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Review

Microbial ecology of the gastrointestinal tract of fish and the potential application of probiotics and prebiotics in finfish aquaculture

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Abstract

World aquaculture is the fastest growing food-producing sector in the world. Globally, aquaculture is expanding into new directions, intensifying and diversifying. With increasing demand for environment friendly aquaculture, the use of alternatives of antibiotic growth promoters in fish nutrition is now widely accepted. Science-based knowledge on probiotics and prebiotics has increased in recent years. No doubt exist that in the last decade we have greatly expanded our knowledge about pro- and prebiotics as important functional ingredients in finfish aquaculture. They have numerous beneficial effects: improved activity of gastro-intestinal microbiota and enhanced immune status, disease resistance, survival, feed utilization and growth performance. As natural products pro- and prebiotics have much potential to increase the efficiency and sustainability of aquacultural production. Therefore, comprehensive research to more fully characterize the intestinal microbiota of prominent fish species, mechanisms of action of pro- and prebiotics, and their effects on intestinal ecosystem, immunity, fish health and performance is warranted. All pro- and prebiotics must be evaluated for their safety before being used in fish nutrition. Also, there is need for establishing dose-response relationships. The application of up to date molecular procedures to study of the gut microbiota as well as the development and validation of research methods, *in vitro*, *ex vivo* and *in vivo* models, have provided important information to understand the mechanisms of action behind the effects. This review summarizes and evaluates current knowledge of microbial ecology of the gastrointestinal tract of fish as well as the potential application and challenges of pro- and prebiotics in finfish aquaculture.

Keywords: Probiotics, Prebiotics, Intestinal microbiota, Finfish, Aquaculture

1. Introduction

World aquaculture has grown tremendously during the last years becoming an economically important industry (Subasinghe et al. 2009). Today it is the fastest growing food-producing sector in the world with the greatest potential to meet the growing demand for aquatic food (FAO 2006). Globally, aquaculture is expanding into new directions, intensifying and diversifying. A persistent goal of global aquaculture is to maximize the efficiency of production to optimize profitability.

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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 (SCAN 2003; 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 the other hand antibiotics inhibit or kill beneficial microbiota in the gastrointestinal (GI) ecosystem but it also made antibiotic residue accumulated in fish products to be harmful for human consumption (WHO 2006). By the above reasons since January 2006 European Union ratified a ban for the use of all sub-therapeutic antibiotics as growth-promoting agents in animal production.

The microbial ecology of the GI tract of variety of freshwater and marine fish has been investigated intensively by many researchers during the last decade (Spanggaard et al. 2000; Ahmed et al. 2004, 2005; Ringø et al. 2006, 2006a; Skrodenyte-Arbaciauskiene et al. 2006; Hovda et al. 2007; Kim et al. 2007; Yang et al. 2007; Zhou et al. 2009). There is evidence that the alimentary tract of fish is a complex ecosystem, containing a large number of microorganisms. Microbial populations in the intestinal contents are much higher than those in the surrounding water. This indicates that the intestines provide favorable ecological niches for these organisms. It is known, mainly from studies of the intestinal microbiota of terrestrial species, that the resident bacterial population of the intestine influences the establishment of pathogenic microorganisms in the intestinal tract and have disease preventive effect (Huber et al. 2004). However, the role that individual microbes play in the health and nutrition of fish is still poorly understood, therefore investigations of the intestinal microbiota are important for finfish aquaculture.

In connection with the ban of antibiotic growth promoters (AGP) 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 recent years, the research of pro- and prebiotics in fish nutrition is increasing with the demand for consumer and environment-friendly aquaculture (Denev 2008). Many published reports demonstrated positive effects of probiotics and prebiotics in feeds for various fish species, including rainbow trout (*Oncorhynchus mykiss*; Dügenci et al. 2003; Aubin et al. 2005; Brunt and Austin 2005; Panigrahi et al. 2004, 2005, 2007; Staykov et al. 2005, 2005a, 2005b, 2006, 2006a, 2007, 2009; Bagheri et al. 2008; Denev 2008; Sealey et al. 2008), Common carp (*Cyprinus carpio*: Yanbo and Zirong 2006; Staykov et al. 2005, 2005c, 2006b, 2007, 2007a, Denev 2008) Indian major carp (*Labeo rohita*; Nayak et al. 2007); Mozambique tilapia (*Oreochromis mossambicus*; Logambal et al. 2000), Nile tilapia (*Oreochromis niloticus*; Francis et al. 2005; Yin et al. 2006; Taoka et al. 2006a; Pirarat et al. 2006; Abdel-Tawwab et al. 2008); Japanese flounder (*Paralichthys olivaceus*; Taoka et al. 2006; Atlantic cod (*Gadus morhua* L.; Skjermo et al. 2006); European Sea bass juveniles (*Dicentrarchus labrax*; Carnevali et al. 2006; Dalmo and Bøgwald 2008). Although application of pro- and prebiotics as environment-friendly alternatives of AGP in fish nutrition seems to be relatively recent, the interest in such dietary supplements is increasing rapidly not only in fish, but also in Shrimp aquaculture (Castex et al. 2006; Farzanfar 2006; Ziaei-Nejad et al. 2006; Balcázar and Rojas-Luna 2007; Balcázar et al. 2007d, Chiu et al. 2007; Dalmo and Bøgwald, 2008; Wang et al. 2008; Zhang et al. 2009; Zhou et al. 2009a). Currently, many pro- and prebiotics are successfully use for growth and health management in the sustainable aquaculture industry.

