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Review Article

Overview of the use of probiotics in aquaculture

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Abstract

Probiotics, which have been widely used in livestock rearing, have recently been applied to aquaculture. Probiotics are defined as live cells or a substrate that provides benefits through stimulation of growth, improved digestion, and improved immune response. Probiotics can also improve water quality. This review summarizes the current understanding of the use of probiotics in aquaculture, including the definition and mechanism of probiotics, and describes their application, and prospects and difficulties associated with their use in aquaculture. This review includes general knowledge of probiotics from previous studies and evaluates the efficacy of probiotics in aquaculture.

1. Introduction

Aquaculture, which is now the fastest growing food-producing sector in the world, is moving in new directions, intensifying and diversifying (Bondad-Reantaso et al., 2005). With the increase in the intensification and commercialization of aquaculture production come many challenges, such as combatting diseases and epizootics, broodstock improvement and domestication, development of appropriate feedstuffs and feeding mechanisms, hatchery and grow-out technology, as well as water-quality management (Subasinghe, 2003). Of these, disease outbreaks are one of the important problems that affect aquaculture production, suppressing both economic and social development in many countries (Qi et al., 2009). Moreover, the availability of feed for aquaculture is another significant challenge in the intensifying aquaculture industry, as feed accounts for up to 70% of operating costs for most aquaculture species (Muzinic et al., 2004). Feed quality and feeding methods therefore need to be thoroughly considered in order to improve growth performance and feed efficiency of the cultured animals. Several previous reports have suggested that probiotic supplementation can reduce disease outbreaks by enhancing the immune system of fish and shrimp (Kim and Austin, 2006; Mohideen et al., 2010; Wang and Gu, 2010), and can decrease culture costs by improving the growth and feed efficiency of fish (Wang and Xu, 2006; Soundarapandian et al., 2010; Faramarzi et al., 2011; Mohapatra et al., 2012; Peterson et al., 2012). In addition, by improving animal physiology, the application of probiotics can lead to an improvement in water quality, as better feed efficiency may result in fish producing less waste (Boyd and Gross, 1998; Velmurugan and Rajagopal, 2009; Ngan and Phu, 2011; Nimrat et al., 2012).

The application of probiotics in aquaculture has been widely used as a means of controlling disease, enhancing immune response, providing nutritional and enzymatic contributions to the digestion of the host, and improving water quality (Qi et al., 2009). Probiotics are also regarded as an environmentally friendly treatment method. The probiotics may be added to feed as live microorganisms to create a balanced indigenous microfloral community in the gastrointestinal tract (Rengpipat, 2005). Moreover, probiotics are being considered for use as therapeutic agents and some farmers are already using them preferentially over antibiotics (Fuller, 1989). 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). This review summarizes studies on probiotics and evaluates further applications of probiotics in aquaculture.

2. Definition of probiotic

The term probiotic has its origins in Greek words meaning "for life" (Gismondo et al., 1999). It was originally used by

Lilley and Stillwell (1965) to describe one of the substances produced by protozoans that stimulates other microorganisms, and it was later used to describe animal feed supplements that benefit the host animal (Fuller, 1989). Fuller (1989) revised the definition to "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". This definition highlights the essential component of probiotics as being live cells and not only "substances". Other definitions used in aquaculture indicate that a probiotic is a live microbial food supplement that confers health benefits or disease resistance to the host (Lara-Flores and Aguirre-Guzman, 2009). The concept of aquatic probiotics is a relatively new one, and methods for evaluating the efficacy of probiotics are needed. Fuller (1989) proposed that a good probiotic has the following characteristics: (1) effectiveness in application; (2) non-pathogenic and non-toxic; (3) existing as viable cells, preferably in large numbers; (4) surviving and being actively involved in the metabolism of the gut environment and (5) being stabilized and remaining viable during long periods of storage and under field conditions.

