium psychrophilum</i>	Korkea-aho et al. (2011)
38.	<i>Lactobacillus rhamnosus</i> GG	From human origin	Nile tilapia (<i>Oreochromis niloticus</i>)	Bacterial pellets	Higher feed absorption and lymphocyte count	Pirarat et al. (2011)
39.	Genera <i>Lactobacillus</i> , <i>Lactococcus</i> and <i>Leuconostoc</i>	Healthy rainbow trout weighing 35–40 g	Rainbow trout	–	Probiotics strains shows good adherence, pathogen antagonism	Perez-Sanchez et al. (2011)
40.	LAB and yeast	Protexin (Probiotics International Ltd., Lopen Head, Somerset, TA13 5JH, UK)	Ornamental fish fingerlings	Mixed with basal feed	Significant boost in total blood count	Firrouzbakhsh et al. (2011)

Table 1 continued

Sl. no.	Probiotic strain	Source	Used on	Method of application	Observation	References
41.	<i>Pseudomonas</i> sp. MSB1	Rainbow trout	–	In vivo	Produced by siderophores	Strom-Bestor and Wiklund (2011)
42.	<i>Lactobacillus farcininis</i> CNCM MA27/6R and <i>Lactobacillus rhannosus</i> CNCM MA27/6B	From dairy products.	–	Heat-killed probiotic mixed with basal feed	Increased egg production and hatching success	Drillet et al. (2011)
43.	<i>Bacillus</i> spp. Quorum-Sensing inhibitor (QSI)-1	Intestine gut of <i>Carassius auratus gibelio</i>	–	<i>Carassius auratus gibelio</i>	Good survival, protection against <i>A. hydrophila</i>	Chu et al. (2011)
44.	<i>Pseudalteromonas salina</i>	Traditional fermented Korean foods	Swimming crab, Zoea	In culture water	Mortality rate reduced	Morya et al. (2013)
45.	<i>Vibrio harveyi</i>	Moribund abalones	–	In vitro gill cell culture	Significant changes in gill cells metabolism and immune response	Pichon et al. (2013)
46.	Customized probiotics (<i>Bacillus mycooides</i> , <i>Shewanella</i> sp., <i>Bacillus subtilis</i>)	–	<i>Cherax tenuimanus</i>	Feed supplement	Immune responses improved against <i>Vibrio mimicus</i>	Ambas et al. (2013)
47.	<i>Shewanella colwelliana</i> WA64; <i>S. olleyana</i> WA65	Commercial product	Abalone	Feed preparation	Immunity and mortality rate reduced against <i>V. harveyi</i> infection	Jiang et al. (2013)
48.	Heat-killed LAB	Mongolian dairy products	Japanese pufferfish (<i>Takifugu rubripes</i>)	In vitro tissue culture	Proinflammatory cytokines genes copy number increased	Biswas et al. (2013)
49.	Inulin and <i>Bacillus subtilis</i>	–	Gilthead sea bream (<i>Sparus aurata</i> L.)	Feed diet preparation	Immunity improved against gut edema	Cerezuela et al. (2013)
50.	<i>Lactobacillus plantarum</i> VSG3	<i>Labeo rohita</i> Gut content	<i>L. rohita</i>	Fed diet preparation	Growth rate, disease resistance improved	Giri et al. (2013)

There is also evidence for the effect of dead probiotic cultures, consisting of a mixture of *Vibrio fluvialis* A3-47S, *Aeromonas hydrophila* A3-51 and *Carnobacterium* BA211, on the control of furunculosis in rainbow trout. For this specific case, the number of leukocytes was greater than with live cells; in fact, the data suggested that cellular immunity more than humoral factors was involved in the benefits of these preparations of inactivated bacterial cells (Irianto and Austin 2003). In shrimp, studies have focused on the evaluation of probiotics such as *Bacillus cereus*, *Paenibacillus polymyxa* and *Pseudomonas* sp. PS-102 as biocontrol agents against pathogens of various *Vibrio* spp. (Ravi et al. 2007; Vijayan et al. 2006). Probiotic strains isolated from the gastrointestinal tract of clownfish (*Amphiprion percula*) have been used to inactivate pathogens such as *Aeromonas hydrophila* and *Vibrio alginolyticus*, existing among different fish species. It has been observed that probiotics in vivo generate a density that allows the production of antimicrobial metabolites; therefore, the bacteria isolated from adult clownfish have the potential to colonize the intestinal mucus and therefore can be used as prophylactic and therapeutic agents (Vine et al. 2004). Furthermore, it has been found that concentrations of 106–108 cells per gram of probiotic promoted the development of healthy microbiota in the GIT of ornamental fishes from the genera *Poecilia* and *Xiphophorus*, decreasing the amount of heterotrophic microorganisms (Ghosh et al. 2008). Peraza-Gomez et al. (2009) reported that use of *Vibrio alginolyticus* strain as a probiotic increased the survival and growth of white shrimp (*Litopenaeus vannamei*) in Ecuadorian shrimp hatcheries, where the production increased by 35 %, while with the use of antimicrobials production was decreased by 94 %. Furthermore, Perez-Sanchez et al. (2011) identified and characterized genera of lactic acid bacteria including *Lactobacillus*, *Lactococcus* and *Leuconostoc*, which were observed to generate lactic acid that was endowed with antimicrobial activity against *Lactococcus garvieae*, a causative agent for hemorrhagic septicemia and meningoencephalitis in several species of fish (Martin-Visscher et al. 2009; Munoz-Atienza et al. 2013).

Growth-promoting effects

Indigenous microbiota are being recognized to have a prominent effect on the structure, function and

metabolism of the digestive tract of aquatic animals, which are needed to sustain the normal physiological functions of an organism, and serve as a source of nutrients, vitamins, enzymes, microbial breakdown of chitin, *p*-nitrophenyl-*N*-acetyl-beta-D-glucosamine cellulose, and collagen (Ringo et al. 1995; Boyd and Gross 1998). In fact, it is still not clear whether feed probiotics increased the appetite or nature itself improved the digestibility, causing the improved appetite. Researchers are inclined to think that it could be both factors; furthermore, it would be important to determine whether probiotics actually taste good to aquaculture species (Irianto and Austin 2002a, b). Studies showed that intestinal anaerobic bacteria can speed up the digestive process, which was corroborated by the better growth of fish and shellfish because of providing a variety of extracellular enzymes, proteases, lipases, carbohydrases, phosphatases, esterases, lipases and peptidases, which facilitated the efficient absorption of nutrients (Hood et al. 1971; Ramirez and Dixon 2003). Prominent levels of enzymes such as cellulase and amylase in pinfish (*Lagodon rhomboids*), ayu (*Plecoglossus altivelis*), carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*), Japanese eel (*Anguilla japonica*), *Cherax tenuimanus* and tilapia (*Oreochromis niloticus*) showed the differences in growth of the above fishes (Luczkovich and Stellwag 1993; Sugita et al. 1997; Ambas et al. 2013; Morya et al. 2013). In general, the enzymatic degrading ability of gut bacteria for *p*-nitrophenyl-*b*-*n*-acetylglucosaminide, chitin, cellulose and collagen indicated their possible involvement in the nutrition and growth promotion of fish (Shcherbina and Kazlawlene 1971; Lindsay and Harris 1980; Lesel et al. 1986; Macdonald et al. 1986; Das and Tripathi 1991; Kar and Ghosh 2008; Giri et al. 2013). In some reports, probiotics were found to enhance bio-growth parameters, and improved the survival and growth of halibut larvae meticulously (Robertson et al. 2000; Bjornsdottir et al. 2010).

The effects of probiotics were experienced in phytoplankton (microalgae), which form the basis of aquatic food chains (Paiva-Maia et al. 2013) because of its nutrient-producing photosynthetic mechanisms; in most cases, higher organisms were unable to synthesize polyunsaturated fatty acids and vitamins (Bonnet et al. 2010; Tang and Suter 2011). Gomez-Gil et al. (2002) assessed the possibility of co-culturing the shrimp probiotic C₇b in the presence of the shrimp larvae food

Chaetoceros muelleri, without affecting microalga. This study proposed that these organisms can be grown together to achieve a high density and fed to shrimp. Rotifers are indispensable as the first live feed for larvae of most cultured aquatic species because of their small size and inherent nutritional dominance compared to copepods (Busch et al. 2011; Nordgreen et al. 2013). Sayed et al. (2011) experimented on Nile tilapia fingerlings (*Oreochromis Niloticus*) with Super Bio-buds, Bio-yeast and Stop stress gold at different levels. The results of the study indicated that Nile tilapia fingerlings fed with probiotics had better growth. Lara-Flores et al. (2003) carried out experiments on the Nile tilapia (*Oreochromis niloticus*) diet, manipulated with a probiotic *Streptococcus* strain, significantly increasing the crude protein and crude lipid contents in the fish; also the weight increased from 0.154 to 6.164 g in 9 weeks of culture. Due to the commercial importance of this species, the effect of supplementing the diet with probiotics produced an increase of 115.3 % when a commercial formulation was used at a concentration of 2 % (El-Haroun et al. 2006). Probiotics have been applied to ornamental fishes as well. Dharmaraj and Dhevendaran (2010) and Ghosh et al. (2008) evaluated the growth performances of ornamental fishes exclusively [swordtail (*Xiphophorus helleri*, *Xiphophorus maculatus*) and guppy (*Poecilia reticulata*, *Poecilia sphenops*)]; their feed was supplemented with *Streptomyces* sp. and *Bacillus subtilis*. The study revealed a boost in the growth and survival of *Xiphophorus* and *Poecilia* after 90 and 50 days of administration, respectively, and could prove the efficacy of probiotics in the growth of the above-mentioned ornamental fish (Ghosh et al. 2008; Dharmaraj and Dhevendaran 2010). Macey and Coyne (2005) also formulated a diet with a mixture of three putative probiotics, two yeasts and one bacterial strain designated SS1, AY1 and SY9, isolated from the digestive tract of abalone (*Haliotis midae*). Each probiont was added to the feed to achieve a final concentration of approximately 107 cells/g of dry feed. In abalone the growth rate of 20 mm and 67 mm was improved by 8 and 34 %, respectively, in 8-month cultures. Furthermore, abalones supplemented with probiotics had a survival rate of 62 % when confronted with the pathogenic bacterium *Vibrio anguillarum* compared to the 25 % survival of untreated animals.

Numerous studies have shown that the application of probiotics can improve the feed conversion, growth

rate and weight gain of carp, salmonids and other fish (Taoka et al. 2006a, b; Bagheri et al. 2008; Wang et al. 2008). Prevalent applications of the probiotics have also shown a promising improvement in the growth of shellfish. Probiotics are used to reduce the incidence and severity of diseases caused by *Vibrio alginolyticus* in *Penaeus vannamei* larvae (Garriques and Arevalo 1995) and led to a significant improvement in the FCR, FER and PER of shrimp larvae fed with *L. plantarum* bioencapsulated Artemia, in harmony with observations made by Suralikar and Sahu (2001). Uma et al. (1999) also obtained the same results when probiotic *L. cremoris* was fed to post larvae of *M. rosenbergii*. In addition, immobilized *Lactobacillus sporogenes* administration was also found to accelerate the growth rate and food utilization of *Labeo rohita* fingerlings as compared to its direct suspension in water or feed (Priya 2006; Jayalakhmi 2006).

Immune system of fish and shellfish

Fish are a heterogeneous group divided into different classes (Zapata et al. 1996). The emergence of new taxonomic categories coincided with the diversification of the immune response. Apparently, vertebrates inherited innate immunity from their invertebrate ancestors (Flajnik and Du Pasquier 2008). However, fish belong to lower vertebrates that have a primitive type of adaptive immune system, and for adaptive immunity fish use a system of receptor proteins including polysaccharides, lipopolysaccharide (LPS), peptidoglycan bacterial DNA, viral RNA and other molecules that are not normally on the surface of multicellular organisms (Uribe et al. 2011). However, generative and secondary lymphoid organs in mammals are also found in fish, except for lymphatic nodules and bone marrow (Zapata et al. 1996). The defense mechanisms of crustaceans are less developed than those in finfish and other vertebrates as they do not have the ability to produce immunoglobulins, so they apparently depend only on the innate defense system of phenol oxidase enzymes (Roch 1999; Gonzalez-Santoyo and Córdoba-Aguilar 2012). Keeping this in view, Rodriguez and Le Moullac (2000) elucidated the application of immunological tools to evaluate those of an inherent health marker, i.e., Po and ROS, and the clinical significance of responses in penaeid shrimp.