This review summarizes and evaluates current knowledge of microbial ecology of the gastrointestinal tract of fish, as well as the potential application and challenges of probiotics and prebiotics in finfish aquaculture.

2. Gastrointestinal microbiota of fishes

The alimentary tract of fishes represents an interface between the external environment and the body. Its complex polymicrobial ecology interacts with the internal and external environment and has an important influence on health and disease. The intestine is a complex multifunctional organ. In addition to digesting and absorbing feedstuff, it is critical for water and electrolyte balance, endocrine regulation of digestion, metabolism and immunity. The GI microbiota of fish is characterized by high population density, wide diversity and complexity of interactions. While all major groups of microbes are represented, bacteria predominate. They are the main constituent of the gut microbiota in fish (Spanggaard et al. 2000; Pond et al. 2006).

Our knowledge of the molecular and cellular bases of host-microbe interactions is limited. The indigenous microflora of fish, particularly the microbial ecology of the GI tract, has been traditionally investigated by culture-dependent methods and identification of the fish microbiota has typically relied on phenotypic and biochemical key characteristics. However, studies on other animal species suggest that only a small fraction of the total microbial community present can be captured using culture-based techniques. Molecular methods that rely on the recovery and analysis of bacterial community DNA directly from samples have been proven useful for studying less cultivable microbial populations. Recently, findings from culture-based methods have been supplemented with molecular ecology techniques that are based on the 16S rDNA gene (Zoetendal et al 2004; Romero and Navarrete 2006; Yang et al. 2007). One of the most popular used method in fish studies is Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE), as the method is reliable, rapid, sensitive and easy to use to study microbial diversity (Brunvold et al. 2007; Liu et al. 2008; Zhou et al. 2007, 2009). The molecular approach provides a more complete picture about bacterial community composition than do cultured-based methods. Molecular methods enable characterization and quantification of the intestinal microbiota, while also providing a classification scheme to predict phylogenetic relationships. They improved understanding microbe-microbe and host-microbe interactions in health and disease, and the potential for manipulation of the fish microbiota by nutritional and environmental factors. Therefore, further studies about the identification, ecological adaptations, benefits and pathogenic potential of GI microbiota which live in close association with healthy fish are required.

2.1. Composition and functions of gastrointestinal microbiota

Aquatic animals, including fishes have a much closer relationship with their external environment. There are the big differences between terrestrial and aquatic species in the level of interaction between the intestinal microbiota and the surrounding environment. On the other hand many variations in morphology of the GI tract exist between various fish species (Ringø et al. 2003). Depending on feeding habits and diet, it is generally accepted to divide fish into carnivores (eating fish and bigger invertebrates), herbivores (consuming mainly plant material), omnivores (mixed diet eaters) and detrivores (feeding largely on detritus). According to Tanaka et al. (2004), Ringo et al. (2006a) and Yang et al. (2007) the type of food is important for composition and activity of the fish GI microbiota.

Fishes possess specific intestinal microbiota consisting of aerobic, facultative anaerobic and obligate anaerobic bacteria. This microbiota has been classified as autochthonous or indigenous (when they are able to colonize the host’s gut epithelial surface) or as allochthonous or transient. Several studies on various fresh- and saltwater fish have demonstrated bacteria in the intestinal lumen and associated with the intestinal epithelium (Ringø and Olsen 1999; Ringø et al. 1995, 1997, 2001, 2002, 2003). Bacteria attached to epithelial surfaces have been demonstrated in the gut of a variety fish species, and it has been suggested that this attachment is an important factor in determining whether a particular organisms colonizes in the intestinal tract. It therefore, seemed likely that the presence of consistently high numbers of beneficial bacteria was dependent on their ability to colonize the intestinal surface. These attached (resident) bacteria are responsible for enteric bacterial antagonism and colonization resistance, since they are associated closely with the intestinal epithelium, and form a barrier, serving as the first defence to limit direct attachment or interaction of fish pathogenic bacteria to the gut mucosa. The equilibrium between species of resident bacteria provides stability in the microbial population within the same individual under normal conditions. From a microbial point of view, it is important to have stable resident intestinal microbiota as a part of the natural resistance of fish to infections.