The difference in the intestinal flora of aquatic and terrestrial animals is a consequence of the differences in the surrounding environment. The intestinal microbiota of aquatic animals, therefore, mostly resembles the microbiota in the water environment. In aquatic animals, probiotic strains with two sources, indigenous and exogenous microbiota, have been isolated. Gram-negative facultative anaerobic bacteria, *Vibrio* and *Pseudomonas,* are the predominant indigenous microbiota of marine fish species. Other major indigenous microbiota of freshwater fish species include *Aeromonas*, *Plesiomonas*, representatives of the family Enterobacteriaceae, and obligate anaerobic bacteria of the genera *Bacteroides*, *Fusobacterium* and *Eubacterium*, but lactic acid bacteria are generally sub-dominant in fishes (Rengpipat, 2005; Balcazar, 2006; Kesarcodi-Watson et al., 2008). However, the population dynamics of the indigenous gut microflora that colonize the gut are very complex, with many interrelationships among different microorganisms and among microorganisms and the host (Fuller, 1989). The maintenance and stability of microbial flora within aquatic animals is related to external environmental factors (Lara-Flores, 2011). This stability is not exhibited in bivalve larvae because of the short time for the transit of bacteria in bivalve larvae (Jorquera et al., 2001). Moreover, the effect of probiotic using on intestinal flora balance was defined and demonstrated only for some cases (Lara-Flores and Aguirre-Guzman, 2009). Tannock (1997) defined probiotics as "living microbial cells administered as dietary supplements with the aim of improving health".

3. Probiotics in aquaculture

3.1 Mechanisms of probiotics

Application of probiotics in aquaculture is recent and is a new concept compared to its use in mammals and other terrestrial livestock, such as cattle, swine, and poultry. The benefits of probiotics were evaluated alone or in combination for each probiotic. Numerous studies have examined the mechanisms by which probiotics improve the feed efficiency, control microbiota, or confer resistance to diseases, including (1) competitive exclusion of pathogenic bacteria through habitat competition, nutrient competition and alteration of enzymatic activities of pathogens; (2) contributing to nutrient availability and improvement of feed digestibility and feed utilization by enzymatic contribution; (3) bacteria-mediated direct uptake of dissolved organic material; (4) enhancement of the immune response against infectious pathogens; (5) antiviral effects (Balcazar, 2006; Kesarcodi-Watson et al., 2008). Furthermore, probiotics are also useful for improving soil and water quality (Boyd and Gross, 1998).

3.2 Methods of application

Probiotics have been supplied directly through feed, in some cases using binders for stabilization (Kolndadacha et al., 2011). Supplementing feed with probiotics is common in aquaculture; the aim of this method is to introduce live cells of probiotics to the host animal gut in order to establish a balanced gastrointestinal microbial flora and to improve digestive function or immune system responses. Probiotics, including bacterial strains, yeast and extracted substances, are generally supplied by this method of application. Probiotics are diverse and are usually derived from the intestines of the host animals (Tovar et al., 2002; Chantharasophon et al., 2011; Chu et al., 2011; Sun et al., 2012), cultured in diverse environments (Wang and Xu, 2006), and come have been developed into commercial products which are also introduced and used (Abraham et al., 2008; Fernandez et al., 2011). Some probiotics that have been supplemented in animal feed include bacterial species, such as *Lactobacillus* spp., *Enterococcus faecium*, *Bifidobacterium thermophilum*, *Streptomyces* spp., *Micrococcus* spp., *Pseudomonas fluorescens*, as well as yeast, such as *Saccharomyces cerevisiae*, as well as herbs and extracted substrates, such as azadirachtin.

Further, probiotics have been applied directly to culture ponds to improve water quality (Boyd and Cross, 1998) and the survival of cultured animals (Moriarty, 1998). The effectiveness of probiotics can be explained by bioaugmentation or biocontrol mechanisms by which the microbial ecology of the water and sediment is improved (Rengpipat, 2005). Several biological products, such as live bacterial inocula, enzyme preparations, and plant substrates extracts have been used as water and soil quality condition improvement factors in aquaculture ponds (Boyd and Cross, 1998). Probiotics include numerous strains of bacteria. In addition, the method of injecting probiotic products into aquatic animals has been used to stimulate the immune response of fish against bacterial pathogenic infection (Anderson and Siwicki, 1994; Sahoo and Mukherjee, 1999). Freeze dried probiotics can also be used as vaccinations in fish (Austin et al., 1995) because the host animal probiotics stimulate the immune system by promoting the activity of antibodies. However, it is difficult to inject probiotics into cultured fish, especially into small animals, and to treat large numbers of fish in this way.