Stimulation of host immunity

Probiotics produce transduction signaling molecules that have the ability to alert the immune system against assaults by pathogenic agents (Rendón and Balcazar 2003) and specific diseases such as gut edema (Cerezuola et al. 2013). These immunostimulants can be applied by immersion and injection; however, the most practical method for the administration of these immunostimulating substances is inclusion in feed. Various studies revealed that probiotic bacteria, commercial probiotics, their supplementation in feed or any sort of inclusion can boost the cellular and humoral component of the innate immune system in several types of fish and shellfish including salmonids and shrimps (Panigrahi et al. 2004; Cerezuola et al. 2013; Goncalves et al. 2011; Song et al. 2006; Rodríguez et al. 2007; Rengpipat et al. 2000; Balcazar et al. 2004; Gullian et al. 2004; Pais et al. 2008; Meena et al. 2013a, b; Biswas et al. 2013). Whether invertebrates are able to mount an immune response with some of the attributes of the vertebrate immune system, viz. memory and specificity, has been a huge matter of debate till now. There was disagreement concerning the existence of any form of immunological memory such as 'specific immune priming' (Watson et al. 2005; Dong et al. 2006; Powell et al. 2011; Roth and Kurtz 2010). Alternatively, in some studies heterogeneous expression of probiotic bacteria in Nile tilapia (*Oreochromis niloticus*) and other fish could increase the existing level of intraepithelial lymphocytes and acidophilic granulocytes, serum lysozyme activity and serum bactericidal activity, which were in accordance with having specificity in fish (Sorroza et al. 2012; Pirarat et al. 2011; Sharma et al. 2013; Meena et al. 2013a, b). Vieira et al. (2010) evaluated the effect of a probiotic-supplemented diet on marine shrimp survival after challenge with *Vibrio harveyi*. The study results showed that the total hemocyte count and serum agglutination activity were higher ($p > 0.05$) in the probiotic-supplemented group after coming in contact with *V. harveyi*. Results showed that the probiotic-fortified diet modified the bacterial microbiota of the shrimp digestive tract, thus increasing resistance to *V. harveyi* infection. Sayed et al. (2011) evaluated the effects of some probiotics on the growth performance, physiological measurements and immune response of Nile tilapia (*Oreochromis niloticus*) fingerlings. The experimental fish were fed nine diets

supplemented with 0.05, 0.10 and 0.15 % Super Biobuds, Bio-yeast and Stop stress gold at 0.5, 1.0 and 1.5 g/kg diet and T_0 as a control group for 70 days. The results indicated that fish groups fed on diets supplemented with probiotics revealed a significant increase in body weight gain and concentrations of serum protein, globulin and enhancing immune responses detected with in vitro phagocyte activity tests. Diaz-Rosales et al. (2009) studied the effects of the dietary administration of two closely related probiotics, *Shewanella putrefaciens* Pdp11 and *Shewanella baltica* Pdp13, on the immunological responses of Senegalese sole (*Solea senegalensis*) and the survival of fish challenged with *Photobacterium damsela* subsp. Piscicida. Respiratory burst activity of fish fed with the Pdp11 diet significantly increased after 60 days of feeding, while this significant increase was not detected in phagocytes from fish fed control and Pdp13 diets. Alternatively, the cumulative percentage of mortality after the challenges with *P. damsela* piscicida was 100 % in the groups fed with control diet, whereas mortality rates observed in the groups fed with diets supplemented with Pdp11 and Pdp13 ranged from 75 to 100 and 65 to 80 %, respectively. These results suggested that an increased respiratory burst activity of the phagocytes was not essential to heighten the protection against the *P. damsela* subsp. Piscicida, and both probiotics improved the growth and the survival against the pathogen in comparison with those fish being fed the control diet. Moreover, Korkea-aho et al. (2012) evaluated the antagonistic effect of *Pseudomonas* M162 against rainbow trout fry syndrome caused by *Flavobacterium psychrophilum*. The results showed that *Pseudomonas* M162 inhibited the growth of *Fl. psychrophilum* in vitro and increased the resistance of the fish against the pathogen, resulting in a relative 39.2 % percent survival. However, the siderophores produced by M162 did not have an inhibitory effect on *Fl. psychrophilum*. In fish fed with M162, the probiotic colonized the gastrointestinal tract and stimulated peripheral blood leucocyte counts, serum lysozyme activity and total serum immunoglobulin levels 3 weeks after the start of feeding. Korkea-aho et al. (2011) used a different strain of *Pseudomonas* M174 against infection in rainbow trout (*Oncorhynchus mykiss*). The results showed that M174 is a potential probiotic against *Fl. psychrophilum* and had several modes of action as compared to the *Pseudomonas* M162 strain.

Antibacterial activity

Probiotics improve the intestinal microflora, which have antagonistic properties, because of the formation of organic acids and bacteriocins (Ringo et al. 2010, 2012); they alter the metabolism of microbiota to produce short-chain fatty acids, increase sodium and water absorption, decrease colonic motility (Sakata et al. 1999) and support the host's good health, providing protection against infections by stimulating the immune system, alleviating lactose intolerance, reducing blood cholesterol levels, and improving weight gain and the feed conversion ratio (Lara-Flores and Aguirre-Guzman 2009; Lara-Flores 2011). The proliferation site of fish pathogens and the mechanisms of antagonism by a probiotic culture influence the choice of a probiotic bacterium (Mette et al. 2004). Probiotic bacteria isolated from the gut would appear promising if the targeted pathogenic bacteria infected through the gastrointestinal tract. However, some fish pathogens may proliferate on the skin surface (Esteban 2012), and probiotic bacteria adapted to the outer surfaces could limit pathogen proliferation. Moriarty (1998) suggested that probiotic cultures could also originate from the general rearing environments; since *Bacillus* spp. generally dwell in the sediment from which shrimps feed, a commercialized *Bacillus* product was added to it and successfully prevented infection by pathogenic vibrios (Moriarty 1998). Marco-Noales et al. (2004) recently demonstrated that levels of *Vibrio vulnificus* in water were suppressed by the presence of other bacteria. Therefore, it is plausible that health-beneficial organisms in fish-rearing systems may be found in several other niches than the fish itself. Lactic acid bacteria are normal flora in the GI tract of healthy animals such as mammals and aquatic animals (Wu et al. 2012); they have probiotic properties with no harmful effects, except in some reports from maricultures in Japan and North America (Eldar et al. 1996; Munoz-Atienza et al. 2013). However, a novel *Weissella* species has been portrayed as an opportunistic pathogen for rainbow trout (Liu et al. 2009a, b). LABs are widely known for their ability to inhibit bacterial pathogens by the production of antimicrobial compounds: organic acids, hydrogen peroxide and ribosomally synthesized peptides referred to as bacteriocins (Gatesoupe 2008; Desriac et al. 2010). Moreover, Lactobacilli ferment lactose to lactic acid, thereby reducing the pH to a level that

harmful bacteria cannot tolerate. Hydrogen peroxide is also produced, which inhibits the growth of gram-negative bacteria. It has also been reported that lactic acid-producing bacteria of *Streptococcus* and *Lactobacillus* sp. produced antibiotics (Klose et al. 2010; Zoumpopoulou et al. 2013). Antimicrobial activity against fish pathogens and in vitro safety of 99 LAB previously isolated from fish, seafood and fish products have been tested recently (Munoz-Atienza et al. 2013). Zapata and Lara-Flores (2013) found that *Leuconostoc mesenteroides* showed more ability to inhibit the growth of fish pathogenic bacteria compared to others and determined that probiotic bacteria can possibly be used in aquaculture.

Antiviral activity

Besides being beneficial bacteria, probiotics also possess antiviral activity. Although vaccination is an age-old practice for controlling viral diseases, its success rate is highly variable, and the duration of immunity against viruses is disputed (McLoughlin and Graham 2007). Exploitation of these probiotic bacteria in the treatment and prevention of viral diseases in aquaculture, particularly in shrimp, is a comparatively novel and efficient method (Lakshmi et al. 2013). By and large, few probiotics have antiviral effects, and the exact mechanism is not yet known (Mohapatra et al. 2012). Strains of *Pseudomonas*, *Vibrio*, *Aeromonas* spp. and groups of *Coryneforms* showed antiviral activity against infectious hematopoietic necrosis virus (IHNV) (Kamei et al. 1988). In shrimps, antiviral immune responses are mediated through the pattern recognition receptors (PRRs), and to date 11 PRPs have been identified in shrimps (Xian-Wei and Jin-Xing 2013). These PRRs are known to trigger effective and appropriate antiviral responses, including the production of various cytokines and induction of inflammatory and adaptive immune reactions (Kawai and Akira 2006). Moreover, feed supplementation with a *B. megaterium* strain has resulted in increasing resistance to white spot syndrome virus (WSSV) in the shrimp *Litopenaeus vannamei* (Li et al. 2009). The viral proteins VP68, VP281 and VP466 were documented to play a very significant role in WSSV infection (Wu et al. 2005; Xu et al. 2007; Liu et al. 2009a, b). Another mechanism in the antiviral immune response is the RNA interference (RNAi) method, which has been applied to silence viral genes

in eukaryotic organisms. It readily silenced the transcription and translation of the viral gene and significantly reduced the mortality rate of the shrimp (Xu et al. 2007).

Antifungal activity

Very few studies are being performed on this aspect particularly in aquaculture. To date, isolated *Aeromonas* media (strain A199) from fresh water in eel culture (*Anguilla australis* Richardson) have offered antagonistic activity against *Saprolegnia* sp. (Lategan et al. 2004). Recently, Heikkinen et al. (2013) reported that utilization of protective bacterial cultures, *Pseudomonas* sp. M162, *Pseudomonas* sp. M174 and *Janthinobacterium* sp. M169, enhanced immunity against saprolegniasis, and the mode of probiotic action was to evoke immunostimulatory effects and siderophore production. *Pseudomonas* sp. M162 also decreased *Flavobacterium psychrophilum*-related mortality, while the probiotic effect resulted mainly from immunostimulation. Atira et al. (2012) assessed the curative action of *Lactobacillus plantarum* FNCC 226 on *Saprolegnia parasitica* A3 in catfish (*Pangasius hypophthalmus*) and reported a decrease in *S. parasitica* A3 infection coinciding with the increment of *L. plantarum* FNCC 226 blockage. This study revealed that *L. plantarum* has the capacity to inhibit *S. parasitica* and that it would be possible to design new biocontrol of this pathogen in catfish. Moreover, Goulden et al. (2012) evaluated the potential of multispecies probiotic treatment and demonstrated that natural microbial communities associated with wild phyllosomas and zooplankton prey support antagonistic bacteria capable of in vivo suppression of a pathogen causing epizootics in phyllosoma culture systems. Ali et al. (2013) isolated probiotic strains from different fermented cheese products, which have been reported to show antifungal activity against the plant pathogens *Rhizoctonia solani* and *Fusarium oxysporum*.

Siderophore production

Siderophores are biosynthetically produced and secreted by many bacteria, yeasts, fungi and plants; they scavenge for ferric iron Fe^{3+} and have an extremely high affinity for binding this trivalent metal

ion (Chu et al. 2010). The iron metabolism plays an exceptionally important role in bacterial infections in fish (Neves et al. 2009). Because levels of free ferric iron in biological systems are always extremely low, there is serious competition for iron and ferric siderophores between the pathogenic bacteria and animal host. The molecular basis for the regulation of iron, which is accomplished by the interaction of several genes including the iron transporter transferrin and iron storage protein ferritin, was reported by Neves et al. (2009). Korkea-aho et al. (2012) confirmed that *Pseudomonas* M162 enhanced immunity against infection in rainbow trout (*Oncorhynchus mykiss*) in iron-rich and -deficit environments. The inhibitory effect of probiotic bacteria on fish pathogens has been demonstrated under iron-depleted conditions, resulting in the production of siderophores by the probiotic bacteria in the absence of iron (Gram et al. 2001; Spanggaard et al. 2000). Siderophores are high-affinity iron acquisition molecules produced by bacteria, giving them a competitive advantage in iron-scarce environments (Miethke and Marahiel 2007). One of the probable modes of action of *Pseudomonas* spp. is thought to be through their ability to produce siderophores (Strom-Bestor and Wiklund 2011).

Modulation in antioxidant enzyme activity and prevention of amine synthesis

The larval stages are critical because of the high energy consumption and possible saturation of the antioxidant defenses (Sole et al. 2004). Aubin et al. (2005) revealed that the imputation of the effects of dietary yeast in sea bass larvae was thus easier than in rainbow trout fry, where autochthonous *D. hansenii* was naturally present. In sea bass larvae, dietary *D. hansenii* promoted their growth and gut maturation, possibly by the influence of endoluminal yeast-secreted polyamines as has been observed previously (Tovar-Ramirez et al. 2004). Guzman-Villanueva et al. (2007) also observed that the larvae group of the spotted sand bass, *Paralabrax maculatofasciatus*, fed *D. hansenii* in their feed had non-inhibition of ornithine decarboxylase (ODC) activity and had precocious digestive maturation compared to *P. maculatofasciatus* larvae fed ODC-inhibited yeast with α -difluoromethylornithine. Ornithine decarboxylase, which catalyzed the formation of putrescine,

was the rate-limiting enzyme in the biosynthesis of polyamines in cells.