Numerous surveys of the bacterial flora in the GI tract of fish have been made during the last twenty years. Many reports have demonstrated that Gram-negative, facultative anaerobic bacteria such as *Acinetobacter, Alteromonas*, *Aeromonas, Bacteroides, Cytophaga, Flavobacterium, Micrococcus, Moraxella, Pseudomonas, Proteobacterium* and *Vibrio* spp. constitute the predominant endogenous microbiota of a variety of species of marine fish (Cahill 1990; Onarheim et al. 1994; Blanch et al. 1997; Ringo et al. 2006; Brunvold et al. 2007; Zhou et al. 2009). In contrast to saltwater fish, the endogenous microbiota of freshwater fish species tends to be dominated by members of the genera *Aeromonas, Acinetobacter, Bacillus, Flavobacterium, Pseudomonas* representatives of the family *Enterobacteriaceae*, and obligate anaerobic bacteria of the genera *Bacteroides, Clostridium* and *Fusobacterium* (Sakata 1990; Huber et al. 2004; Kapetanovic et al. 2005; Hovda et al. 2007; Kim et al. 2007). Various species of lactic acid bacteria (LAB) (*Lactobacillus, Lactococcus, Streptococcus, Leuconostoc*, and *Carnobacterium* spp.) have been also demonstrated to comprise part of this microbiota (Ringø and Gatesoupe 1998;

Syvokiene and Mickeniene 1999; Asfie et al. 2003; Hagi et al. 2004; Vendrell et al. 2006; Hovda et al. 2007, Balcázar et al. 2007, 2007b, 2008; Vijayabaskar and Somasundaram 2008). They are not dominant in the normal intestinal microbiota of fish, but some strains can colonize the gut (Ringø and Gatesoupe 1998; Balcázar et al. 2007b) or to inhibit adhesion of several fish pathogens (Balcázar et al. 2006c, 2008). LAB has become a major source of concern for aquaculture in recent decade. In addition to true pathogenic species of worldwide significance, such as *Streptococcus iniae* and *Lactococcus garvieae*, several species have been reported to produce occasional fish mortalities in limited geographic areas, and many unidentifiable or ill-defined isolates are regularly isolated from fish or fish products (Michel et al. 2007). Yeasts have been commonly isolated in the intestinal microenvironment as well. They constitute a significant part of the intestinal microbiota and can stimulate the immune response, metabolism and growth (Andlid et al. 1998; Gatesoupe 2007).

One of the most important features of GI microbiota in fish is variability. Many investigations have demonstrated variation in the microbial flora in different fish species depending of nutrition, intestinal microenvironment, age, geographical location, environmental factors, stress and etc. (Verschuere et al. 2000; Refstie et al. 2006; Skrodenyte-Arbaciauskiene et al. 2008; Yang et al. 2007; Kesarcodi-Watson et al. 2008). The regulations of bacterial populations in the GI tract of fish are complex processes that are not yet fully understood. Little is known about the early steps of colonization of the GI tract of fish, the establishment of normal microbiota and its stability. Understanding some aspects of microbial ecology in aquaculture systems, such as knowing the types, numbers, and sources of bacteria commonly associated with different developmental stages, could be useful for manipulating microbiota as a strategy to prevent pathogenic infection or to improve nutrition (Romero and Navarrete 2006).

According to Hansen and Olafsen (1999) and Balcázar et al. (2006) the colonization of the GI tract of fish larvae starts immediately after hatching and is completed within a few hours. Colonizing bacteria can modulate expression of genes in the digestive tract, thus creating a favorable habitat for them and preventing invasion by other bacteria introduced later into the intestinal ecosystem. Some investigations have reported that bacteria present in the hatchery environment may influence the composition of GI microbiota (Ringo and Birkbeck 1999). Using a culture-based approach, these results suggest that bacteria present in the GI tract generally seem to be those from water or the diet, and which can survive and multiply (Olafsen, 2001). Furthermore, larvae may ingest substantial amounts of bacteria by grazing on suspended particles and egg debris (Beveridge et al. 1989). Hence, it is tempting to suppose that egg microbiota would also affect the primary colonization of the fish larvae.

The intestinal microbiota has important and specific metabolic, trophic, and protective functions (Denev et al. 2000; Guarner and Malagelada, 2003). The normal (resident) microbiota of the gut confers many benefits to the intestinal physiology of the host. Some of these benefits include the metabolism of nutrients, contribution of the colonization resistance, antagonistic activity agains pathogens, immunomodulation and etc. (Denev, 1996; Denev et al. 2000). The intestinal microbiota has a profound impact on the anatomical, physiological and immunological development of the host (Rawls et al. 2004). Thus, establishing a healthy microbiota plays an important role in the generation of immuno-physiologic regulation by providing crucial signals for the development and maintenance of the immune system (Salminen et al. 2005). Understanding how the fish immune system generally responds to gut microbiota may be an important basis for targeting manipulation of the microbial composition. This might be of special interest to design adequate strategies for fish disease prevention and treatment (Gomez and Balcázar 2008).

The intestinal microbiota possesses antagonistic activity against many fish pathogens and participates in infection-protective reactions (Gutowska et al. 2004; Saha et al. 2006; Skrodenyte-Arbaciauskiene et al. 2006; Sugita and Ito 2006). Yoshimizu and Ezura (1999) reported that fish intestinal bacteria such as *Aeromonas* and *Vibrio* spp. produced antiviral substances.

The bacterial flora of the GI tract of fishes in general, represents a very important and diversified enzymatic potential. It is capable of producing proteolytic, amylolytic, cellulolytic, lipolytic, and chitinolytic enzymes, which is important for digestion of proteins, carbohydrates, cellulose, lipids and chitin (Bairagi et al. 2002; Gutowska et al. 2004). The enzyme producing microbiota can be beneficially used as probiotic supplements while formulating the fish diet, especially in the larval stages. It presents a scope for fish nutritionists to use the enzyme producing isolates as a probiotic in formulating cost-effective fish diets. However, much more research should be conducted to determine if the addition of such isolates to fish feeds do, in fact, provide some kind of benefit to the fish involved before advocating their use (Bairagi et al. 2002).