4. Functions of probiotics

Numerous studies have demonstrated the benefits of probiotics for aquatic animals, such as the stimulation of growth or to improve feed digestion, immune responses and water quality control.

4.1 Growth and digestive process promoter

Supplementing the diet fish with probiotics can reduce the use of antibiotics and synthetic chemicals in the feed (Fuller, 1989). Consequently, the addition of probiotics to fish diets has become widespread on aquaculture farms. The application of probiotics results in reduced feed costs, which plays an important role in determining the practices of aquaculture. Interestingly, previous research findings have shown that the beneficial effects of probiotics can manifest as enhanced feed utilization of cultured aquatic animals through the supplementation of digestive enzymes, improved feed efficiency and higher growth, the prevention of intestinal disorders and the pre-digestion of anti-nutritional factors present in mixed feed (Balcazar et al., 2006; Suzer et al., 2008). The function of probiotics in the improvement of growth and feed utilization in fish was noted as related to the improvement of nutrient digestibility (Faramazi et al., 2011). Most probiotics colonize the host and affect the digestive processes through increased numbers and production of microbial enzymes, improving the intestinal microbial balance and consequently the digestibility and absorption of feed and feed utilization (El-Haroun et al., 2006; Mohapatra et al., 2012). After transitioning through the stomach, the microbes colonize the intestines and utilize a large number of sugars (carbohydrates) for growth and to produce a range of digestive enzymes (amylase, protease and lipase) (El-Haroun et al., 2006). However, it is important to consider the treatment processes for feed preparation in order to avoid deactivating or killing useful probiotic species in the culture (Mohapatra et al., 2012).

Table 1. Probiotics used to stimulate aquatic animal growth and digestive processes

Probiotic genus or species	Target host(s)	Reference
Bacillus	*Oncorhynchus mykiss*	Bagheri et al. (2008) Merrifield et al. (2010a) Merrifield et al. (2010b)
	Catla catla	Bandyopadhyay and Mohapatra (2009) Mohideen et al. (2010)
	Acipencer persicus	Faramarzi et al. (2011)
	Cyprinus carpio	Wang and Xu (2006)
	Carassius auratus, *Xiphophorus helleri*	Abraham et al. (2008)
	Epinephelus coioides	Sun et al. (2010)
	Litopenaeus vannamei	Geovanny and Shen (2008) Nimrat et al. (2012)
	Penaeus monodon	Rengpipat et al. (1998)
	Penaeus vannamei	Zhou et al. (2009)
	Macrobrachium rosenbergii	Deeseenthum et al. (2007)
	Babylonia areolata	Thao and Ngan (2011)
Lactobacillus	*Oreochromis niloticus*	Jatoba et al. (2011)
	Epinephelus coioides	Son et al. (2009) Sun et al. (2012)
	O. mykiss	Faramazi et al. (2011)
	Sparus aurata	Suzer et al. (2008)
Enterococcus faecium	*O. niloticus*	Wang et al. (2008)
Psychrobacter sp.,	*E. coioides*	Sun et al. (2011)
Carnobacterium divergens	*Gadus morhua*	Gildberg et al. (1997)
Pseudomonas pseudoalcaligenes	*Labeo rohita*	Chaudhary and Qazi (2007)
Micrococcus	*O. niloticus*	Osman et al. (2010)
Streptomyces	*X. helleri*	Dharmaraj and Dhevendaran (2010)
L. acidophilus, *Streptococus cremoris*, *L. bulgaricus*- 56, *L. bulgaricus*-57	*Peaneus indicus*	Fernandez et al. (2011)
B. subtilis, *L. lactis*, *S. cerevisiae*	*L. rohita*	Mohapatra et al. (2012)
Saccharomyces cerevisiae	*C. carpio*	Mazurkiewicz et al. (2005)
Debaryomyces hansenii, S. cerevisiae	*Dicentrarchus labrax*	Tovar et al. (2002)
Lactococcus lactis, E. faecium	*E. coioides*	Sun et al. (2012)
Lactic acid bacteria MM1 and MM4	*E. coioides*	Yang et al. (2010)
Biogen (El-Salam Veterinary Trading Company, Taiwan)	*O. niloticus*	El-Haroun et al. (2006) Khalafalla (2010)
Mannanoligosaccharide, fructooligosaccharide, galactooligosaccharide	*Salmo salar*	Grisdale-Helland et al. (2008)
Inulin	*Huso huso*	Reza et al. (2009)