Tovar-Ramirez et al. (2010) found that dietary probiotic live yeast modulated the antioxidant enzyme activities and gene expression of sea bass (*Dicentrarchus labrax*) larvae. Moreover, coliform bacteria decarboxylate amino acids to produce amines, which irritate the gut; they are toxic and cause diarrhea. If desirable bacteria prevent coliform proliferation, then amine production will also be prevented (McDonald et al. 2002; Ige 2013). Probiotics may alter the metabolism of the hindgut bacterial ecosystem to increase production of short chain fatty acids and other organic acids and decrease production of ammonia and isovaleric acid; this is very likely done by increasing the breakdown of hard-to-degrade carbohydrates, thereby reducing the net breakdown of protein. Such changes in bacterial metabolism seem to be responsible for the antidiarrheic actions of probiotic preparations (Modesto et al. 2009; Ige 2013).

Prevention of colonization provoked by pathogenic microorganisms

Bacterial antagonism is a common phenomenon in nature (Qi et al. 2009); therefore, microbial interactions play a major role in the equilibrium between competing beneficial and potentially pathogenic microorganisms. However, the composition of microbial communities can be altered by husbandry practices and environmental conditions that stimulate the proliferation of selected bacterial species. Adhering to adhesion sites along the wall of the gut is an important colonization factor, and many intestinal pathogens rely on adhesion to the gut wall to prevent being swept away by the peristaltic movement of food along the intestinal tract (Denev et al. 2009). Hence, an important function of these probiotic bacteria is to prevent or limit the growth and colonization of potentially pathogenic bacteria such as *E. coli*, *Salmonella*, *Listeria*, *Campylobacter* and *Clostridia* within the gut (Pandiyar et al. 2013). Where the gut microflora is well balanced, the beneficial microorganisms colonized within the gut can help to reduce the risk of pathogenic challenge. Detrimental bacteria, such as *E. coli*, need to become attached to the gut wall to exert their harmful effects. Attachment is achieved by means of hair-like structures, called fimbriae, on the bacterial surface

(McDonald et al. 2002). The fimbriae are made up of proteins, called lectins, that recognize and selectively combine with specific oligosaccharide receptor sites on the gut wall. Lactobacilli successfully compete for these attachment sites (Barth et al. 2009; Lalles et al. 2007; Ringo et al. 2010, 2012; Gatesoupe 1999). The ability to inhibit pathogen adhesion appears to depend on the specific probiotics and pathogens and on the mucosal site (Collado et al. 2007). Probiotics were attributed antagonistic properties against harmful bacteria entering the intestinal tract (Ige 2013). The first step of intestinal infections is mediated by the adhesion of pathogenic bacteria to mucosal surfaces and disruption of the microbiota. The protective role of probiotic bacteria might be mediated through adhesion and colonization of the mucosal simulation (Cruz et al. 2012). The ability to adhere to the intestinal mucosa is considered an important prerequisite for microorganisms intended for probiotic use; this is related to many of the health benefits attributed to probiotics (Collado et al. 2007; Vendrell et al. 2008; El-Haroun et al. 2006). The gastric pathogen has to overcome or colonize the mucus layer in order to attach, interact with and infect the host epithelium (Merrifield et al. 2010a). The intestinal mucosal barrier, including the epithelial cells, tight junctions controlling the paracellular pathways and a superficial mucous layer, forms an effective physical barrier that separates the individual from the complex microbial populations that constitute the normal intestinal microflora (Ringo and Birkbeck 1999; Ringo et al. 2012). The ability of some of probiotics to adhere to intestinal mucus may block the intestinal infection route common to many pathogens (Ringo et al. 2010; Gatesoupe 1999). The interrelationship among gut mucosal epithelial cells, mucus, antimicrobial products, commensal organisms resident in the gut and immune cells in the mucosa or submucosa was found to be vital for the health and well-being of the fish (Ringo et al. 2012). Thus, microbiota stimulated intestinal epithelial proliferation and influenced enterocyte morphology (Merrifield et al. 2010c).

Competitive segregation for essential nutrients, enzymes and energy

Inside the gut, beneficial as well as pathogenic microorganisms utilize the same types of nutrients

Table 2 Feed probiotics used in aquaculture

Sl. no.	Identity of the probiotics (applied in feed)	Species	Effects	Reference (s)
1.	<i>Bacillus subtilis</i> and <i>Bacillus licheniformis</i>	Rainbow trout (<i>Onchorhynchus mykiss</i>)	Increased resistance to <i>Yersinia ruckeri</i>	Raida et al. (2003)
2.	<i>Bacillus subtilis</i> <i>Lactobacillus delbriueckii</i>	Gilthead seabream	Stimulated cellular innate immune response	Salinas et al. (2005)
3.	<i>Bacillus</i> spp. Photosynthetic bacteria	Common carp (<i>Cyprinus carpio</i>)	Better digestive enzyme activities; better growth performance and feed efficiency	Wang and Xu (2006)
4.	<i>Bacillus</i> spp.	Rainbow trout (<i>Onchorhynchus mykiss</i>)	Better growth performance and survival	Bagheri et al. (2008)
5.	<i>Bacillus subtilis</i> (ATCC 6633), <i>Lactobacillus acidophilus</i>	Nile tilapia (<i>Oreochromis niloticus</i>)	Stimulated the gut immune system; enhanced the immune and health status; increased the survival rate and body-weight gain	Salah Mesalhy et al. (2008)
6.	<i>Carnobacterium</i> spp.	Atlantic salmon (<i>Salmo salar</i> L.)	Inhibited <i>A. salmonicida</i> , <i>V. ordalii</i> and <i>Y. ruckeri</i> ; reduced disease outbreak	Robertson et al. (2000)
7.	<i>Carnobacterium maltaromaticum</i> B26, <i>Carnobacterium divergens</i>	Rainbow trout (<i>Onchorhynchus mykiss</i>)	Enhanced the cellular and humoral immune responses	Kim and Austin (2006)
8.	<i>Carnobacterium divergens</i> 6251	Atlantic salmon (<i>Salmo salar</i> L.)	<i>Carnobacterium divergens</i> is able to prevent pathogen-induced damage in the foregut to some extent	Ringo et al. (2007)
9.	<i>Lactobacillus rhamnosus</i>	Rainbow trout (<i>Onchorhynchus mykiss</i>)/feed	Increased resistance to <i>Aeromonas salmonicida</i> ; reduced mortality	Nikoskelainen et al. (2001)
10.	<i>Lactobacillus rhamnosus</i> (ATCC 53103)	Rainbow trout (<i>Onchorhynchus mykiss</i>)/feed	Enhanced immune parameters; stimulated immune response	Nikoskelaine et al. (2003)
11.	<i>Lactobacillus rhamnosus</i> (JCM 1136)	Rainbow trout (<i>Onchorhynchus mykiss</i>)/feed	Increased the serum lysozyme and complement activities	Panigrahi et al. (2004)
12.	<i>Lactobacillus rhamnosus</i>	Rainbow trout (<i>Onchorhynchus mykiss</i>)/feed	Stimulated immune response	Panigrahi et al. (2005)
13.	<i>Lactobacillus delbrueckii</i> subsp. <i>delbrueckii</i> (AS13B)	European sea bass (<i>Dicentrarchus labrax</i> L.)/feed	Positive effects on welfare and growth; increased body weight	Carnevali et al. (2006)
14.	<i>Lactobacillus rhamnosus</i> GG	Tilapia (<i>Oreochromis niloticus</i>)/feed	Enhanced the growth performance and immunity	Pirarat et al. (2008)
15.	<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	Rainbow trout (<i>Onchorhynchus mykiss</i>)/feed	Enhanced humoral immune response	Tukmechi et al. (2007)
16.	<i>Lactobacillus rhamnosus</i> (ATCC 53103); <i>Bacillus subtilis</i> , <i>Enterococcus faecium</i>	Rainbow trout (<i>Onchorhynchus mykiss</i>)/feed	Modulated cytokine production; stimulated immune response	Panigrahi et al. (2007)
17.	<i>Lactococcus lactis</i> subsp. <i>lactis</i> ; <i>Lactobacillus sakei</i> ; <i>Leuconostoc mesenteroides</i>	Rainbow trout (<i>Onchorhynchus mykiss</i>)/feed	Stimulated phagocytosis; enhanced the non-specific immunity	Balcazar et al. (2006)
18.	<i>Lactococcus lactis</i> ssp. <i>lactis</i> ; <i>Lactobacillus sakei</i> , <i>Leuconostoc mesenteroides</i>	Brown trout (<i>Salmo trutta</i>)/feed	Modified the intestinal microbiota; stimulate the humoral immune response	Balcazar et al. (2007)

Table 2 continued

Sl. no.	Identity of the probiotics (applied in feed)	Species	Effects	Reference (s)
19.	<i>Leuconostoc mesenteroides</i> CLFP 196; <i>Lactobacillus plantarum</i> CLFP 238	Rainbow trout (<i>Onchorhynchus mykiss</i>)/feed	Reduced fish mortality	Vendrell et al. (2008)
20.	<i>Micrococcus luteus</i>	Nile tilapia (<i>Oreochromis niloticus</i>)/feed	Enhanced the non-specific immune parameters; improved resistance against <i>Edwardsiella tarda</i> infection	Taoka et al. (2006a, b)
21.	<i>Micrococcus luteus</i> <i>Pseudomonas</i> sp.	Nile tilapia (<i>Oreochromis niloticus</i>)/feed	Higher growth performance, survival rate and feed utilization; enhanced fish resistance against <i>Aeromonas hydrophila</i> infection	Abdel-Rhman et al. (2009)
22.	<i>Streptococcus faecium</i> , <i>Lactobacillus acidophilus</i>	Nile tilapia (<i>Oreochromis niloticus</i>)/feed	Better growth performance and feed efficiency	Lara-Flores et al. (2003)
23.	<i>Saccharomyces cerevisiae</i>	Nile tilapia (<i>Oreochromis niloticus</i>)/feed	Better growth performance and feed efficiency	Lara-Flores et al. (2003)
24.	Live yeasts	European sea bass (<i>Dicentrarchus labrax</i>)/feed	Better growth performance and feed efficiency	Tovar-Ramirez et al. (2004)
25.	<i>Saccharomyces cerevisiae</i> strain NCYC Sc 47 (Biosaf [®] Sc 47)	Rainbow trout (<i>Onchorhynchus mykiss</i>)/feed	No significant effect on enzyme activities	Waché et al. (2006)
26.	<i>Saccharomyces cerevisiae</i> var. <i>boulardii</i> CNCM I-1079 (Levucell [®] SB20)	Rainbow trout (<i>Onchorhynchus mykiss</i>)/feed	Stimulated enzyme activities	Waché et al. (2006)
27.	<i>Saccharomyces cerevisiae</i> (DVAQUA [®])	Hybrid tilapia (<i>Oreochromis niloticus</i> ♀ × <i>O. aureus</i> ♂)/feed	Inhibited potential harmful bacteria; stimulated beneficial bacteria; enhanced the non-specific immunity; no significant effects on growth performance and feed efficiency	He et al. (2009)
28.	<i>Bacillus</i> sp.	Gilthead sea bream (<i>Sparus aurata</i> , L.)	Increase the specific activities of alkaline and acid protease; improved the husbandry parameters and nutritional condition in larvae of <i>S. aurata</i>	Arig et al. (2013)
29.	<i>Bacillus pumilus</i> and <i>Bacillus clausii</i>	Grouper <i>Epinephelus coioides</i>	Improved growth performance and immune responses of <i>E. coioides</i>	Yun-Zhang et al. (2010)
30.	<i>Bacillus</i> strain isolated from soil or intestine of channel catfish (<i>Ictalurus punctatus</i>) intestine	Channel catfish (<i>Ictalurus punctatus</i>)	Good potential to mitigate the enteric septicemia of catfish (ESC)	Ran et al. (2012)
31.	<i>Bacillus subtilis</i> C-3102	Koi carp (<i>Cyprinus carpio</i>)	Improved growth, feed utilization and modulating intestinal microbial community	He et al. (2011)
32.	<i>B. coagulans</i> and <i>B. subtilis</i>	<i>Artemia nauplii</i>	Produced antimicrobial activity against the pathogenic <i>Vibrio</i> species, including <i>V. alginolyticus</i>	Mahdhi et al. (2011)
33.	<i>Lactobacillus acidophilus</i>	Cat fish (<i>Mystus montanus</i>)	Better food conversion, survival, growth rate	Veni et al. (2012)
34.	Mixture of <i>B. subtilis</i> , <i>L. plantarum</i> and <i>Saccharomyces cerevisiae</i>	Nile tilapia (<i>Oreochromis niloticus</i>)	Acid phosphatase activity, lysozyme activity and total immunoglobulin activity	Magda et al. (2011)
35.	<i>Kocuria</i> SM1	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Successfully controlled vibriosis in rainbow trout	Sharifuzzaman and Austin (2010)

Table 2 continued

Sl. no.	Identity of the probiotics (applied in feed)	Species	Effects	Reference (s)
36.	<i>Bacillus subtilis</i> AB1	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Controls <i>Aeromonas</i> infection in rainbow trout (<i>Oncorhynchus mykiss</i>)	Newaj-Fyzul et al. (2007)
37.	<i>Bacillus subtilis</i>	Orange spotted grouper (<i>Epinephelus coioides</i>)	Increase the innate immunity and intestinal microbial population	Purwandari and Chen (2013)
38.	<i>L. plantarum</i> , <i>L. salivarius</i> and <i>L. rhamnosus</i>	<i>P. pelagicus</i>	Lowered pH and increased protease and amylase activity	Talpur et al. (2012)
39.	<i>Bacillus subtilis</i> (isolated from gut of juvenile freshwater prawn)	Freshwater prawn (<i>Macrobrachium rosenbergii</i>)	Growth and survival enhancement	Mehran and Masoumeh (2013)
40.	<i>Lactobacillus plantarum</i>	Nile tilapia (<i>Oreochromis niloticus</i>)	Improved growth rates, protection against <i>Pseudomonas fluorescens</i> compared to the control	Iman et al. (2013)

(Sommer and Backhed 2013); therefore, there is a general competition for these nutrients to grow and reproduce. The more the gut is flooded with beneficial microorganisms, the more competition is created between beneficial and pathogenic microorganisms.