3. Probiotic - The concept

In recent years, there has been an upsurge in research into probiotics, as well as growing commercial interest in the probiotic concept. This increased research has resulted in significant advances in our understanding and ability to characterize specific probiotic organisms, as well as attempts to verify their attributed health benefits.

3.1. History and definitions

The term "probiotic" comes from the Greek words “pro” and “bios" meaning '*for life*'. It is opposed to the term "antibiotic" meaning '*against life*' (Hamilton-Miller et al. 2003). The concept of probiotics was introduced in the early 20th century, when Nobel Prize-winning Elie Metchnikoff hypothesized that the long healthy lives of Bulgarian peasants were the result of their consumption of fermented milk (yogurt). In his bestseller “The Prolongation of Life“ Metchnikoff (1907) was probably the first to advocate, or rather postulate the health benefits of LAB associated with yogurt, as protector of the intestine from the damaging effects of harmful bacteria. Since the early observations by Elie Metchnikoff - the first scientist who proposed the therapeutic use of LAB, a wealth of experiments have described the use of selected microorganisms, mainly belonging to the LAB family, for the prevention or treatment of several pathological conditions (Mercenier et al. 2003).

The term "probiotic" was probably first proposed by Werner Kollath (1953). He suggested the term to denote all organic and inorganic food complexes as probiotics, in contrast to harmful antibiotics, for the purpose of upgrading such food complexes as supplements. He defined the term "probiotic" as "Active substance that is essential for a healthy development of life". After that, in his publication "Antu-und Probiotika" Vegrin (1954) compared the detrimental effects of antibiotics with favorable factors "probiotics" on the gut microbiology. Lilly and Stillwell (1965) described probiotics as "Substances secreted by one organism that stimulate the growth of another". Some years later the term probiotic was used in the context of animal feeds by Parker (1974) “Organisms and substances that have a beneficial effect on the host of animal by contributing to its intestinal microbial balance”.

The definition of the term “probiotic” has evolved through the years. Several definitions were successfully proposed during the last two decades (Fuller 1989; Havenaar and Huis In't Veld 1992; Schaafsma (1996; Berg 1998; Naidu et al. 1999; FUFOSE 1999; Salminen et al. 1999; Schrezenmeir and de Vrese 2001; FAO/WHO 2001). The most widely quoted definition was made by Fuller (1989). He defined probiotics as “A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”. This definition is still widely referred to, despite continual contention with regard to the correct definition of the term. The next most appropriate definition was published by an Expert Consultation at a meeting convened by the FAO/WHO in October, 2001: “Live microorganisms which when administered in adequate amounts confer a health benefit on the host”.

Probiotic bacteria belong to the natural flora of intestinal ecosystem with low or no pathogenicity and show functions that are important for the health and well-being of the host. Therefore, the maintenance of this ecological flora is important to prevent diseases, especially infections of the GI tract. Since the last decade there has been an increased awareness of the beneficial effects of probiotics.

Bengmark (1998) elegantly described the reasons for this interest in microbial interference treatment.

The concept of probiotic activity has its origins in the knowledge that active modulation of the GI ecosystem could confer antagonism against pathogens via production of antagonistic compounds and competition for attachment sites or nutrients; help development and stimulation of the immune system; assist to maintenance of mucosal integrity; provide nutritional and health benefits; improve alteration of enzymatic activity and food/feed utilization (Vaughan et al. 2002; Holzapfel 2006; Gómez et al. 2007; Denev 1996, 2000, 2008).

Probiotics are attractive biological products with extremely interesting characteristics. Their using in animal and human nutrition is well documented (Denev 1996; Fioramonti et al. 2003; Goktepe et al. 2006). In recent years, considerable benefits have been established also in terrestrial animals by feeding potentially beneficial bacteria or adding probiotic supplements to the diet to alter the intestinal environment and favor the establishment of certain beneficial microorganisms. The use of probiotics have been studied most extensively in pigs (Sakata et al. 2003; Gardiner et al. 2004; Denev 2008), poultry (Sotirov et al. 2000, 2001; Nisbet 2002; Patterson and Burkholder 2003; Denev 2004, 2006a, 2008), calves (Fonty and Chaucheyras-Durand 2006; Nader-Macías et al. 2008) and other animals (O’Mahony et al. 2009). Today, probiotics are quite common place in health promoting “functional foods” for humans, and natural and ecological alternative of AGP in animal production (Ouwehand et al. 2002; Sullivan and Nord 2002; Senok et al. 2005; Denev 2008).

3.2. What are aquatic probiotics?

The concept for aquatic probiotics is a relatively new. When looking at probiotics intended for an aquatic usage it is important to consider certain influencing factors that are fundamentally different from terrestrial based probiotics. Aquatic animals have a 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. On the other hand, potential pathogens are able to maintain themselves in the external environment of the aquatic organisms and proliferate independently of the host (Hansen and Olafsen 1999; Verschuere et al. 2000; Kesarcodi-Watson et al. 2008). 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).