Some microorganisms, such as *Agrobacterium* sp., *Pseudomonas* spp., *Brevibacterium* spp., *Microbacterium* spp., and *Staphylococcus* spp., may contribute to nutritional processes in Arctic charr (*Salvelinus alpinus*) (Ringo et al., 1995). The microbiota may serve as a supplementary source of food and microbial activity in the digestive tract and may be a source of fatty acids, vitamins (Sakata, 1990) and essential amino acids (Balcazar et al., 2006). Practically, a variety of microorganisms and substrates have been reported to have stimulatory function as a probiotic in specific growth rate, feed digestibility and utilization efficiencies and survival of the aquatic animal species (Table 1). In fact, the digestive organs are very sensitive to food composition and affect immediate changes in the activities of the digestive enzymes (Mohapatra et al., 2012). In the case of enzymes, secreting proteases breaks peptide bonds and produces free amino acids that can then be absorbed by the host (Mohapatra et al., 2012). However, using higher concentrations of probiotic does not always lead to improved growth performance (Son et al., 2009). Different probiotics have different functions in promoting growth and nutrient utilization in different fish species (Mohapatra et al., 2012). Thus, the effectiveness of probiotics in the culturing of aquatic animals depends on factors such as hydrobiont species, body temperature, enzyme level, and genetic resistance of the host, and water quality (Cruz et al., 2012). Moreover, host life stage also plays an important role in the evaluation of the efficiency of probiotic implementation. This was clearly evident in the attempts to introduce beneficial bacteria in bivalve larvae, as the transit time of bacteria in bivalve larvae was too short and it seemed to be difficult to establish bacterial populations (Jorquera et al., 2001; Kesarcodi-Watson et al., 2008).

In addition, prebiotics are known as non-digestible food ingredients that are beneficial in stimulating the growth of health-promoting bacteria in the intestinal tract in order to improve the balance of the host's intestinal bacterial population (Gatesoupe, 2005). Nevertheless, some researchers have noted that the supplementation of prebiotics had no positive effect on growth and feed digestibility in fish (Grisdale-Helland et al., 2008; Akrami et al., 2009). To some extent, the continuous supply of substrates in the intestine carries the risk that the pathogens could metabolize the compounds in the intestine (Gatesoupe, 2005). Thus, more studies on the effectiveness of using prebiotics for aquaculture diets are needed before implementing the application of prebiotics in farms and hatcheries for fish or shrimp.

4.2 Immune system promoters

The use of beneficial bacteria to displace pathogens through competition is being used in the animal industry as a preferable method to administering antibiotics and is now gaining acceptance for the control of pathogens in aquaculture (Moriarty, 1999; Nikoskelainen et al., 2003). Among the protective microflora that has been reported to be effective for forming a barrier against infectious pathogens (Rengpipat et al., 2000; Rengpipat et al., 2008; Wang et al., 2008; Bandyopadhyay and Mohapatra, 2009; Dhanasekaran et al., 2010; Yang et al., 2010; Sun et al., 2012) and for the production of regulatory factors, such as short-chain fatty acids and bacteriocins, in the fish digest tract (Herich and Levkut, 2002). The immune systems of larval fish, shrimps and other invertebrates are less well developed than they are in the adult stages. Consequently, larvae are typically more dependent on nonspecific immune responses for their resistance to infection. However, recent studies have demonstrated that the non-specific immune responses of the species listed in the table above can be stimulated by the supplementation of probiotics to the diet or to the culture water. The colonization rate of bacteria in the digestive tracts has been reported to depend on the level of bacteria in the feed (Bagheri et al., 2008). Other studies have tried to clarify the different mechanisms by which probiotics modulate the immune system of fish, including the stimulatory effect of pro-inflammatory cytokines on the activity of immune cells, antibodies, acid phosphatase, lysozymes, complement, and antimicrobial peptides, in response to invasive pathogens (Pirarat et al., 2006; Lara-Flores and Aguirre-Guzman, 2009). Generally, probiotics actively inhibit the colonization of potential pathogens in the digestive tract by antibiosis or by competition for nutrients and space, as well as alteration of the microbial metabolism and stimulating host immunity (El-Haroun et al., 2006). Nevertheless, the efficiency of different probiotics against pathogens differs depending on the defense mechanism of the fish species to different pathogens and the pathogenic mechanisms of the pathogen (Son et al., 2009).