Availability of energy and essential nutrients can play an important role in the composition of the GI microbiota for nutrient competition in the culture water of aquatic species (Tinh et al. 2008; Wu et al. 2012). Due to the increase in the relative numbers of probiotic bacteria, nutrients and energy are consumed that would otherwise be available for the growth of pathogenic bacteria (Verschuere et al. 2000; Denev et al. 2009). Prebiotic oligosaccharides such as inulin and oligofructose are fermented in the colon where they promote the growth of bacterial populations associated with a healthy, well-functioning colon. This selective stimulation occurs because oligosaccharides are readily fermented by beneficial types of colonic bacteria and are not used effectively by potentially pathogenic bacterial species (Yousefian and Amiri 2009). Competition for iron has been reported to be an important factor for growth in marine bacteria, but is generally limited in the tissues and body fluids of animals and in the insoluble ferric Fe³⁺ form (Verschuere et al. 2000; Oke et al. 2013). Iron-binding agents, siderophores, allow acquisition of iron suitable for microbial growth. Siderophore production is a noted mechanism of virulence in some pathogens (Denev et al. 2009). Equally, a siderophore-producing probiotic could deprive potential pathogens of iron

under iron-limiting conditions. Mohideen et al. (2010) revealed that a culture supernatant of *Pseudomonas fluorescens*, grown in iron-limited conditions, inhibited the growth of *Vibrio* spp, whereas the supernatant from iron-accessible cultures did not reveal the same. Ringo et al. (2010) examined the effects of enhanced nutrition on the host and the modulation of interactions with the environment and development of a beneficial immune response (Verlhac and Viswanath 2004). Dimitoglou et al. (2011) appraised microbial manipulations to improve fish health and production in the Mediterranean area; in Mediterranean teleosts, applying probiotics, they could stimulate immune responses and enhance growth performance, feed utilization, digestive enzyme activities, antioxidant enzyme activities, gene expression, disease resistance, larval survival, gut morphology, and modulate GI microbiota and mediate the stress response (Tables 2, 3, 4).

Probiotic bacteria ferment food derived from indigestible carbohydrates to produce short chain fatty acid in the gut (Ige 2013). Short chain fatty acids cause a decrease in the systemic levels of blood lipids by inhibiting hepatic cholesterol synthesis from the plasma to liver, indicating a better health status in fish (Tukmechi et al. 2007; Soccol et al. 2010).

Water probiotics

The indiscriminate use of antibiotics and chemotherapeutics for improving the quality of the health and

Table 3 Synbiotics used in aquaculture

Sl. no.	Species	Probiotic/prebiotic	Increases	Decreases	References
1	<i>O. mykiss</i>	<i>E. faecalis</i> /MOS, PHB	Body weight, SGR, total blood count, innate immune response	Mortality	Rodriguez-Estrada et al. (2009)
2	<i>P. olivaceus</i>	<i>B. clausii</i> /MOS, FOS	Body weight, WGR, lysozyme, crude protein and lipid, protease and amylase activities	TG and LDL-C, FCR, CF	Ye et al. (2011)
3	<i>O. mykiss</i>	Biomim IMBO (<i>E. faecium</i> /FOS)	Body weight, SGR, FCE, SR, serum protein		Mehrabi et al. (2011)
4	<i>L. crocea</i>	<i>B. subtilis</i> /FOS	SGR, FER, lysozyme, SOD	Mortality	Ai et al. (2011)
5	<i>R. canadum</i>	<i>B. subtilis</i> /chitosan	SGR, lysozyme, ACP, innate immunity		Geng et al. (2011)
6.	European lobster (<i>Homarus gammarus</i>) larvae	<i>Bacillus</i> sp. and MOS	Survival and provides the added benefits of improved growth performance	Larval mortality	Daniels et al. (2010)
7.	<i>Litopenaeus vannamei</i>	<i>Bacillus</i> sp. and MOS	Disease resistance, improved growth		Li et al. (2009)
8.	<i>O. fasciatus</i>	<i>Lactobacillus sakei</i> (BK19) and herb (<i>Scutellaria baicalensis</i>)	Enhancing the innate immunity in <i>O. fasciatus</i> against <i>Edwardsiella tarda</i>	Mortality	Harikrishnan et al. (2011b)
9.	Olive flounder, (<i>Paralichthys olivaceus</i>)	<i>Lactobacillus sakei</i> (BK19) and herb (<i>Scutellaria baicalensis</i>)	Enhancing the innate immunity against <i>Streptococcus parauberis</i> , growth, blood biochemical constituents		Harikrishnan et al. (2011a)
10.	<i>Penaeus vannamei</i> larviculture	<i>Vibrio alginolyticus</i> and β -1,3/1,6-glucans	Enhanced survival during the first 0–52 h post-WSSV challenge period	Negative effects on O ₂ generation	Rodriguez et al. (2007)

rearing water quality in fish has led to the development of drug-resistant strains of pathogenic microorganisms (Amabile-Cuevas et al. 1995; Thomas and Nielsen 2005; Akinbowale et al. 2006). Water probiotics contain multiple strains of bacteria such as *Bacillus acidophilus*, *B. subtilis*, *B. licheniformis*, *Nitrobacter* spp., *Aerobacter* and *Saccharomyces cerevisiae* (Kolndadacha et al. 2011). Application of water probiotics in lentic water bodies may improve fish health by improving several water quality parameters, since they modify the bacterial composition of the water and sediments (Ashraf 2000; Venkateswara 2007). Probiotics applied in culture water are considered prone to multiply and outgrow the pathogenic organisms present in the water. Chaucheyras-Durand and Durand (2010) described the most important benefits of yeast and bacterial probiotics on the gastrointestinal microbial ecosystem in ruminants

and monogastric animals. Nowadays, a number of probiotic preparations are commercially available and have been introduced to fish, shrimp and mollusk farming as feed additives (Wang et al. 2005). Iribarren et al. (2012) used LCA to appraise the potential environmental effects of probiotics used in turbot aquaculture. This LCA study showed that the potential environmental impacts associated with probiotic production are generally offset by the impact reductions linked to lower consumption levels and reduced waste and emission generation rates within the hatching and nursing subsystem (Iribarren et al. 2012). In line with this, Venkateswara (2007) reported that probiotic bacteria are generally called water probiotics and can improve the water quality of aquaculture and inhibit the pathogens in water; thus the increased production and disease control are interdependent and linked to the

Table 4 Probiotics produced used as antimicrobials

Sl. no.	Name of probiotics	Substance	Inhibitory strain	References
1	<i>Pseudomonas</i> isolate MSB1	Siderophores	<i>Flavobacterium psychrophilum</i>	Strom-Bestor and Wiklund (2011)
2	Genera <i>Lactobacillus</i> , <i>Lactococcus</i> and <i>Leuconostoc</i>	Lactic acid	<i>Lactococcus garvieae</i>	Perez-Sanchez et al. (2011)
3	<i>Pseudomonas</i> M174	Siderophores	<i>Flavobacterium psychrophilum</i>	Korkea-aho et al. (2011)
4	<i>Pseudomonas</i> M162	Siderophores	<i>Flavobacterium psychrophilum</i> (in vitro)	Korkea-aho et al. (2012)
5	Lactic acid bacteria	Class I/II/Lantibiotic	–	Oppegard et al. (2007) and Martin-Visscher et al. (2009)
6	<i>Vibrio anguillarum</i> AVP10	Vibriocin AVP10	<i>Escherichia coli</i> , <i>Vibrio anguillarum</i> AVS91	Zai et al. (2009)
7	<i>Vibrio mediterranei</i>	BLIS	<i>V. parahaemolyticus</i> , <i>V. mediterranei</i> 5	Carraturo et al. (2006)
8	<i>Vibrio harveyi</i> VIB 571	BLIS	<i>Vibrio harveyi</i> 1, <i>V. fischeri</i> , <i>V. gazogenes</i> , <i>V. parahaemolyticus</i>	Prasad et al. (2005a, b)
9	Bacteriocinogenic strain marine strain ZM81 (gram positive) pleomorphic strain)	Bacteriocins/BLIS	Marine bacterial strain ZM19	Pirzada et al. (2004)
10	<i>Pseudomonas</i> strains	Siderophores	<i>V. anguillarum</i>	Gram et al. (1999)
11	<i>Aeromonas media</i>	BLIS:bacteriocin-like inhibitory substance	<i>Aeromonads</i> and <i>vibrios</i>	Gibson et al. (1998)
12	Lactic acid bacteria	Hydrogen peroxide and CO ₂	–	Naidu et al. (1999)
13	<i>Pseudoalteromonas</i> species strain X153	Antibiotic protein P-153	<i>Ichthyopathogenic Vibrio</i> 1, <i>Staphylococcus epidermidis</i> , <i>Propionibacterium acnes</i> , <i>Propionibacterium granulosum</i>	Longeon et al. (2004)
14	<i>Lactobacillus rhamnosus</i> L60 and <i>Lactobacillus fermentum</i> L23	Organic acids, bacteriocins and, in the case of L60, hydrogen peroxide also	Antifungal activity of lactobacilli strains against feed used for animal production	Gerbaldo et al. (2012)

microbial activities in the aquaculture system. Inhibition of pathogens in the digestive tract by the probiotic bacteria has been reported by several authors (Kabir et al. 2005; Trachoo and Boudreaux 2006; Anukam 2007; Nenci et al. 2007; Raj et al. 2008; Hung et al. 2008; Radfar and Farhoomand 2008; Capcarova et al. 2008; Soundarapandian and Sankar 2008; Vamanu et al. 2008; Vijayabaskar and Somasundaram 2008; Abdelhamid et al. 2009; Vali 2009; Vamanu and Vamanu 2010; Bansal et al. 2011; Dahiya et al. 2012a, b; Ran et al. 2012; Cruz et al. 2012; Sihag and Sharma 2012). Gatesoupe (1999) applied the concept of water

probiotics to aquatic animals, which are quite different from land animals. Moriarty (1998) extended the application of probiotics to aquaculture by the addition of live bacteria to the tanks and ponds in which the animals live. The health of the animals was improved by the elimination of pathogens or at least minimizing the effect of pathogens by improving the water quality. Moriarty (1998) suggested that probiotics could be used in aquaculture not only as feed supplements, but also as water additives. Verschuere et al. (2000) claimed that there is an intensive interaction between the culture environment and aquatic organisms. In addition, Fuller