The resident microbes benefit from a fairly constant habitat in the GI tract of man and terrestrial livestock, whereas most microbes are transient in aquatic animals (Moriarty 1990). These animals are poikilothermic and their associated microbiota may vary with temperature changes. Salinity changes in the rearing environment will also affect the microbiota and marine finfish are obliged to drink constantly to prevent water loss from the body. A consequence of the specificity of aquatic microbiota is that the most efficient probiotics for aquaculture may be different from those of terrestrial species (Gatesoupe 1999; Kesarcodi-Watson et al. 2008).

Defining probiotics is a challenge – even more so for aquaculture application. Historically, probiotics were defined according to their expected benefits or improvement to the host's intestinal balance. Being concerned with humans and terrestrial animals, probiotics were generally Gram-positive obligate or facultative anaerobes, mostly LAB.

Based on the intricate relationship an aquatic organism has with the external environment when compared with that of terrestrial animals, the definition of probiotics for aquatic animals was modified at the end of the last century. Gatesoupe (1999) redefined probiotics for aquaculture as "Microbial cells that are administered in such a way as to enter the GI 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) defined aquatic probiotics as "Live microorganisms that have a beneficial effect on the host by modifying the microbial community, associated with the host, 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". This implies a much wider range of microorganisms being used as probiotics for aquaculture animals that for terrestrial animals. The above definition is a more holistic and most appropriately defines probiotics for aquaculture.

During the last ten years there is a discussion about what an aquatic probiotic actually is, because the above definitions differ to that of Fuller (1989). Gatesoupe (1999) and Verschuere et al. (2000) didn't include information about improving intestinal microbial balance. On the other hand, the Fuller's definition could not be accepted for aquaculture probiotics since the definition does not encompass the role of environment.

Probiotics that currently used in aquaculture industry include a wide range of taxa – from *Lactobacillus, Bifidobacterium, Pediococcus, Streptococcus* and *Carnobacterium* spp. to *Bacillus*, *Flavobacterium, Cytophaga, Pseudomonas, Alteromonas, Aeromonas, Enterococcus, Nitrosomonas, Nitrobacter,* and *Vibrio* spp., yeast (*Saccharomyces*, *Debaryomyces*) and etc. (Irianto and Austin 2002; Burr et al. 2005; Sahu et al. 2008).

Aquatic probiotics are mainly of two types: 1) gut probiotics which can be blended with feed and administrated orally to enhance the useful microbial flora of the gut and, 2) water probiotics which can proliferate in water medium and exclude the pathogenic bacteria by consuming all available nutrients. Thus, the pathogenic bacteria are eliminated through starvation (Nageswara and Babu 2006; Sahu et al. 2008). The first type probiotics are using mainly in finfish aquaculture and the second type in shrimp aquaculture. Commercially available probiotics include pure strains, defined mixture of specific strains, but also consortia of strains and undefined mixtures. Generally, probiotics proposed as biological control agents in fish aquaculture are applied in the feed or as a water additive supplement.

Aquatic probiotics are marketed in two forms: 1) Dry forms: the dry probiotics that come in packets can be given with feed or applied to water. They have many benefits, such as safety, easy using, longer shelf life and etc. (Decamp and Moriarty 2007); 2) Liquid forms: the hatcheries generally use liquid forms which are live and ready to act. These liquid forms are directly added to hatchery tanks or blended with farm feed. The liquid forms can be applied any time of the day in indoor hatchery tanks, while it should be applied either in the morning or in the evening in outdoor tanks. Liquid forms give positive results in lesser time when compared to the dry and spore form bacteria, though they are lower in density (Nageswara and Babu 2006). There are no reports of any harmful effect for probiotics but it is found that the biological oxygen demand level may temporarily be increased on its application; therefore it is advisable to provide subsurface aeration to expedite the establishment of probiotics organisms. A minimum dissolved oxygen level of 3% is recommended during probiotics treatment.

The development of suitable probiotics for aquaculture is not a simple task. It requires empirical and fundamental research, full-scale trials as well as the development of appropriate monitoring tools and production under stringent quality control. A performing mixture of probiotic strains can be designed after evaluating the ability of individual strains to grow in low/high salinity under micro-aerophilic or anaerobic conditions, produce various enzymes, and more importantly, produce a range of inhibitory compounds (Decamp 2004).

3.3. Modes of action of probiotics in aquaculture

Probiotic agents exert a beneficial effect via a wide array of actions. These include competition for adhesion sites and resistance to colonization; competition for essential nutrients; production of antagonistic compounds against pathogens; enhancement of the immune response and diseases resistance. In addition, probiotics improving enzyme activity feed digestibility and feed utilization, fish health and performance (Ringø and Gatesoupe 1998; Verschuere et al. 2000; Balcázar et al. 2004, 2006b; Das et al. 2008; Gómez and Balcázar 2008; Kesarcodi-Watson et al. 2008).