Effects of probiotics on immune response and bacterial loading in aquatic organisms and the environment are well documented. It has been demonstrated that oral administration of *Clostridium butyricum* bacteria to rainbow trout enhanced their resistance to vibriosis by increasing the phagocytic activity of leucocytes (Sakai et al., 1995). The administration of probiotics by live food and/or culture water dramatically decreased bacterial activity in some teleosts such as *Sparus aurata*, *Paralichthys dentatus*, *Scophthalmus maximus* and *Salmo salar* (Suzer et al., 2008). The rod-shape beneficial bacteria, lactic acid bacteria (strains MM1 and MM4) were reported along with the secretion of hydrogen peroxide and bacteriocin-like substances, which have strongly inhibitory activities against pathogens of gram-negative *Vibrio metschnikovii* and *V. harveyi*, and gram-positive *Staphylococcus aureus* that infects orange-spotted grouper (*E. coioides*) (Yang et al., 2010); further, the phagocytic activity and phagocytic index, serum lysozyme activities, serum complement C3 levels and serum IgM levels of *E. coioides* were high in *Bacillus*-treated fish groups (Sun et al., 2010). Nikoskelainen et al. (2003) showed that administration of lactic acid bacterium *Lactobacillus rhamnosus* (strain ATCC 53103) at a level of about 10^5 cfu g^{-1} feed stimulated the respiratory burst in rainbow trout (*O. mykiss*).

Unlike higher vertebrates, which have an acquired immune response, shrimp have an innate immune response (Rengpipat et al., 1998; Rengpipat et al., 2000; Zhou et al., 2009; Ismail and Soliman, 2010; Soudarapandian et al., 2010; Wang and Gu, 2010; Zokaeifar et al., 2012; Purivirojkul, 2013). Due to the relatively simple immune system, probiotics were expected to play an important role in the stimulation of the immune response in shrimp (Lakshmi et al., 2013). Rengpipat et al. (2000) determined that the use of *Bacillus* sp. (strain S11) provided disease protection by activating both cellular and humoral immune defenses in tiger shrimp (*P. monodon*). The administration of a mixture of bacterial strains (*Bacillus* spp. and *Vibrios* spp.) positively influenced the growth and survival of white shrimp juveniles and gave a protective effect against pathogens *V. harveyi* and white spot syndrome virus. This protection was attributed to the stimulation of the immune system by increasing phagocytosis and antibacterial activity (Balcazar, 2006). *Bacillus* spp. was used to improve and control the *Vibrio* spp. infection to penaeid shrimp (Moriarty, 1998; Moriarty, 1999). In addition, *Vibrio* spp. cells, lipopolysaccharide (LPS), peptidoglycan, β-1-3 glucan, fucoidan, laminaria, yeast glucans have been experimentally tested in small-scale cultures, and the results suggest that these can be used as important elements in the control of disease in shrimp or crustacean through immunostimulation (Lara-Flores and Aguirre-Guzman, 2009). Moreover, the probiotic bacteria *L. plantarum* and *B. subtilis* (strains L10 and G1) were reported to promote immune responses and gene expression in white shrimp (*L. vannamei*) when supplied in the diet. *L. plantarum* improved phenoloxidase, prophenoloxidase (proPO), respiratory bursts and superoxide dismutase activity, and clearance efficiency of *Vibrio alginolyticus*, peroxinectin (PE) mRNA transcription, and survival rate after challenge with *V. alginolyticus* for 168 h when supplied to shrimp at 10^{10} cfu probiotics/kg diet (Chiu et al., 2007). The *B. subtilis* (strains L10 and G1) enhanced the up-regulation of immune-related genes comprising proPO, PE, LPS- and β-1,3-glucan-binding protein and serine protein after challenge with *V. harveyi* for eight weeks when probiotics were supplied at 10^5 and 10^8 cfu/g feed to shrimp (Zokaeifa et al., 2012).