Table 5 Commonly used water probiotics in aquaculture

Sl. no.	Putative probiotics	Origin	Action on water quality and observation	Doses	References
1.	<i>Bacillus</i> sp., <i>Saccharomyces</i> sp.	Commercial product	Total nitrogen and ammonia concentrations lower up to a lower extent Water transparency was highest during the initial phase of culture but gradually declined	$10^8 + 10^5$ cfu/ml	Matias et al. (2002)
2.	<i>Bacillus</i> sp., <i>S. cerevisiae</i> , <i>Nitrosomonas</i> sp.	Commercial product	Reduced concentrations of nitrogen and phosphorus, and increased yields of shrimp	10^4 – 10^9 cfu/ml	Wang et al. (2005)
3.	<i>Streptomyces</i> sp.	Estuary sediment	Experimental culture tanks provided with <i>Streptomyces</i> had better water quality parameters	2–10 g of dry mat/kg feed	Das et al. (2006)
4.	Gram-positive <i>Bacillus</i> sp.	–	Probiotics maintained optimum transparency and low organic load	–	Dalmin et al. (2001)
5.	Mixed bacillus	Commercial product	Levels of pH, ammonia and nitrite were significantly decreased	–	Nimrat et al. (2012)
6.	<i>B. subtilis</i> and <i>B. megaterium</i> with soyabean meal	Commercial product	Stress tolerance and hemolymph metabolites also showed the best performance in this treatment	1.2×10^4 cfu/g	Olmos et al. (2011)
7.	BZT®BIO-AQUA	Commercial product	Significant improvement in water quality, growth performance	–	Mohamed et al. (2013)
8.	<i>Bacillus</i> sp. and <i>Lactobacillus</i> , yeasts	Commercial product	Increased the percentage values of Pyrrophyta concentration, improving the environmental quality of the sediment and water in ponds with closed recirculation systems	$5.749 \pm 0.67 \times 10^4$ cfu/g	Paiva-Maia et al. (2013)
9.	<i>Bacillus circulans</i> and <i>B. licheniformis</i>	Commercial product	Reduced fish culture risks by improving of growth and health of cultured fish	1×10^6 cfu/ml	Sahandi et al. (2012)
10.	<i>Enterococcus faecium</i> ZJ4	Nile tilapia (<i>Oreochromis niloticus</i>)/water	Increased growth performance; improved immune response	–	Wang et al. (2008)
11.	<i>Bacillus pumilus</i> , <i>B. licheniformis</i> , and <i>B. subtilis</i>	Marine water and soil samples	Reduced TAN; better growth and survival in shrimp PL without water exchange	–	Devaraja et al. (2013)
12.	Lactic acid bacteria	Rotifer, <i>B. plicatilis</i>	Better survival rate of larval turbot, <i>Scophthalmus maximus</i>	10^7 and 2×10^7 cfu/ml	Gatesoupe (1994)

(1989) found that many probiotics are obtained from the culture environment itself rather than directly from feed (Table 5).

Bioaugmentation

This technology basically consists of adding microorganisms able to degrade or remove polluting compounds, especially organic matter and nutrients, to refine

the heavily loaded pond or lake ecosystem in an environmentally friendly manner (Lopes et al. 2011). For this purpose, the use of beneficial bacteria as the bioaugmentor or probiotics has begun to play a role in the bioaugmentation process, which has been found to increase the production efficiency in fish culture ponds (Tucker et al. 2009). In addition to this, Sayeda et al. (2011) evaluated *Azotobacter* and *Azospirillum* biofertilizers as probiotics in *Oreochromis niloticus*

Table 6 Commonly used stress probiotics in aquaculture

Sl. no.	Putative probiotics	Used on	Observation	Doses	References
1	<i>Lactobacillus delbrueckii</i>	Juvenile of European sea bass (<i>Dicentrarchus labrax</i>)	Cortisol level was chosen as a stress marker and growth was evaluated	–	Carnevali et al. (2006)
2	<i>Alteromonas</i> sp. strain Pdp 11	Juvenile gilthead sea bream (<i>Sparus auratus</i>)	Improved stress tolerance under high stocking density	10^9 cfu/g ⁻¹	Varela et al. (2010)
3	<i>B. subtilis</i> , <i>L. acidophilus</i> , <i>S. cerevisiae</i>	<i>Paralichthys olivaceus</i>	Providing them a higher resistance against stress (heat stress) conditions and pathogen <i>Vibrio anguillarum</i>	<i>Bacillus subtilis</i> ($> 1.6 \times 10^7$ cfu/g), <i>Lactobacillus acidophilus</i> ($> 1.2 \times 10^8$ cfu/g), <i>Clostridium butyricum</i> ($> 2.0 \times 10^7$ cfu/g) and <i>Saccharomyces cerevisiae</i> ($> 1.6 \times 10^7$ cfu/g)	Taoka et al. (2006a, b)
4	<i>Pediococcus acidilactici</i>	<i>Litopenaeus stylirostris</i>	Reduction of the infection level and/or an enhancement of the antioxidant status of the shrimps	10^7 cfu/g	Castex et al. (2009)
5	<i>Nitrosomonas</i> and <i>Nitrobacter</i>	<i>Pangasius sutchi</i> , <i>Catla catla</i> and <i>Labeo rohita</i>	Lower pathogenic bacterial loads in fish ponds	1.62 and 0.82 kg/ha, respectively	Padmavathi et al. (2012)
6	<i>Vibrio</i> sp. strain OY15	Oyster larvae (<i>Crassostrea Virginica</i>)	Significantly improved survival of oyster larvae to metamorphosis when challenged with pathogen B183 in pilot scale	10^3 cfu/ml	Kapareiko et al. (2011)
7	<i>Shewanella putrefaciens</i> Pdp11	Senegalenses sole (<i>Solea senegalensis</i>)	Improves the immune response, gastrointestinal health and nutrition	–	Tapia et al. (2012)
8	<i>Lactobacillus delbrueckii</i>	Juvenile European sea bass (<i>Dicentrarchus labrax</i>)	Cortisol level (cortisol, a hormone directly involved in stress responses, was chosen as a stress marker) and growth were evaluated	–	Carnevali et al. (2006)

aquaculture. Although the mixed bacterial treatment improved some water parameters, it showed higher ALT and AST levels and severe histopathological lesions in fish compared to those with single treatment. Therefore, [Sayeda et al. \(2011\)](#) suggested a single inoculation of *Azotobacter* bacteria biofertilizer as a suitable probiotic that might be effective in *Oreochromis niloticus* aquaculture. Water probiotics involve the addition of specifically formulated microorganisms to water to improve its microbial ecology by a bioaugmentation process ([Rengpipat 2005](#)). Water quality parameters such as dissolved oxygen, ammonia (NH₃),

nitrite (NO₂⁻) and sulfide were improved. To perform this process, *Bacillus* spp. was found to have an excellent water purification capability as gram-positive bacteria are better converters of organic matter back to CO₂ than gram-negative bacteria ([Moriarty 1997](#); [Anik et al. 2011](#)). *Bacillus* spp. improved water quality, survival and growth rates well, improved the health status of juvenile *Penaeus monodon* and reduced the amount of pathogenic vibrios ([Dalmin et al. 2001](#); [Devaraja et al. 2013](#)). For edible fish, *Bacillus* spp. could reduce the produced chemical load of high concentrations of nitrogen in trout production ([Maillard et al.](#)

2005) and reduce the total ammonia in tilapia production recirculating systems, (Rafiee and Saad 2005). Moreover, commercial probiotics made from *Bacillus licheniformis* and *B. subtilis* were used in Nile tilapia *Oreochromis niloticus* farming to optimize the dissolved oxygen level (El-Haroun et al. 2006). *Bacillus* from *Cyprinus carpio* was used to minimize the pathogenicity of *Aeromonas hydrophila* in ornamental fish culture (Lalloo et al. 2007), but in catfish *Ictalurus punctatus*, no significant difference was found between the treated and control group of fishes for the examined water quality variables (Queiroz and Boyd 1998). This may be due to the intrinsic capability of catfish, enabling the fish to sustain themselves even at the threshold limit. Commercial probiotics formulated from mixed cultures of bacteria and yeast could provide better growth, survival and water quality parameters in a closed recirculating system of Japanese flounder (*Paralichthys olivaceus*) (Taoka et al. 2006a, b). These results were in good accordance with the findings of Queiroz and Boyd (1998). Commercial products made from *Bacillus* spp., *Saccharomyces cerevisiae*, *Nitrosomonas* spp. and *Nitrobacter* spp. were found to increase the beneficial bacterial microbiota in a culture pond for *Penaeus vannamei* shrimp farming (Wang et al. 2005).

Bioremediation

Bioremediation is a branch of biotechnology that provides possible options to destroy and modify the contaminated soil, air and water prior to discharge into the receiving waters of sensitive areas with the help of microassays and microorganisms (Jones et al. 2001; Strong and Burgess 2008). In a competitive and changing field, aquaculture industries require new and constantly emerging technologies to increase the production yield. Application of and research on probiotics as bioremediators has increased the demand for ecofriendly aquaculture (Ali 2006; Lakshmanan and Soundarapandian 2008; Sreedevi and Ramasubramanian 2010; Dimitroglou et al. 2011; Cruz et al. 2012; Iribarren et al. 2012; Wang et al. 2005; Li et al. 2008; Guo et al. 2009; Peraza-Gomez et al. 2009; Shen et al. 2010; Silva et al. 2012, 2013; Souza et al. 2011; Zhang et al. 2011).

Bacterial species belonging to the genera *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Cellulomonas*, *Rhodospseudomonas*, *Nitrosomonas* and *Nitrobacter* are

known to be potent bioremediators for organic wastes (Thomas et al. 1992). These probiotic bacteria regulate the microflora of aquaculture water and control pathogenic microorganisms to enhance the decomposition of undesirable organic substances in the water and sediment because of the improved ecological environment of aquaculture (Xiang-Hong et al. 2003; Venkateswara 2007). Paiva-Maia et al. (2013) studied the effect of a commercial probiotic on the bacterial and phytoplankton concentrations in intensive shrimp farming of *Litopenaeus vannamei* with a recirculation system. The results indicated that probiotics caused marked changes in the total heterotrophic bacteria in the sediment and percentage values of *Pyrrophyta* concentrations, improving the environmental quality of the sediment and water in ponds with closed recirculation systems. Padmavathi et al. (2012) appraised the efficacy of two probiotic bacteria as bioremediators in three earthen ponds cultured with *Pangasius sutchi*, *Catla catla* and *Labeo rohita* for a 1-year period. The results indicated that concentrations of ammonia, nitrite and phosphates were low in treated ponds compared to the control pond. This may be due to bioremediating properties of the above-mentioned bacteria. Devaraja et al. (2013) isolated indigenous *Bacillus pumilus*, *B. licheniformis* and *B. subtilis* from marine water and soil samples and investigated them for their potential bioremediation ability in *Penaeus monodon* culture. The study revealed that bacillus spp. secreted protease, amylase and lipase and inhibited the pathogenic *Vibrio* spp. without distressing the shrimp post larvae, and bacillus spp. has been recommended as a bioremediator for *Penaeus monodon* culture systems (Devaraja et al. 2013). In situ bioremediation has also been widely applied in aquaculture through bioaugmentation using indigenous or exogenous probiotics, which ameliorate water quality (Wang et al. 2005).

Biomonitors

These are groups of organisms whose behavior, growth, survival and histopathological changes could be monitored to assess environmental changes. Bio-monitoring offers an appealing tool for the assessment of metal pollution in aquatic ecosystems (Zhou et al. 2008). Bioindicators including algae, macrophytes, zooplanktons, insects, bivalve mollusks, gastropods, fish, amphibians and others have been enumerated and

compared concerning their advantages and disadvantages for the practical biomonitoring of aquatic metal pollution (Zhou et al. 2008). Shrimp aquaculture ponds are often impacted by acid sulfate soils, typically resulting in increased disease and mortality of cultured organisms, which can be traced by macroalgal biomonitors (Gosavi et al. 2004). Aquatic and pulmonate snails were evaluated for their suitability as biomonitors for habitat recovery following an experimental oil spill in a freshwater marshland to assess the impacts of crude oil (Lee et al. 2002), rates of natural recovery and the efficacy of bioremediation treatments to enhance the bacterial degradation of residual oil in the sediments (Ronald and Terry 2011). The use of *Chondrilla nucula* sponges for marine bioremediation in a farming scenario has been investigated (Milanese et al. 2003). *C. nucula* exhibited a marked ability to retain high quantities of bacteria. A 1-m² patch of this sponge can filter up to 14 l/h of seawater, retaining up to “7 × 10” bacterial cells. Zhang et al. (2010) reported that the marine sponge *Hymeniacidon perlevis* is highly efficient in removing bacterial pollution in the intensive mariculture water system of the turbot *Scophthalmus maximus*.

Bioreporters

Bioreporters are prokaryotic bacteria and eukaryotic organisms, specially bacteria, fungi, algae and animals that can be exploited as living sensors for priority environmental pollutants and chemicals of toxicological concern (Tingting et al. 2013). The underlying principle is that the microbial cells and eukaryotic cell lines can detect specific analytes and report an analytically useful signal, and its performance can be enhanced by improving the transcriptional regulation, molecular signaling, microbial physiology and detection methodology (Hynninen et al. 2010; Muller and Fetzner 2013).

