

Review

The use of probiotic in aquaculture: an overview

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In last decade, the total world fishery production decreased slightly and the human consumption for aquatic product increased. The reduction in capture fisheries was partly compensated for the fast growth of aquaculture industry. The need for enhanced disease resistance, feed efficiency, and growth performance of cultured organisms is substantial for various sectors of this industry. If growth performance and feed efficiency are increased in commercial aquaculture, the costs productions are likely to be reduced. Also if more aquatic organisms are able to resist diseases and survive the subsequent cost of medication and overall production costs would be reduced. Hormones, antibiotics, ionophores and some salts compounds have been used at some extent to prevent disease and as growth promoters; however, their inadequate application can produce adverse disorders, such as hormone imbalance, poisoning and predisposition to disease development. In the search of new options, several studies have been carried out to test new compounds, from which the aquaculture industry has developed the concept of "functional additives". Among these additives, the additions of microorganisms to diets, named probiotics, has shown to improve the energy expenditure derived from other sources such as carbohydrates and increase the incorporations of protein for growth; increase the immunity and disease resistance of host organism. The use of probiotics in aquaculture just begun, since that gastrointestinal microbiota of aquatic organisms has been poorly characterized; and their effects not be study extensive. This review summarizes and evaluates current knowledge of use and the action of probiotic in fish culture; and the potential for further application in aquaculture production.

Keywords: *probiotic, bacteria, growth promoters, disease control, aquaculture*

INTRODUCTION

Aquaculture is a fast-growing and rapidly expanding multibillion dollar industry. Marine capture fisheries and aquaculture supplied the world with about 104 million tons of fish in 2004 (FAO, 2007). Of this total, marine aquaculture accounted for about 18%, where shrimp from aquaculture continues to be the most important commodity traded in terms of value (2.4 million tons). Worldwide, the aquaculture sector has been expanding at an average compounded rate of 9.2% per year since 1970, compared with only 1.4% for capture fisheries and 2.8% for terrestrial-farmed meat production systems.

With the increasing intensification and commercialization of aquaculture production, disease is a major problem in the fish farming industry (Bondad-Reantaso et al., 2005). Although vaccines are being developed and marketed, cannot be used as a universal

disease control measure in aquaculture. During the last decades, antibiotics used as traditional strategy for fish diseases management but also for the improvement of growth and efficiency of feed conversion. However, the development and spread of antimicrobial resistant pathogens were well documented (Kim et al., 2004; Cabello, 2006; Sørum, 2006). There is a risk associated with the transmission of resistant bacteria from aquaculture environments to humans, and risk associated with the introduction in the human environment of nonpathogenic bacteria, containing antimicrobial resistance genes, and the subsequent transfer of such genes to human pathogens (FAO, 2005). On other hand antibiotics inhibit or kill beneficial microbiota in the gastrointestinal ecosystem it also made antibiotic residue accumulated in fish products to be

harmful for human consumption (WHO, 2006). Considering these factors, as well as the fatal effect of residual antibiotics of aquaculture products on human health, the European Union and USA implemented bans on, or restricted the use of antibiotics (Kesarcodi-Watson et al., 2008). The norms are stringent and there are many events of returning consignments to the exporting countries for not maintaining the prescribed standards.

In connection with the ban of antibiotic growth promoters new strategies in feeding and health management in fish aquaculture practice have received much attention (Balcázar et al., 2006). In addition, the global demand for safe food has prompted the search for natural alternative growth promoters to be used in aquatic feeds. There has been heightened research in developing new dietary supplementation strategies in which various health and growth promoting compounds as probiotics, prebiotics, synbiotics, phytobiotics and other functional dietary supplements have been evaluated (Denev, 2008).

In this context, microbial intervention can play a vital role in aquaculture production, and effective probiotic treatments may provide broad spectrum and greater nonspecific disease protection (Rengpipat et al., 2000; Panigrahi and Azad, 2007). The range of probiotic microorganisms examined for use in aquaculture includes both Gram-negative and Gram-positive bacteria, bacteriophages, yeasts, and unicellular algae (Irianto and Austin, 2002). The selection for probiotic candidate organisms was based on in vitro antagonism (Vershuere et al., 2000), as well as on the results of adhesion, colonization, and growth in intestinal mucus (Irianto and Austin, 2002; Vine et al., 2004).