3.3.1. Competition for adhesion sites

Bacterial adherence is an important prerequisite for colonization by pathogenic microorganisms and virulence manifestations (Bengmark 1998; Ouwehand et al. 1999). Adhesion capacity and growth on or in intestinal or external mucous has been demonstrated also *in vitro* for many fish pathogens like *Vibrio anguillarum* and *Aeromonas hydrophila* (Krovacek et al. 1987). The beneficial bacteria with probiotic properties are also capable of adhering to the epithelial wall of the GI tract of fish (Ringø et al. 2003; Balcázar et al. 2008). The host-specific adhesion of probiotic bacteria to mucosal surfaces is crucial in the competitive exclusion of pathogenic microorganisms and merits special attention (Bengmark 1998). The adhesion of some pathogens as *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Vibrio anguillarum* and *Yersinia ruckeri* to intestinal mucus of fish was significantly reduced by *Lactococcus lactis*. In addition, *Lactobacillus plantarum* significantly reduced the adhesion of *A. hydrophila* and *A. salmonicida*. With the exception of *V. anguillarum*, adhesion of all pathogenic strains to intestinal mucus was significantly reduced by *Lactobacillus fermentum* and by mixture of the three LAB strains (Balcázar et al. 2008). A recent study suggests also that intestinal colonization by endogenous LAB (*Lactobacillus sakei*, *Lactococcus. lactis* and *Leuconostoc mesenteroides*) prevents the development of furunculosis in rainbow trout (Balcázar et al. 2007c). These results are in agreement with those of several other authors, who have demonstrated that probiotic microorganisms reduce the proliferation of pathogens by competing for attachment sites (Rinkinen et al. 2003; Chabrillón et al. 2005).

The ability of bacteria to colonize the GI tract is dependent on 1) bacterial factors that permit the organism to survive in the GI environment; 2) host resistance factors which mitigate against maintenance of colonization by enteropathogens; and 3) on the interactions of the colonizing enteropathogen with indigenous normal microflora that compete with, or in some manner inhibit, the ability of a given enteropathogen to survive within the GI tract. By applying a high number of beneficial bacteria (probiotic), harmful bacteria (pathogens) are not able to adhere and thus cannot proliferate. Adhesion to the intestinal mucosa has been suggested to enhance the ability to stimulate the immune system (Nikoskelainen et al. 2003). More recently have been observed a correlation between the colonization ability of endogenous LAB and nonspecific humoral responses such as alternative complement pathway activity and lysozyme activity in brown trout (Balcázar et al. 2007a).

Although competition for adhesion sites has been widely suggested as a mode of action, there is a little evidence in the literature to demonstrate this. There are studies reporting an adhesion of certain bacteria to intestinal mucus *in vitro*, but transferee of these to *in vivo* models has not produced supporting results. Attachment ability of potential probiotics seen *in vitro* cannot be assumed to demonstrate the real effect *in vivo* (Kesarcodi-Watson et al. 2008). Additionally, while studies to date have demonstrated the ability of certain bacteria to adhere to intestinal mucus *in* *vitro* (Garcia et al. 1997; Jöborn et al. 1997), they failed to assess a competitive exclusion effect. Recently, Vine et al. (2004) demonstrated a competitive exclusion effect with five probiotics versus two pathogens on fish intestinal mucus. They found that the presence of one of the probiotics on the mucus inhibited the attachment of one of the pathogens tested. Interestingly, pre-colonization with the other probiotics encouraged attachment of the two pathogens. However, the general trend from their study showed that post treatment with the probiotics displaced the pathogen.

The adhesion ability of probiotic bacteria is very important for colonization resistance. It is the ability of the normal GI flora of fish to protect against unwanted colonization of the GI tract by pathogens. Colonization resistance is achieved by complex interactions between the different resident bacteria of the mucosal microbiota. If normal GI flora of fish is altered for any reason, the ability to prevent pathogenic overgrowth will be compromised. The beneficial effects of probiotics might in part result from enhancement of colonization resistance by the direct suppression of harmful enteropathogens and the stimulation of beneficial microbiota.

3.3.2. Competition for nutrients

Competition for nutrients or available energy can play an important role in the composition of the GI microbiota or in the culture water of aquatic species (Tinh et al. 2008). Microbial competition for organic and inorganic substances and energy sources in the GI tract of fish means that by increasing the relative numbers of probiotic bacteria, nutrients and energy are consumed which would otherwise be available for the growth of pathogenic bacteria (Verschuere et al.1999, 2000).

Competition for iron has been reported as an important factor in marine bacteria for their growth, but is generally limited in the tissues and body fluids of animals and in the insoluble ferric Fe^{3+} form (Verschuere et al. 2000). Iron-binding agents, siderophores, allow acquisition of iron suitable for microbial growth. Siderophore production is a noted mechanism of virulence in some pathogens (Gram et al. 1999). Equally, a siderophoreproducing probiotic could deprive potential pathogens of iron under iron limiting conditions. This was shown by Gram et al. (1999), who found that a culture supernatant of *Pseudomonas fluorescens*, grown in iron-limited conditions, inhibited growth of *V. anguillarum*, whereas the supernatant from iron-available cultures did not.