A substantial number of genetically modified bioreporter bacteria are being designed to measure concentrations of polluting and toxic chemicals (Diplock et al. 2010; Eltzov and Marks 2011; Ripp et al. 2011; vander Meer and Belkin 2010). Moreover, whole-cell bacterial bioreporters can be used for the assessment of bioavailability and toxicity of metals in different sample milieus (Bondarenko et al. 2008; Brandt et al. 2006; Ivask et al. 2011; Hynninen and Virta 2010). Whole-cell bioreporters most commonly

incorporate reporter genes that code for signaling elements that emit bioluminescent, fluorescent or colorimetric endpoints, with bioluminescence being derived from the bacterial (*lux*) gene (Close et al. 2010) and firefly (*luc*) gene (Close et al. 2009); fluorescence from the green fluorescent protein (*gfp*) gene (Hever and Belkin 2006) and its other colored variants, and colorimetric end points relying upon the galactosidase (*lacZ*) gene (Ron and Rishpon 2010).

As microorganisms are living beings, their ‘measurements’ reflect a bioavailable concentration rather than the total concentration that can be assessed by many chemical methods (Trogel et al. 2012). The relationship between bioreporter sensing and bioavailability under environmental influences is a complex process that depends on the bioreporter’s physiology, growth rate, membrane composition and transport mechanisms, and the contaminant binds to the receptor and turns on the reporter gene in bacteria. The expression intensity is measured by a fluorescence microscope or by PCR amplification methods (Harms et al. 2006; Sochor et al. 2011).

Growth promoters

One of the expected activities of water probiotics is a direct growth-promoting effects on fish by direct involvement in nutrient uptake by providing nutrients or vitamins. However, it has been demonstrated experimentally that probiotics indeed may enhance the growth of fish. The ability of organisms to outgrow the pathogens either in favor of the host or to improve the growth of the host, and so far no side effects on the host have been noted; these are some of the most important criteria for these organisms being true probiotics. Yassir et al. (2002) attempted to use probiotic bacteria as growth promoters in tilapia (*Oreochromis niloticus*); the highest growth performance was recorded with *Micrococcus luteus*, a probiotic, and the best feed conversion ratio was observed with the same organism. Therefore, *M. luteus* may be considered as a growth promoter in fish aquaculture. Lactic acid bacteria were also reported to have growth promoting effects in juvenile carp, whereas seabass seemed to be unaffected by LAB (Noh et al. 1994). *Enterococcus faecium* has been used in feed to improve fish growth (Bogut et al. 2000). Irianto and Austin (2002a, b) reported that probiotics can stimulate the appetite and improve nutrition by

producing vitamins, detoxifying compounds in the diet and by breaking down indigestible components. *Streptococcus facium* improved the growth and feed efficiency of Israeli carp (Noh et al. 1994). Growth-promoting effects of commercial probiotics in Nile tilapia (*Oreochromis mossambicus*) have been reported (Eid and Mohamed 2008). Sahandi et al. (2012) investigated the effects of direct inoculation of probiotic bacilli on the growth rate of *Cyprinus carpio* and *Ctenopharyngodon idella* being fed with *Artemia* in the rearing tanks. The results of the study show that probiotics reduced fish culture risks because of the improved growth and health of cultured fish. Mohamed et al. (2013) assessed the effects of probiotic application on the natural food, water quality and growth performance of saline tilapia (*Oreochromis mossambicus*) cultured in concrete tanks. The study pointed out that *O. mossambicus* with probiotics added to their tanks had significantly higher effects ($p < 0.05$) on the final weight, % weight gain, SGR% and FCR than with the control treatment, respectively. *O. mossambicus*' average body weight was higher ($p < 0.05$), and they tended to grow with time for the overall treatments with better performance in probiotic-treated fish. Water quality was better in *O. mossambicus* treated with probiotics, and it provided control of the water quality, growth performance and phytoplankton production. *Bacillus* spp. has been found to provide promising growth and survival in a *Penaeus monodon* shrimp culture system without water exchange (Devaraja et al. 2013). Probiotics therefore can be regarded as growth promoters in aquaculture organisms in addition to other various benefits (Kolndadacha et al. 2011; Cruz et al. 2012; Oke et al. 2013).

Inhibition of quorum sensing

Waters and Bassler (2005) illustrated that quorum sensing is a process of bacterial cell-to-cell communication. The disruption of quorum sensing (QS) is a new anti-infective strategy in aquaculture (Defoirdt et al. 2004). Defoirdt et al. (2005) reported that an AI-2 (autoinducer-2)-mediated system is responsible for the virulence of *V. harveyi* toward the gnotobiotic *Artemia franciscana* (Defoirdt et al. 2005), despite the fact that both HAI-1 (*Harveyi* autoinducer-1) and AI-2-mediated systems were involved in the growth-retarding

effect of this bacterium toward the gnotobiotic *Brachionus plicatilis* (Tinh et al. 2007). This might have suggested the existence of host-dependent QS in *V. harveyi*. It has been found that marine red algae (*Delisea pulchra*) act as very good QS antagonists (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 probiotic bacteria that can act as QS-disrupting agents in aquaculture systems needs to be evaluated. Therefore, the determination of the concentration of QS molecules in vivo would provide better knowledge about the importance of QS in vivo and also help to clarify the mechanism of action of the QS-disrupting bacteria.

Biological regulation

The use of probiotics, which control pathogens through a variety of mechanisms, is increasingly viewed as an alternative to antibiotic treatment (Verschuere et al. 2000). Generally, probiotics are fed as fortified live or attenuated microorganisms, although, nutritional effects are also often attributed to probiotics, especially for filter feeders, and found to function as biological controllers of fish disease and activators of nutrient regeneration (Yasuda and Taga 1980). Since then the research effort has continually increased. The majority of probiotics proposed as biological control agents in aquaculture are lactic acid bacteria (*Lactobacillus*, *Carnobacterium*), of the genus *Vibrio* (*Vibrio alginolyticus*), *Bacillus* or *Pseudomonas*, although other genera or species have also been evaluated (Table 1). Qi et al. (2009) reviewed the probiotic status in relation to Chinese aquaculture and suggested that because of the characteristic lifecycle of *Bdellovibrio*, which are gram-negative δ -proteobacteria (Jurkevitch 2007), they as a group can utilize any of a wide variety of gram-negative bacteria as a substrate cell, making them attractive candidates for a number of applications concerning reducing or modulating bacterial populations, i.e., biological control of pathogens, water purification and biofilm control (Yair et al. 2003). Recently, Karim et al. (2013) screened and

characterized the marine bacterial isolates *Phaeobacter* sp. S4 and *Bacillus pumilus* RI06-95 as potential agents to prevent larval and juvenile mortality by the oyster pathogens *Vibrio tubiashii* and *R. crassostreae*. In the experiment, pretreatment of larval and juvenile oysters for 24 h with 10^2 – 10^6 cfu/ml of *Phaeobacter* sp. S4 or *B. pumilus* RI06-95 protected larval oysters challenged with *R. crassostreae* and *V. tubiashii* (RPS range, 9–56 %), and for juvenile oysters the rate against *V. tubiashii* RPS was reported to be 37–50 %. Moreover, the protection conferred to larvae by probiotic isolates against bacterial pathogens was short-lived, lasting only for 24 h after removal of the probiotics from the incubation water, and it had no negative impact on oyster survival. These results suggested the potential of the marine bacterial isolates *Phaeobacter* sp. S4 and *B. pumilus* RI06-95 to serve as biocontrol agents to reduce the impact of bacterial pathogens in the culture of *Crassostrea virginica* (Karim et al. 2013).

Water productivity enhancement

Water probiotics have been reported to regulate microflora, control pathogens, enhance the decomposition of undesirable organic substances and improve the ecological environment by minimizing toxic gases such as NH_3 , N_2O , H_2O_2 and methane (Venkateswara 2007). It is suggested that by maintaining high levels of probiotics in production ponds, fish farmers can minimize the accumulation of dissolved and particulate organic carbon during the growing season. In addition, probiotics can balance the production of phytoplankton (Balcazar et al. 2006). However, this hypothesis could not be confirmed by assessments carried out during cultivation of shrimps and channel catfish, using one or more species of *Bacillus*, *Nitrobacter*, *Pseudomonas*, *Enterobacter*, *Cellulomonas* and *Rhodopseudomonas*. Thus, published evidence for improving water quality is limited, except for nitrification (Verschuere et al. 2000; Cruz et al. 2012). However, aerobic denitrifiers are considered to be good candidates to reduce nitrates and nitrites to N_2 under aerobic conditions in aquaculture water. Liao et al. (2006) isolated the new aerobic denitrifying strain X0412 from shrimp ponds, which was identified as *Stenotrophomonas maltophilia*. This denitrifying strain was found to contain the nitrite reductase gene *nirS*. Later, 27 denitrifying bacterial strains were

isolated from a shrimp pond. 16S rDNA sequence analysis revealed that the 27 bacterial strains belonged to 11 genera, including *Pseudomonas*, *Halomonas*, *Acinetobacter*, *Paracoccus*, *Arthrobacter*, *Microbacterium*, *Cellulosimicrobium*, *Bacillus*, *Stenotrophomonas* and *Sphingobacterium* (Wang et al. 2007a, b). In the case of edible fish, trout production farms generate high concentrations of nitrogen ranging from 0.05 to 3.3 mg/l of total Kjeldahl nitrogen and up to 6.4 mg/l after 7 months of monitoring (Maillard et al. 2005). For tilapia production in recirculating systems, concentrations of total ammonia ($\text{NH}_4 + \text{NH}_3$) increased from 4.73 to 14.87 mg/l in a 21-day experiment, while the nitrite concentration increased from 3.75 to 9.77 mg/l (Rafiee and Saad 2005). Due to the high concentrations of produced nitrogen compounds, especially the highly toxic total ammonia nitrogen, the use of probiotics is recommended as they may improve the water quality (Devaraja et al. 2013). El-Haroun et al. (2006) supplemented the food of Nile tilapia (*Oreochromis niloticus*) with a commercial probiotic made from *Bacillus licheniformis* and *B. subtilis* in 17 weeks of culture. Assessment of water quality parameters showed an acceptable range for fish cultivation: 5.7–6.3 mg/l for the dissolved oxygen concentration, 0.36–0.42 mg/l for the ammonia concentration, and pH between 6.3 and 8.2 (El-Haroun et al. 2006). Laloo et al. (2007) isolated several strains of *Bacillus* from *Cyprinus carpio* and carried out tests to improve the water quality and inhibit the growth of *Aeromonas hydrophila* in ornamental fish culture. Three out of nine isolates showed a high capacity to inhibit the pathogens with a relative incidence rate of 78; moreover, concentrations of ammonia, nitrate and phosphate were lowered to rates of 74, 76 and 72 %, respectively. Contrarily, Queiroz and Boyd (1998) examined the effects of a commercial probiotic in catfish (*Ictalurus punctatus*), noting a significantly higher survival and net fish production when the probiotic was applied. However, very few differences were significant for the determined water quality variables—ammonia, chemical oxygen demand, nitrates, soluble reactive phosphorus and dissolved oxygen between the treated and control ponds. Taoka et al. (2006a, b) studied the effects of commercial probiotics formulated from mixed cultures of bacteria and yeast on survival in Japanese flounder (*Paralichthys olivaceus*) and water quality in a closed recirculating system. The probiotic-treated groups showed

significantly greater survival rates compared to the control group at the end of the rearing experiment in a 50-day culture period, and water quality parameters were significantly lower in the probiotic diet groups. In the interim, Wang et al. (2005) showed that a commercial product made from *Bacillus* sp., *Saccharomyces cerevisiae*, *Nitrosomonas* sp. and *Nitrobacter* sp. had the ability to increase the beneficial bacterial microbiota of *Penaeus vannamei* shrimp, further reducing the concentrations of inorganic nitrogen from 3.74 to 1.79 mg/l and phosphate from 0.1105 to 0.0364 mg/l (Jiqui et al. 2006). Dalmin et al. (2001) showed that *Bacillus* spp. improved water quality and growth rates and increased the health status of juvenile of *Penaeus monodon* during the culture period (Verschuere et al. 2000). Hena et al. (2008) studied the effects of bacterial products during the culture period of black tiger shrimp (*Penaeus monodon*). The results showed the improvement in the organic matter and total sulfur (TS) in pond sediments during the culture period. The organic matter content in the ponds was low (3.95 ± 0.56 %), probably because of the decomposition activity of bacteria during the mineralization process. The lower (1.58 ± 0.33 %) concentration of TS in the culture pond sediments suggested that heterotrophic bacteria utilized the superficial soil sulfate compounds, which were converted into sulfur and its related compounds. The water quality was within the suitable range for shrimp growth and did not cause stress. The higher concentrations of major macronutrients such as Ca, Na, Mg and K in pond sediments could be partly attributed to nutrient loading and accumulation from soil pore water during drying over time and pond age (Forsius et al. 2012). None of the elements accumulated in the ponds reached harmful concentrations for pond health and the cultured shrimp species.