This review summarizes and evaluates current knowledge of the use and the action of the probiotic in aquaculture; and the potential for further application of this in production.

Definition of probiotic

The term probiotic means “for life,” originating from Greek words “pro” and “bios” (Gismondo et al., 1999). The concept of probiotic was originally used by Lilley and Stillwell (1965) to mean a substance (s) that stimulates growth of other microorganisms (Chukeatirote, 2002). Parker in 1974 modified the definition to “organisms and substances which contribute to intestinal balance”. Fuller (1992) revised the definitions as “ A live microbial feed supplement which beneficially effects the host animal by improving its intestinal microbial balance”. This definition has put forward the importance of live cells as the essential component of a potential probiotic and its clears the confusion created by the use of term “substance”. However, an effect in intestinal microbial balance has been defined and demonstrated only in few cases. This

noted by Tannock (1997), and he proposed the following definition “living microbial cells administered as dietary supplement with the aim of improving health”.

The concept for aquatic probiotic is a relatively new. When looking at probiotics for an aquatic usage it is important to consider certain influencing factors that are fundamentally different from terrestrial based probiotics. Aquatic animals have much closer relationship with their external environment. There are the big differences between terrestrial and aquatic animals in the level of interaction between the intestinal microbiota and the surrounding environment. Potential pathogens are able to maintain themselves in the external environment of the animal (water) and proliferate independently of the host animal (Hansen and Olafsen, 1999; Verschuere et al., 2000). These potential pathogens are taken up constantly by the animal through the processes of osmoregulation and feeding. The bacterial community composition of the intestinal tract of aquatic animals is different from that found in terrestrial animals, which the probiotic concept was developed. Man and terrestrial livestock undergo embryonic development within an amnion, whereas the larval forms of most fish and shellfish are released in the external environment at an early ontogenetic stage. These larvae are highly exposed to gastrointestinal microbiota-associated disorders, because they start feeding even though the digestive tract is not yet fully developed (Timmermans, 1987), and though the immune system is still incomplete (Vadstein, 1997). Thus, probiotic treatments are particularly desirable during the larval stages (Gatesoupe, 1999). A study with Atlantic halibut (*Hippoglossus hippoglossus*) showed the transition from a prevailing *Flavobacterium spp.* intestinal flora to an *Aeromonas spp.* and *Vibrio spp.* dominant flora occurred when first feeding commenced (Bergh et al., 1994). This study highlighted the impact that the external environment and feeding had on the microbial status of fish. However, the same study also found that larvae did maintain a specific intestinal flora different to that of the external tank flora. This showed that, although there were ever-present external environmental factors influencing the microbial flora inside an aquatic animal, they could still maintain a host specific flora at any given time. It was suggested that this ability did not apply to bivalve larvae (Jorquera et al., 2001). Their work demonstrated that the transit time of bacteria in bivalve larvae was too short to allow the establishment of a bacterial population different from that of surrounding water.

Based on the intricate relationship an aquatic organism has with the external environment when compared with that of terrestrial animals, the definition of a probiotic for aquatic environments needs to be modified. Gatesoupe (1999) redefined probiotics for aquaculture as “Microbial cells that are administered in such a way as to enter the gastrointestinal tract and to be kept alive, with the aim of