Several studies have documented the nutritional effect of probiotic bacteria on the growth and survival of fish and shellfish larvae (for review see Tinh et al. 2008). Hence, successful application of the principle of competition to natural situation is not easy and this remains as a major task for microbial ecologists (Sahu et al. 2008).

3.3.3. Production of anagonistictic compounds

Antagonistic compounds are defined as chemical substances produced by bacteria that are toxic or inhibitory towards other microorganisms. Most probiotics have the capability to produce substances which have direct antimicrobial action. Organic acids, hydrogen peroxide, lysozyme, siderophores and bacteriocins (Braun and Braun 2002; Yoshida et al. 2002; Vazquez et al. 2005; Sahu et al. 2008) are among the known products with inhibiting effects on many gram-positive and gram-negative pathogens. These substances can either reduce the number of pathogenic organisms in GI ecosystem directly, or in some instances can alter the metabolism of pathogens (Vine et al. 2006). Recently Balcázar et al. (2007b) studied five LAB for use as probiotics based on their competitive adhesion and production of antagonistic substances against some fish pathogens. Based on mucus adhesion, competitive exclusion, and suppression of fish pathogen growth by production of antagonistic compounds, they concluded that the selected LAB strains are very promising alternative of chemotherapeutic agents in the fish industry. Vijayabaskar and Somasundaram (2008) reported that bacteriocin producing LAB, isolated from the GI tract of fresh water fishes posses antagonistic activity against *Aeromonas hydrophila*, and may be use as probiotic. There were also a few studies on the possible use of marine actinomycetes with antimicrobial activity against aquatic pathogens in disease prevention in marine aquaculture (Das et al. 2006; Kumar et al. 2006; You et al. 2005, 2007).

Some bacteria used as candidate probiotics have antiviral activities. Though 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 extracellular agents of bacteria. It has been reported that strains of *Pseudomonas*, *Vibrio*, *Aeromonas* spp. 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) also reported that a marine bacterium, tentatively classified in the genus *Moraxella*, showed antiviral capacity, with high specificity for poliovirus. Yoshimizu and Ezura (1999) demonstrated that intestinal bacteria such as *Aeromonas* and *Vibrio* spp. also produced antiviral substances.

Although production of an antagonistic or inhibitory compounds has been shown to work very well in probiotics and this screening method has identified very good probiotics in aquaculture (Irianto and Austin 2002a; Lategan and Gibson 2003; Lategan et al. 2004 a, b; Vaseeharan et al. 2004; Balcázar et al. 2007), but there are some limitations to this approach. The positive results *in vitro* fail to determine the real *in vivo* effect. The production of antagonistic compounds against any other microflora *in vitro* is no guarantee that the potential probiotic will be effective *in vivo* (Kesarcodi-Watson et al. 2008). This means that not only i*n vitro* but *in vivo* models are needed to clarify the antagonistic activity of probiotics in aquaculture.

3.3.4. Enhancement of the immune response

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, transferrin, pentraxins, lectins, antiproteases and natural antibodies, whereas nonspecific cytotoxic cells and phagocytes (monocytes/macrophages and neutrophils) constitute innate cellular immune effectors. Cytokines are an integral component of the adaptive and innate immune response, particularly IL-1β, interferon, tumor necrosis factor-α, transforming growth factor-β and several chemokines regulate innate immunity (Gomes and Balcázar 2008).

The demonstration that the gut microbiota is an important component of mucosal barrier has resulted in the promotion of the use of beneficial probiotics. There is increasing evidence that many bacteria have been shown to modulate the innate humoral responses, including innate host resistance, and thereby facilitate the exclusion of potential pathogens. In a previous studis have been demonstrated that oral administration of *Clostridium butyricum* bacteria to rainbow trout (*Oncorhynchus mykiss*) enhanced the resistance of fish to vibriosis, by increasing the phagocytic activity of leucocytes (Sakai et al. 1995). Rengpipat et al. (2000) reported that the use of *Bacillus* spp. (S11) has provided disease protection by activating both cellular and humoral immune defenses. Nikoskelainen et al. (2003) showed that administration of *Lactobacillus rhamnosus* (ATCC 53103) at a level of 10^5 Cfu/g feed stimulated the respiratory burst in rainbow trout.

Probiotics can modify the immune response of the host by interacting with epithelial cells and by modulating the secretion of anti-inflammatory cytokines, which could result in a reduction of inflammation. Recently, studies showed that IL-1β, IL-8, TNF-α, and TGF-β expression was not induced in rainbow trout gut cells following administration of the probiotic bacteria *Carnobacterium maltaromaticum* B26 and *Carnobacterium divergens* B33. However, detection of significantly higher IL-1β and TNF-α expression in head kidney cells indicates induction of an antiinflammatory effect (Kim and Austin 2006b).

During the past five years have been demonstrated that many probiotic agents stimulate immune response and diseases resistance, and have a positive effect on fish health (Brunt and Austin 2005; Salinas et al. 2005; Kim and Austin 2006a, 2006b; Taoka et al. 2006a; Pirarat et al. 2006; Panigrahi et al. 2004, 2005, 2007; Balcázar et al. 2006a, 2007a, 2007c; Gomes and Balcázar 2008; Mesalhy et al. 2008; Vendrell et al. 2008; Wang et al. 2008a).