Stress probiotics in aquaculture

Stress is defined as physical or chemical factors causing bodily reactions that may contribute to disease and death (Rottmann et al. 1992). Biological stress is also known as a “non specific response of the body” to any demand made upon it, which could be evoked from a variety of different kinds of stressors (Selye 1973, 1985). Global climate change is suggested to potentially affect the aquaculture sector by

lowering productivity in wild fish populations and in intensive aquaculture systems worldwide (Ficke et al. 2007). Particular attention has been drawn to stocking density as one of the key factors influencing the perceived level of stress in fish (Turnbull et al. 2005; North et al. 2006). As fish are poikilothermic animals, any alterations in water parameters beyond the optimum level have a profound influence on the physiological and behavioral activities. Fish may encounter different types of stress: thermal (LeBlanc et al. 2011; Logan and Somero 2011; Das et al. 2005; Akhtar et al. 2011, 2012a), nutrition, stocking density (Lupatsch et al. 2010), anoxia, hypoxia, chemical and pesticide (DeMicco et al. 2010; Prusty et al. 2011; Vani et al. 2011). However, in nature fish are often resistant to these pathogens; many potential fish disease pathogens are continually present in the water, soil, air or fish (Rottmann et al. 1992; Smith et al. 2012) (Table 6).

To avoid these stressful conditions, intervention with immunostimulants, vaccines and probiotic bacteria, either as a feed supplement or in water, could trigger the defense system and thus ameliorate the harmful effects mediated by different stress factors (Sarma et al. 2009; Akhtar et al. 2010; Rio-Zaragoza et al. 2011; Ringo et al. 2012; Maqsood et al. 2011; Meena et al. 2013a, b, Akhtar et al. 2012b; Tejpal et al. 2009).

Mechanism of action of stress probiotics

Cortisol level and osmoregulation equilibrium

Commercialization of probiotics has increased their accessibility on the market for use in post-stocking management (Gatesoupe 1999; Gomez-Gil et al. 2000). These products are primarily composed of highly concentrated bacteria, vitamins and nutrients (Verschuere et al. 2000). Nowadays, blood glucose, serum cortisol and the RNA/DNA ratio of the different tissues are deployed as reliable biochemical stress markers or fleshiness indicators to study the fish stresses, growth and health status at various levels (Marcel et al. 2009; Smith and Buckley 2003; Mukherjee and Jana 2007; Sivaraman et al. 2012; Meena et al. 2013a, b). These probiotic bacteria were found to reduce the biochemical stress levels. One of the first formal reports on this aspect was supplementation of *Lactobacillus delbrueckii* spp. in the diet of European sea bass (*Dicentrarchus labrax*) at time

intervals of 25–59 days; it concluded that cortisol levels obtained in the treated fishes were significantly lower than those in the controls (Carnevali et al. 2006). Gomes et al. (2008, 2009) noted that incorporation of *Bacillus* spp. during transport reduced handling stress by reducing the cortisol levels. Furthermore, cortisol has been found to play important roles in osmoregulation and to mitigate salinity stress via control of Na^+ , K^+ -ATPase activity, ionocyte size and density and drinking rate (Varsamos et al. 2005) and was necessary for hypo-osmoregulation by larval summer flounder (*Paralichthys dentatus*) in seawater (Veillette et al. 2007).

Amendment in pH of culture system

A buffering system to avoid wide pH swings is essential in aquaculture (Swann 1990). Without some means of storing carbon dioxide released from plant and animal respiration, pH levels may fluctuate in ponds from approximately 4–5 to over 10 during the day (Boyd 1979). The acceptable pH range for fish culture is normally between 6.5 and 9.0. Abrupt spacious deviation from this range may cause mass mortality in fishponds. The quantity of hydrogen ions (H^+) in water will determine whether it is acidic or basic. The pH may vary in correspondence with the other water parameters also. In lentic or lotic water bodies, complex ligand formation of numerous chemicals with either H^+ ion uptake or release of other alkaline compounds leads to pH variability at different time intervals. This has been studied by many researchers along with combinations of probiotic bacteria as feed supplements or separately (Millero 2002). These differences probably resulted from the direct uptake of ammonia by probiotic bacteria, resulting in differing H^+ concentrations. In control shipping bags, increased total ammonia–nitrogen concentrations could have led to increased uptake of free H^+ by NH_3 , thus yielding the observed higher pH. *Bacillus* spp. have long been recognized to utilize multiple nitrogen sources, including both NH_3 and NH_4^+ , for catabolism of proteins. Without buffer utilization, significant differences between initial and final pH were anticipated. This is readily explained by the metabolic production of CO_2 and subsequent carbonic acid (H_2CO_3) formation from the reaction with water molecules (Millero 2002; Estudillo and Duray 2003; Benetti et al. 2007; Colburn et al. 2008;

Brownell 1980). Brauner (2008) reported that exchange of HCO_3^- as a waste product of respiration reduced the pH, which led to an increase in anhydrase activity as a physiological stress indicator.

Refinement in total ammonia nitrogen concentration

Water quality in both recirculating and confined culture systems is determined by the DO concentration, combining ammonia nitrogen, nitrite and CO_2 . Concentration levels of nitrates, pH and alkalinity of water are important water quality parameters (Cristea et al. 2002). Elena et al. (2011) analyzed the use of a probiotic feed containing 30 % protein and varying concentrations of the probiotic BioPlus 2B[®], consisting of *Bacillus licheniformis* (DSM 5749) and *Bacillus subtilis* (DSM 5750) at a ratio of 1:1. This was enough to maintain the recycled water quality in the allowable range of culture supported by the *cyprinus carpio* juveniles. Padmavathi et al. (2012) suggested that in intensive and semi-intensive aquaculture practices, high stocking densities of fish along with intense feeding and fertilization often lead to the deterioration of water quality and proliferation of pathogens. They can be treated with *Nitrosomonas* spp. to keep water quality in an optimal range, which might be because of the various roles played by the probiotic bacteria. Furthermore, new approaches to ammonia mitigation have evolved in the past decade; Anammox bacteria are obligate anaerobic chemolithoautotrophs, belonging to planktomycetes group, and are extremely slow growing in nature with 11 days for doubling time. The new approaches include processes such as combined the SHARON–Anammox process (single reactor system for high ammonium oxidation, nitrite-anaerobic ammonia oxidation) and CANON (completely autotrophic nitrogen removal over nitrite) as detailed by Paredes et al. (2007).

Regulation of the pollution mediated oxidative stress

Oxidative stress is defined as a situation when the steady-state, reactive oxygen species (ROS) concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation, damaging cellular constituents (Jia et al. 2011; Lushchak 2011; Livingstone 2001). ROS production is closely

matched by antioxidant responses (Lesser 2006). Fish are particularly threatened by aquatic pollution, and the environmental stresses frequently activate the endogenous production of ROS, most of which are generated as byproducts of tissue respiration (Padmini 2010; Gerschman et al. 1954; Cadenas 1989). It has reported that pesticides have a great impact on the oxidative status of fish in open water systems. Elevated lipid peroxidation (LPO) in fish from heavily polluted field sites was observed (Ferreira et al. 2005; Farombi et al. 2007; Sanchez et al. 2007). Stress conditions such as temperatures and pesticide levels beyond a critical limit cause hypoxia, which leads to increased production of ROS, resulting in oxidative stress inside and outside the cell (Kassahn et al. 2009; Wells 2009; Catarina et al. 2012). Hypoxia, whatever the cause, was found to cause a significant reduction in appetite and result in poor food ingestion and growth in several fishes, especially salmonids (Wang et al. 2009; Foss et al. 2003). In contrast, environmental hyperoxia is beneficial to fish growth, especially at high ammonia levels and stocking densities (Dong et al. 2011). In addition, hydrological changes, hydromorphological degradation and invasive species can also contribute to the stress factors (Amado et al. 2006; Sureda et al. 2006; dos Anjos et al. 2011). However, although it has been proposed that dietary supplementation of products with antimicrobial and antioxidant properties may be a promising disease prevention option for increasing resistance of shrimps to pathogens (Mathew et al. 2007), very few studies have been performed to elucidate this. For instance, Chiu et al. (2007) reported that administering *Lactobacillus plantarum* can enhance the antioxidant status of *Litopenaeus vannamei* and could lead to an increase in resistance to *V. alginolyticus* infection. Castex et al. (2009) evaluated the effect of *Pediococcus acidilactici* MA 18/5 in the antioxidative response of the shrimp *Litopenaeus stylirostris* to oxidative stress. The results showed high activity levels of the antioxidant enzyme superoxide dismutase were found in the control group compared to the treated one, plausibly because of the counter action of the probiotic. However, except for sodium oxidase dismutase (SOD) activity (Hsieh et al. 2008), to date, no study has been found reporting a significant effect of such dietary products, including probiotics, on the antioxidant response in shrimps following pathogen challenges.

Thermal stress tolerance enhancement

Fish generally experience stress, with the consequent suppression of the immune system when the temperature is inappropriate and chronically close to their maximum tolerance or fluctuates suddenly, which may lead to reductions in immune function, appetite, growth and reproduction, as well as susceptibility to disease and ultimately death (Pankhurst and King 2010; Daw et al. 2009). In addition, the number of fish forced to live at temperatures higher than their natural range is rising as a result of climate change, causing them to show signs of stress (Prowse et al. 2009). A characteristic feature of fish is the rapid release of stress hormones (Fabbri et al. 1998), and a generalized stress response system exists at the cellular level, which includes the actions and functions of various heat shock proteins (Goligorsky 2001; Iwama et al. 2004; Mao et al. 2005; Multhoff 2007; Keller et al. 2008). The functions of Hsps affect various aspects of fish physiology, including development and aging, stress physiology and endocrinology, immunology, environmental physiology, stress tolerance and acclimation (Basu et al. 2003). These proteins have been classified into several families based on their molecular weight, for example, Hsp90 (85–90 kDa), Hsp70 (68–73 kDa), Hsp60, Hsp47 and small Hsps (12–43 kDa) (Park et al. 2007; Hallare et al. 2004). To cope with stress, fish need to be nutritionally prepared to meet the demand and return to normal physiological conditions (Teixeira et al. 2012).

Some reports have suggested that stress tolerance can be increased by using probiotics; Japanese flounder (*Paralichthys olivaceus*) were grown in a recirculating system to assess the stress in fish, which involved subjecting them to heat shock (Taoka et al. 2006a, b). Stress tests were carried out until half the population had died, thus allowing the calculation of the mean lethal time (LT₅₀) in the absence of any stress probiotics and with the addition of a commercial probiotic containing *Bacillus subtilis*, *Lactobacillus acidophilus*, *Clostridium utyricum* and *Saccharomyces cerevisiae*. The group treated with probiotics showed greater tolerance in the stress test than the control group; the LT₅₀ was 40 and 25 min, respectively. Lactate and plasma glucose levels are considered appropriate indicators of stress as they increased as secondary responses during periods of stress to cover high energy requirements induced by this

situation. Furthermore, [Varela et al. \(2010\)](#) have conducted studies on the gilt-head bream (*Sparus auratus*); the glycogen and triglycerides reserves in the livers of the control group were significantly decreased in relation to concentrations obtained when the fish feed was supplemented with the probiotic *Alteromonas* spp. strain Pdp 11. [Tapia et al. \(2012\)](#) raised the possibility of preparing the fish in advance with probiotic treatment to counteract the conventional aquaculture stresses created by handling during transport, changes in water temperature and periodic manipulations.

Methods of probiotic administration and preservation

Generally, probiotics are applied in the feed, added to the entire tank or pond water to confer protection against infection ([Verschuere et al. 2000](#)). It is essential to investigate the best way means of introduction and the optimal dose, and technical solutions are required, especially to keep the probiotics alive in dry pellets ([Gatesoupe 1999](#)). This is a very important aspect as great losses in viability during processing and storage are generally reported ([Havenaar and Huisin't Veid 1992](#)).