improving health". The definition of Gatesoupe is focuses on the oral delivery of the probiotic and its ability to improve the health of the host as a result of its presence in the digestive tract. Verschuere et al. (2000) suggested the definition "a live microbial adjunct which has a beneficial effect on the host by modifying the host associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment". A part from the requirement of the probiotic to be a live culture, this definition is a lengthy way of describing a probiotic as defined by Irianto and Austin (2002) thus "a probiotic is an entire or components of a microorganism that is beneficial to health of the host". Other definitions in aquaculture show that probiotic is a live microbial food supplements that are consumed with the aim of providing health benefit to the host by contributing to an improved microbial balance within the intestinal microbiota (Gram et al., 1999; Crittenden et al., 2005), are biologically active components or single or mixed cultures of microorganism capable of improving the health of the host (Salminen et al., 1999; Ochoa-Solano and Olmos-Soto, 2006), live microorganisms and/or disease resistance (Tacon, 2002), live microorganisms administered in adequate amounts that confer a health effect on the host (Gomez et al., 2007). These definitions reflect the use of microorganism or their products (microbial cells element or cell free supernatant factors) to tanks and ponds in which animals live, as biological control or their capacity of modified the bacterial composition of aquatic animal's intestine, water and sediment, or used with feed as health supplement and/or biological control.

Criteria of probiotic selection in aquaculture

The initial, major, purpose of using probiotics is to maintain or reestablish a favorable relationship between friendly and pathogenic microorganisms that constitute the flora of intestinal o skin mucus of aquatic animals. Since, successful probiotic is expected to have a few specific properties in order to certify beneficial effects (Ali, 2000).

Generally, probiotic strains have been isolated from indigenous and exogenous microbiota of aquatic animals. Gram-negative facultative anaerobic bacteria such as *Vibrio* and *Pseudomonas* constitute the predominant indigenous microbiota of a variety of species of marine animals (Onarheim, 1994). In contrast to saltwater organisms, the indigenous microbiota of freshwater animals tends to be dominated by member of the genera *Aeromonas*, *Plesiomonas*, representatives of the family Enterobacteriaceae, and obligate anaerobic bacteria of the genera *Bacteroides*, *Fusubacterium*, and

Eubacterium (Sakata, 1990). Lactic acid producing bacteria, which are prevalent in the mammal or bird gut, are generally sub-dominant in fishes and represented essentially by the genus *Carnobacterium* (Ringo and Vadstein, 1998).

Ideally, microbial probiotics should have a beneficial effect and not cause any harm to the host. Therefore, all strains have to be non-pathogenic and non-toxic in order to avoid undesirable side-effects when administrated to aquatic animals.

Some research and products talk about the multifactorial action of the probiotics (Gomez et al., 2007; Tuohy et al. 2003) on aquatic animals. However, the multifactorial effect is not agreed with evidence or is overestimate. Sometimes, this type of publicity about of those products really affects the perspective of real probiotic designed for aquaculture industry.

Different modes of action or properties are desire on the potential probiotic like antagonism to pathogens (Ringo and Vadstein, 1998; Gram and Melchirosen, 1996), ability of cells to produce metabolites (like vitamins) and enzymes (Ali, 2000), colonization or adhesion properties (Olsson et al., 1992) enhance the immune systems (Perdigon et al., 1995) and other.

Competitive exclusion

Competitive exclusion as it applies to the gastrointestinal tract is a phenomenon whereby an established microflora prevents or reduces the colonization of a competing bacterial challenge for the same location on the intestine. This microflora begins to form in the gut of aquatic animals during the hatching process and shortly thereafter form bacteria in the environment.

The aim of probiotic products designed under competitive exclusion is obtain stable, agree and controlled microbiota on culture based on competition for attachment sites on the mucosa, competition for nutrients, and production of inhibitory substance by the microflora which prevents replication and/or destroys the challenging bacteria and with this reduce its colonization (Moriarty, 1998; Verschuere et al., 2000). Different strategies are displayed in the adhesion of microorganism to those attachment sites as passive forces, electrostatic interactions, hydrophobic, steric forces, lipoteichoic acids, adhesions and specific structures of adhesion (Salyers and White, 2002).

The aquaculture industry display some probiotics products designed to adhesion on mucosal surface by a collection of microorganisms based on the competitive exclusion factors (Verschuere et al., 2000; Farzanfar, 2004). Those factors are important for adhesion to intestinal epithelial cells or in the activation of immune system, and help to the health of the organisms, intestinal homeostasis, and digestion (Aguirre-Guzman, 1992;

Farzanfar, 2004). These types of probiotic are extensively study in fish since these products were initiated development for vertebrate animals, and show interesting results.