4.3 Water quality improvement

The susceptibility of cultured aquatic species to high concentrations of nitrogenous compounds, such as ammonia, nitrite and nitrate, is generally species-specific but high concentrations of these compounds affect animals in aquaculture and likely cause high mortality. The application of gram-positive *Bacillus* spp. is generally more efficient than the application of gram-negative bacteria species for converting organic matter back to CO_2, which results in the conversion of a greater percentage of organic carbon to bacterial biomass or slime (Verschuere et al., 2000). The effectiveness of aerobic gram-positive endospore-forming bacteria, such as *Bacillus* spp., for improving water quality by affecting the composition and abundance of waterborne microbial populations associated with farmed species was evaluated (Bandyopadhyay and Mohapatra, 2009). *Bacillus* spp. were associated with improvement of water quality, reduction of pathogenic vibrios in culture environment, enhancement of survival and growth rate, and the improved health status of juvenile *Penaeus monodon* (Dalmin et al., 2001; Ngan and Phu, 2011). In parallel, other beneficial bacterial species in the genera *Nitrobacter, Pseudomonas, Enterobacter, Cellulomonas* and *Rhodopseudomonas*, and probiotics derived from plant sources, including yucca extract, potassium ricinoleate, tannic acid and citrus seed extract were also reported to have been used in culture systems noted to have considerable improvement in water quality (Boyd and Cross, 1998; Verschuere et al., 2000). The requirement for the use of candidate probiotics in aquaculture ponds is the enhanced decomposition of organic matter, reduced nitrogen and phosphorus concentrations, improved algal growth, improved availability of dissolved oxygen, suppressed cyanobacteria blooms, controlled ammonia, nitrite, and hydrogen sulfide concentrations, lower incidences of disease, greater survival, and improved production (Boyd and Cross, 1998).

5. Prospects and challenges

The use of probiotics in aquaculture is becoming more popular. As described herein, the use of probiotics confers many advantages, such as improved growth, feed efficiency, enhanced immune system response, as well as improved water quality. Further studies are needed to thoroughly understand the mechanisms of probiotics. Probiotics are more effective when used in the early stages of culture. As aquatic animals are in direct contact with their environment, supplementation to the water can be an effective. For example, exposure to probiotics in the feed at the larval stage may lead to the development of a positive transient intestinal flora that may become established at later stages. Further, regular application of probiotics through feed to animals reared in captivity can be used to maintain the microbial population in the gastrointestinal tract at a level that can express sufficient functionality.

In principle, probiotic bacteria were directly isolated from the gastrointestinal tract and were then applied to this host species, but recently, many commercial probiotics have been developed and used. Probiotics have the potential to positively or negatively impact both the animals in aquaculture and the surrounding environment. The identity of the bacteria strain and host is extremely important and determines the characteristics of the relationship. Therefore, the selection and source of probiotics play an important role; in particular, optimization of the probiotic application is important to avoid unnecessary expense. Moreover, mutations may occur in the natural environment; thus, dominant populations of supplied probiotics may become pathogenic and may be harmful to host animals, which are stressed or weakened state of health.

A number of probiotic products have been researched as evidenced by their efficacy in aquaculture. Beneficial bacterial inocula that are species-specific probiotics have become widely available to the aquaculture industry. These preparations have been refined to have more effective function as applied probiotics. Further, the quality control of probiotic products should be thoroughly considered. The application of new analysis methods, including molecular methods, for the evaluation of probiotic products and for *in vivo* validation, is expected to significantly improve both the quality and functional properties of probiotics.

6. Conclusions

In conclusion, the specific functions of probiotics in aquaculture may not be denied. Probiotics confer benefits of increased disease resistance, improved nutrient digestibility and growth in the host animals, and they also improve culture water quality. Although numerous reports have demonstrated the efficiency of probiotics, most of these studies were conducted and evaluated under laboratory conditions. Therefore, the application of probiotics under culture conditions is necessary in order to accurately evaluate their use. In particular, the consideration of species, source, quality and application methods will be needed to evaluate the use of probiotics.

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