Dietary administration

Probiotics used for dietary supplementation are mainly in the form of spores. [Fuller \(1989\)](#) emphasized the dietary administration of probiotics to improve the host's intestinal microbial balance ([Purwandari and Chen 2013](#)). Probiotics can be directly incorporated into feed pellets at a suitable temperature ([Gatesoupe 1999](#); [Magda et al. 2011](#); [Korkea-aho et al. 2012](#); [Diaz-Rosales et al. 2009](#)). Although dietary administration of probiotics is easy, the viability should be checked in feed pellets, and if it is less, the number of incorporations can be increased accordingly ([Prasad et al. 2005a, b](#)). Probiotics are usually added to feed as freeze-dried cultures, which are sometimes mixed with lipids to be added as top dressings in the feed ([Robertson et al. 2000](#); [Nikoskelainen et al. 2001](#)). Convective drying has been suggested by certain researchers as another means to preserve lactic acid bacteria starters using less expensive equipment than for freeze drying ([Linders et al.](#)

[1996](#); [Kets et al. 1996](#)). Another attractive possibility is to dry and preserve lactic acid probiotics added to the feed ([Galindo 2003](#)). [Mohapatra et al. \(2012\)](#) used *B. subtilis*, *L. lactis* and *S. cerevisiae* as feed probiotics given to *Labeo rohita* fingerlings, which exhibited a higher growth, protein efficiency ratio, nutrient retention and digestibility. Studies on ornamental fish, *Astronotus ocellatus* fingerlings, and juveniles of *M. rosenbergii* and *Litopenaeus vannamei*, using commercial probiotics products, obtained the same results ([Firouzbakhsh et al. 2011](#); [Rahiman et al. 2010](#); [Nimrat et al. 2012](#)). Antagonistic activity of probiotic bacteria against various pathogenic strains of bacterium in salmonids, gilthead sea bream (*S. aurata*), sea bass (*D. labrax*), sole (*S. solea*) and meagre (*A. regius*) has been reported ([Korkea-aho et al. 2012](#); [Sorroza et al. 2012](#)). Furthermore, [Olmos et al. \(2011\)](#) prepared a feed supplemented with alternative economical nutrient sources as an option to increase the yield and profits and to reduce water pollution.

Bioencapsulation

Encapsulation is a process allowing small molecules to pass in, forming a continuous coating around an inner matrix wholly contained within the capsule wall as a core of encapsulated material ([Vidhyalakshmi et al. 2009](#)). Bioencapsulation or bioenrichment is a process that can improve the nutritional status of live food organisms by either feeding or incorporating them together with various kinds of nutrients ([Imelda 2003](#)). Probiotic encapsulation technology (PET) has the potential to protect microorganisms and to deliver them into the gut ([Gbassi and Vandamme 2012](#)). Nevertheless, inoculation of probiotics through bioencapsulation in live food such as microalgae, rotifers and Artemia is an interesting approach, even though the process of administration through fortified live food seems not to be economically viable and is practically difficult in large-scale aquaculture practices. [Avendano and Riquelme \(1999\)](#) suggested that it is feasible to use microalgal cultures as vectors for the introduction of bacterial antagonists to bacterial pathogens in aquaculture. [Munro et al. \(1993\)](#) observed that the influence of bacteria brought by live food organisms was particularly dramatic during the first feeding. [Faramarzi et al. \(2011\)](#) assessed the effects of different concentrations of probiotic *Bacillus* spp. at different bioencapsulation times on the growth performance and

survival rate of Persian sturgeon (*Acipenser persicus*) larvae. The study revealed that sturgeon larvae were being fed at 30 % of their body weight for five times a day. This study showed a significant conversion efficiency ratio (CER), specific growth rate (SGR), food conversion ratio (FCR), condition factor (CF) and daily growth coefficient (DGC) ($p < 0.05$). However, survival of all groups was not significantly different after 28 days (Faramarzi et al. 2011). Skjermo and Vadstein (1999) described a technique for controlled transfer of immunostimulants to marine larvae through incorporation of *Artemia* and rotifers in live feed. Tamaru et al. (2003) reported that with 12- to 24-h enrichment of newly hatched *A. franciscana* with a lipid source, a significant increase in the highly unsaturated fatty acid (HUFA) content is detectable. Esteban (2012) reported that *Bacillus subtilis* and *Lactobacillus plantarum* bioencapsulated in *Artemia nauplii* achieved good results against vibriosis. Pirarat et al. (2011) studied the bacteria *Lactobacillus rhamnosus* GG from human origin and used it on Nile tilapia (*Oreochromis niloticus*) to study the growth performance, gut mucosal immunity, and humoral and cellular immune response, and a feeding trial was done by incorporating the bacteria directly into commercial dry pellets.

Immobilized probiotics

Entrapment of cells released in a gel matrix of alginates around the core substance is known as the wall of immobilization (Champagne et al. 1994). Probiotic immobilization is a new technology used extensively in the dairy and pharmaceutical industries, applied to LAB (Denkova et al. 2004). In particular, cell immobilization has been reported to offer many advantages for biomass and metabolite production compared with free cell systems, such as high cell density and very high volumetric productivity (Doleyres et al. 2004a), biocatalysts, high process stability over long fermentation periods (Lamboley et al. 1997), retention of plasmid-bearing cells (D'Angio et al. 1994; Huang et al. 1996), improved resistance to contamination, uncoupling of biomass and metabolite production, stimulation of production and secretion of secondary metabolites, and physical and chemical protection of cells (Doleyres et al. 2004b; Doleyres and Lacroix 2004; Lacroix et al. 2004). These benefits, however, are not yet being applied to aquaculture

probiotics. Immobilization for probiotics was suggested because viable and biologically active microorganisms are required at the target site in the host (Hussein and Kebary 1999; Sun and Griffiths 2000; Picot and Lacroix 2002). For human probiotics, in order to avoid the effects of peristalsis, which tends to flush out bacteria with food, immobilization was suggested (Suvarna and Boby 2005).

Evaluation, quality control and diffusion of probiotics in aquaculture

Evaluation should include consideration of both the end product formulation and mechanism of action, since these can induce adverse effects in some subjects or counteract the positive effects in total. 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). To date, various molecular fingerprinting techniques, using different genetic markers, have proven useful in subspecies discrimination or strain differentiation. A number of studies have evaluated the bacterial composition of commercial probiotic products for human consumption (Fasoli et al. 2003; Temmerman et al. 2003; Huys et al. 2006).

As the research on probiotic bacteria continues, novel species and species-specific strains of probiotic bacteria are constantly being 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, the safety of probiotics has become of utmost importance and therefore safety considerations for putative probiotics should be a prerequisite in the process of development and marketing (Mohapatra et al. 2013). Commercial probiotic production should take into account beneficial traits of strains useful during industrial processing. To overcome the problem of inactivation during the manufacturing process, aquaculture industries try to improve the technology by screening for more resistant strains or alternatively by protecting the probiont through micro-bioencapsulation. By monitoring probiotics and the microbial community structure and dynamics in the manufacturing process and in vivo culture system, the viability

and effects of probiotics can be documented in detail. For this purpose, nucleic acid-based techniques have been used. Highly discriminative molecular methods such as 16S rRNA gene sequencing and oligonucleotide probes can also be used for accurate probiotic species labeling, which is important for responsible quality control efforts, to build consumer confidence in product labeling and for safety considerations (Yeung et al. 2002).

Generally, the common methods for probiotic selection from the GIT of bony fishes are in vitro assay for NaCl tolerance, quantification of organic acid and determination of the pH value, assay for bile tolerance, assay for gastric juice resistance, assay for sensitivity to antibiotics, hemolytic activity, bacterial resistance to pepsin and pancreatin, sensitivity to antimicrobial agents and characterization by different molecular approaches (Itoi et al. 2008; Hosseini et al. 2009; Hovda et al. 2007; Leyva-Madriral et al. 2011; Nayak 2010). Generally, at the end of the previous phase, a pool of isolates results, which must be screened and preselected to obtain a restricted number of isolates for further examination. The application of in vitro tests to screen the acquired bacterial strains is a well-known mode of action to select the appropriate in vitro test. Since the evidence on the possible modes of action of probiotics is still equivocal, preference should be given to in vivo tests in the search for probiotics. The use of the target organism in the screening procedure provides a stronger basis.

Colonization and adhesion

The ability of a strain to colonize the gut or an external surface of the host and adhere to the mucus layer may be a good criterion for preselection among the putative probiotics. This involves the viability of the potential probiotic within the host and within its culture environment, adherence to host surfaces and the ability to prevent the establishment of potentially pathogenic bacteria. It is true that a candidate probiotic should either be supplied on a regular basis or be able to colonize and persist in the host or in its ambient environment. Examination of adhesion properties using intestinal cells has become a standard procedure for selecting new probiotic strains for human applications (Salminen et al. 1996), but it is less common in aquaculture (Wu et al. 2012).

Conclusion and future perspectives

In aquaculture, probiotics are administered by feed and water additives. In aquatic animals, it is difficult to be certain whether the beneficial effects are due to supplemented exogenous probiotic bacteria or intrinsic bacteria. The direct use of a probiotic in culture water is a special focus of environmental research. They are commonly foreign or exogenous strains, and represent a possible risk of microorganism pollution, especially with the use of strains with genetic modification, specific adhesions or colonization niches, antibiotic production and synergistic action. An environmental impact assessment study and an appraisal with regard to beneficial effects are prime prerequisites to introducing new probiotics into aquaculture (Lara-Flores 2011). However, supplementation of probiotics through feed is a better method to ensure the efficiency of the probiotic bacteria in the GIT of fish without interacting with the surrounding medium. However, their use in fish feed production is still rare. Generally, probiotics have proven their promising growth results in fish (Al-Dohail et al. 2009; Saenz de Rodriguez et al. 2009) by enhancing the feed conversion efficiency, as well as conferring protection against harmful bacteria by competitive exclusion, production of organic acids, hydrogen peroxide and several other compounds (Zhou et al. 2009; Rahiman et al. 2010; Abdullah et al. 2011; Youping et al. 2011; Tapia et al. 2012; Lin et al. 2012; Zhang et al. 2012). Although the role of probiotics in aquaculture is well known, the route of administration that is feasible in organisms other than fish and shellfish needs to be examined. For probiotic doses, the processes of transmission have yet to be standardized for better economic and sustainable uses and to augment the aquaculture production. Bacterial strains used as probiotics are also equally significant. As described by several authors, *Bacillus* spp. are commonly used as probiotic bacteria in animal nutrition, such as *Bacillus cereus*, *B. toyoi*, *B. licheniformis* and *B. subtilis* (Balcazar et al. 2006; Avella et al. 2012; Wu et al. 2012; Mohapatra et al. 2012). Even though probiotic applications in mariculture have been found to be limited, there has been increasing interest in the possible use of probiotics in marine species such as summer flounder, *Paralichthys dentatus* (Eddy and Jones 2002), common dentex, *Dentex dentex* (Hidalgo et al. 2006), and Japanese flounder, *Paralichthys*

olivaceus, (Taoka et al. 2006a, b). Still, exploration is essential to elucidate the beneficial effects of probiotic bacteria as stress probiotics, which may be of a great importance from the ecofriendly production and natural resource management point of view. Probiotics can also effectively trigger the piscine immune system. For all these reasons, their use will definitively increase in the future. All probiotics must be evaluated for their safety before being used in fish nutrition. Also, dose–response relationships need to be established (Denev et al. 2009). In the same way, the application of probiotics for fish has garnered much interest, and today a large number of studies have demonstrated their potential benefits to aquatic hosts. However, these studies were generally laboratory based or conducted in small-scale aquarium facilities, and thus efficacy at the industrial farm level needs to be determined. Additionally, as many of the underlying molecular mechanisms and signaling pathways are poorly understood, as is the impact on indigenous microbes, the reproducibility of these applications is often problematic (Merrifield 2012). Alternatively, there is a need to increase studies on microbial ecology in aquaculture systems, correlating it to microbial communities. Nowadays, a variety of probiotic strains present in the GIT of aquatic animals and nitrifying bacteria from biofilters have been isolated and characterized using biochemical, morphological and molecular techniques (Burr et al. 2007; Schulze et al. 2006; Sugita et al. 2012; Ling et al. 2012). The development of molecular techniques such as PCR (polymerase chain reaction), FISH (fluorescent in situ hybridization), DGGE (denaturing gradient gel electrophoresis) and generation of genomic libraries has started to unveil the diversity present in aquaculture systems. Currently, next-generation sequencing methodologies offer great potential for phylogenetic identification of probiotic microorganisms without using conventional cultivation techniques (Cruz et al. 2012). The application of up-to-date molecular procedures to study the gut microbiota and the development and validation of research methods, in vitro, ex vivo and in vivo models, have provided important information to help understand the mechanisms of action behind the effects. Synbiotics, as a combination of probiotics and prebiotics, have been studied to determine the synergistic effects. However, further investigations on the interaction between probiotics and other functional additives at the molecular level are warranted in

aquaculture. Furthermore, in this review it is highlighted that although probiotic preservation is essential, preservation techniques are yet to be established and standardized in the aquaculture sector.

Acknowledgments The authors are greatly indebted to Dr. S. Ayyappan, Director General, Indian Council of Agricultural Research New Delhi, India, for his encouragement and constant support. We extend our gratitude to Mr. Asim Kumar Jana for assisting the authors in typing the literature materials.

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