Antagonisms

Control of microbial communities with high diversity has been regarded as difficult (Maeda et al., 1997). Such types of microbial communities can disperse the effect caused by the invasion or addition of certain extrinsic pathogenic organisms. Bacterial antagonism is a common phenomenon in nature; therefore, microbial interactions play a major role in the equilibrium between competing beneficial and potentially pathogenic microorganisms (Balcazar et al., 2004). In addition, microorganisms can be sources of a variety of bioactive natural products of basic research and commercial interest that have inhibitory effects on microbial growth (Das et al., 2006).

Antagonistic compounds are defined as chemical substances produced by microorganisms (in this case bacteria) that are toxic (bactericidal) or inhibitory (bacteriostatic) towards other microorganisms. The presence of bacteria producing antibacterial compounds in the intestine of the host, on its surface, or in its culture water is thought to prevent proliferation of pathogenic bacteria and even eliminate these. The antibacterial compounds can be divided into compound with a direct or indirect effect on the pathogen. Lactic acid bacteria often produce bacteriocins, however, these are often only active against closely related species (Klaenhammer, 1993) and most pathogens involved in aquaculture are Gram-negative and bacteriocins from lactic acid bacteria may therefore not inhibit fish pathogenic bacteria.

Probiotic bacteria suggested as probiotic treatment in aquaculture can produce both proteinaceous and non-proteinaceous substrates. The structure of the antibacterial compound is often not elucidated and their mode of action has not been reported. Furthermore none of these reports demonstrate that the antibacterial compound is produced in vivo. This will be of significant importance if production of these compounds is the mode of action. If the production of antibacterial compound is the only mode of action, it is possible that the pathogen eventually will develop resistance towards the compound. This will result in an ineffective treatment. The risk of the pathogen to develop resistance against the active compound has to be evaluated, to assure a stable effect of the probiotic bacterium.

In the other hand, the origin of probiotic strain is an important element in the antagonisms test. The microorganism present different physiologies or

biochemical activities along their development and based on environments (fresh, seawater) and original source. These characteristics affect the probiotic potential for attachment sites (Vanbelle et al., 1990) and may create a false impression of the ability of probiotics to inhibit in vivo test. The probiotics screening preferably requires different strategy of selection as antagonism, production of beneficial compounds, attachment and growth on various environments (Vine et al., 2004).

Immunity stimulation

The immune systems of fish and higher vertebrates are similar and both have two integral components: 1) the innate, natural or nonspecific defense system formed by a series of cellular and humoral components, and 2) the adaptive, acquired or specific immune system characterized by the humoral immune-response through the production of antibodies and by the cellular immune response which is mediated by T-lymphocytes, capable of reacting specifically with antigens. The normal microbiota in the GI ecosystem influences the innate immune system, which is of vital importance for the disease resistance of fish and is divided into physical barriers, humoral and cellular components. Innate humoral parameters include antimicrobial peptides, lysozyme, complement components, transferring, pentraxins, lectins, antiproteases and natural antibodies, whereas nonspecific cytotoxic cells and phagocytes constitute innate cellular immune effectors. Cytokines are an integral component of the adaptive and innate immune response, particularly IL-1 β , interferon, tumor necrosis factor- α , transforming growth factor- β and several chemokines regulate innate immunity (Gomez and Balcazar, 2008).

The non-specific immune system can be stimulated by probiotics. It has been demonstrated that oral administration of *Clostridium butyricum* bacteria to rainbow trout enhanced the resistance of fish to vibriosis, by increasing the phagocytic activity of leucocytes (Sakai et al. 1995). Rengpipat et al., (2000) mentioned that the use of *Bacillus* sp. (strain S11) provided disease protection by activating both cellular and humoral immune defenses in tiger shrimp (*Penaeus monodon*). Balcazar (2003) demonstrated that the administration of a mixture of bacterial strains (*Bacillus* and *Vibrio* sp) positively influenced the growth and survival of juveniles of white shrimp and presented a protective effect against the immune system, by increasing phagocytosis and antibacterial activity. In addition, Nikoskelainen et al. (2003) showed that administration of a lactic acid bacterium *Lactobacillus rhamnosus* at a level of 10⁵ cfu/g feed, stimulated the respiratory burst in rainbowtrout.

Antiviral effects

Some bacteria used as candidate probiotics have antiviral effects. Although the exact mechanism by which these bacteria do this is not known, laboratory tests indicate that the inactivation of viruses can occur by chemical and biological substances, such as extracts from marine algae and extracellular agents of bacteria. It has been reported that strains of *Pseudomonas sp.*, *Vibrio sp.*, *Aeromonas sp.*, and groups of coryneforms isolated from salmonid hatcheries, showed antiviral activity against infectious hematopoietic necrosis virus (IHNV) with more than 50% plaque reduction (Kamei et al., 1988). Girones et al. (1989) reported that a marine bacterium, tentatively classified in the genus *Moraxella*, showed antiviral capacity, with high specificity for poliovirus. Direkbusarakim et al. (1998) isolated two strains of *Vibrio spp.* from a black tiger shrimp hatchery. These isolates displayed antiviral activities against IHNV and Oncorhynchus masou virus (OMV), with percentages of plaque reduction between 62 and 99%, respectively.

Adhesion

Probiotics make up part of the resident microflora and contribute to the health or well-being of their host (Gatesoupe, 1999). The ability of some strain of adhesion to mucus, gastrointestinal tract, epithelial cell and other tissues is a common characteristic in the probiotic selection because it is associated with bacteria colonization (Verschuere et al., 2000; Farzanfar, 2004; Crittenden et al., 2005).

Colonization of the gastrointestinal tract of animals by probiotics is possible only after birth, and before the definitive installation of a very competitive indigenous microbiota. After this installation, only the addition of high doses of probiotic provokes its artificial and temporary dominance. In mature animals, the population of probiotic organisms in the gastrointestinal tract shows a sharp decrease within days after the intake had stopped (Fuller, 1992). According to Conway (1996), a microorganism is able to colonize the gastrointestinal tract when it can persist there for a long time, by possessing a multiplication rate that is higher than its expulsion rate.

The process of colonization is characterized by attraction of bacteria to the mucosal surface, followed by association within the mucous gel or attachment to epithelial cells. Adhesion and colonization of the mucosal surfaces are possible protective mechanisms against pathogens through competition for binding sites and nutrients (Westerdahl et al., 1991), or immune modulation (Salminen et al., 1999).

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The principal objective of adhesion is obtain a significant level of bacteria in the host and prevents them from being flushed out by the movement of food through the digestive tract. By attaching to the intestinal mucosa, probiotics can extend their time within the gut thereby influence the gastrointestinal microflora of their host (Andlid et al., 1998; Ouwehan et al., 2000; Rengpipat et al., 2003; Alavandi et al., 2004).

The attachment ability of some bacteria have been tested *in vitro* and *in vivo* and their results suggest that the pathogen was displaced by the potential probiotic, based on the ability of probiotic to attach to the mucus, where growth of the pathogen in the digestive tract might be suppressed by the candidate probiotic presence (Aguirre-Guzman, 1992; Verschuere et al., 2000; Farzanfar, 2004; Vine et al., 2004). This characteristic is associated with the competition for essential nutrient, space, etc. (Verschuere et al., 2000). Different strains of acid lactic bacteria, like *Enterococcus faecium* and *Lactobacillus sp.*; and other groups of bacteria Gram-positive and Gram negative as *Bacillus sp.*, *Vibrio sp.*, have been tested and *posteriori* used as probiotic for the ability of adhesion (Irianto and Austin, 2002, Rengpipat et al., 2003; Ajitha et al., 2004; Vine et al. 2004).

Digestive process

Many studies on probiotics in aquaculture have used *in vitro* models of specific bacteria as antagonists of pathogens (Vine et al., 2004; 2006), measured the survival of probiotics in the fish gut (Andlid et al., 1998) or evaluated the effect of probiotic on health management, disease resistance and immune response of fish (Li and Gatlin III, 2004; Shelby et al., 2006). But other important effect of probiotic that it is not extensively study, but demonstrated, an important effect is the feed efficiency and the growth promotion of aquatic animals by probiotic supplements (Gatesoupe, 2002; Lara-Flores et al., 2003).

The probiotic after transit through the stomach, attached in the intestine and use a large number of carbohydrates for their growth and produce relevant digestive enzymes (amylase, protease and lipase) that increase the digestibility of organic matter and protein, produce a higher growth, prevent the intestinal disorders and produce or/and stimulate a predigestion of secondary compounds present in plant protein sources (Lara-Flores et al., 2003; El-Haroun et al., 2006).

In fish the use of probiotics demonstrated beneficial effects on the growth performance, feed efficiency and digestibility of organic matter and protein, when used acid

lactic bacteria and yeast (Vazquez-Juarez et al., 1993; Noh et al., 1994; Bogout et al., 1998; Ringo and Gatesoupe, 1998; De Schrijver and Ollevier, 2000; Lara-Flores et al. 2003). In some case this effect attributed to the capacity of the probiotic to stimulate and/or produce some enzymes in the intestinal tract. For example, in fish it has reported that *Bacteroides* and *Clostridium* sp. have contributed to the host's nutrition, especially by supplying fatty acids and vitamins (Sakata, 1990). Some microorganisms such as *Agrobacterium* sp., *Pseudomonas* sp., *Brevibacterium* sp., *Microbacterium* sp., and *Staphylococcus* sp. may contribute to nutritional processes in Arctic charr (*Salvelinus alpinus* L.) (Ringo et al, 1995). Lara et al. (2010) observed a high activity of alkaline phosphatase in Nile tilapia (*Oreochromis niloticus*) when administered probiotics in the diet, the result shoe a high activity reflected a possible development of brush border membranes of enterocytes that can be stimulated by the probiotic and this it can be a indicator of carbohydrate and lipid absorption and explain the higher weight and the best feed conversion.

In addition, some bacteria may participate in the digestion processes of bivalves by producing extracellular enzymes, such as proteases, lipases, as well as providing necessary growth factors (Prieur et al., 1990). Similar observations have been reported for the microbial flora of adult penaid shrimp (*Penaeus chinensis*), where a complement of enzymes for digestion and synthesize compounds that are assimilated by animal (Wang et al., 2000). Microbiota may serve as a supplementary source of food and microbial activity in the tract digestive may be a source of vitamins or essential amino acids (Dall and Moriarty, 1983).

CONCLUSION

The efficient of probiotics was related with the strain multiplications and/or their presence on environment after application, and this attribute was associated with strain colonization on host and some benefic effect on health. Those are not agree with all probiotics products and help to obtain contradictories results about their effect on aquatic organisms. The evolution of probiotic is associated with the better understanding of the intestinal ecology application of this type of products, properties, and the specific strain-host.

The direct use of a probiotic on water (from fresh to seawater of farms and laboratories) is a special point of environment research consideration. Those products (probiotic) are commonly foreign or exogenous strain, and represent a possible risk of microorganism pollution, especially with the use of strain with genetic modification, specific adhesions or colonization niche, antibiotic production, synergistic action. The use and environment effect of those new probiotics generation it's necessary to

understand before massive application on aquaculture.

However, a number of probiotic products have been thoroughly researched, and evidenced their efficacy a possible use on aquaculture. Beneficial bacterial preparations that are species-specific probiotics have become more widely available to the aquaculture community. These preparation show specific benefic effect as disease prevention and offer a natural element to obtain a stable healthy gut environment and immune system. The establishing of strong disease prevention program, including probiotic and good management practice can be beneficial to raise aquatic organism production.

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