3.3.5. Effects on fish health and productivity

The use of probiotics for disease prevention and improved nutrition in aquaculture was relatively recent, but during the last decade, it is becoming increasingly popular due to an increasing demand for environment-friendly aquaculture (Vine et al. 2006; Wang et al. 2008b). Since the first use of probiotics in aquaculture (Kozasa 1986), a growing number of studies have demonstrated their beneficial effects in fish diet. Several new studies have reported that probiotics are effective in improving immunity, health status, feed efficiency and growth performance of fish species (Table 1). As biological control agents probiotics provide nutritional benefits and protection against pathogens in the GI ecosystem. They are vital in modulating interactions with the environment, the development of beneficial immune responses, and in supporting the health. The supplementation of beneficial probiotic bacteria in the fish diet is important for enzyme activity, feed digestion, feed utilization and growth performance. By the above reasons, the use of microbial probiotics in finfish aquaculture is now widely accepted (Wang and Xu 2006; Balcázar et al. 2006, 2007a; Wang et al. 2008).

3.4. Developing, evaluating and safety of probiotics for aquaculture

It has been widely published that an aquatic probiotic must possess certain important properties (Verschuere et al. 2000). These properties were proposed in order to aid in correct establishment of new, effective and safe products. The properties include: 1) The probiotic should not be harmful to the host it is desired for; 2) It should be accepted by the host, e.g. through ingestion and potential colonization and replication within the host; 3) It should reach the location where the effect is required to take place; 4) It should actually work *in vivo* as opposed to *in vitro* findings; 5) It should preferably not contain virulence resistance genes or antibiotic resistance genes. The list of these requisites is given to allow step-wise examination of potential probiotics. However, the sum of many of these properties could be tested quickly via *in vivo* experimentation with the target animal. In essence, these properties are describing one simple question, "does the potential probiotic provide an overall health benefit when given to the animal?" It was stated previously that there are inherent limitations with the past and current *in vitro* screening procedures and problems with changing the initial screening phase to *in vivo* experiments. Despite this, the possibility of being able to answer the above question in the screening phase offers great simplicity, directness and an all-encompassing allowance for probiotics acting by any mode of probiotic action to be identified. For these reasons, the prospect of including test animals in initial screening by means of challenge tests is very appealing (Kesarcodi-Watson et al. 2008).

According to Sahu et al. (2008) the development of probiotics for commercial use in aquaculture is a multidisciplinary process requiring both empirical and fundamental research, full-scale trial and an economic assessment of its uses. Many of the failures in probiotic research can be attributed to the selection of inappropriate probiotic strains. Selection steps have been defined, but they need to be adapted for different host species and environments. It is essential to understand the mechanisms of probiotic action and to define selection criteria for potential probiotics. General selection criteria are mainly determined by biosafety considerations, methods of production and administration of the probiotics, and the location in the body where the microorganisms are expected to be active (for reviews see Sahu et al. 2008).

Currently there are many defining criteria of microorganisms that can be considered probiotics. A probiotic should: 1) Be of fish origin; 2) Be nonpathogenic in nature; 3) Be resistant to destruction by gastric acid (low pH), bile salts and proteases; 4) Be able to colonize (adhere) to intestinal epithelial cells to reduce or prevent colonization of pathogens; 5) Produce antimicrobial compounds like organic acids, hydrogen peroxide, bacteriocins, siderophores; 6) Modulate immune response; 7) Influence GI enzyme and metabolic activities and etc. (Sotomayor and Balcázar 2003; Vine et al. 2004, 2004a; Balcázar et al. 2006; Sahu et al. 2008). By systematically conducting *in vitro* tests on a large number of potential probiotics, less-promising candidates can be excluded, thereby reducing the number of *in vivo* trials required to validate the effectiveness of the probiont (Vine 2004).

Selection criteria for probiotics for larvae differ from those used for adult fish in that initially the pH of the larval GI tract is alkaline (Tanaka et al. 1996; Ronnestad et al. 2000; Vine 2004). As the larval digestive tract is immature at hatching, the gall bladder has yet to develop and subsequently bile is not secreted until later on during development (Govoni et al. 1986). Therefore, the probiotic is not required to move through an acidic environment en route to the gut and, unlike a probiont designed for adults, does not need to be resistant to acid and bile. Probiotics selected for use during the early stages of larval development do thus not need to be screened for this characteristic (Vine 2004). Colonization of the larval gut by bacteria generally increases at the onset of exogenous feeding, resembling the microflora similar to that of the live food as opposed to that of the surrounding environment. To maximize the competitive advantage of probiotics, early delivery seems to be best (Ringø et al. 1996; Ringø and Vadstein 1998; Gatesoupe 1999; Vine 2004), since bacteria colonizing the intestine before first feeding may be able to persist (Hansen and Olafsen 1999; Olafsen 2001). Opportunistic fish pathogens are commonly introduced along with mass-cultured live food due to concentrated feeding levels combined with the rapid growth rate of the bacterial microflora (Skjermo and Vadstein 1993) enforcing the concept that probiotic delivery should occur during the early stages of larval development prior to exogenous feeding.

Table 1. Probiotics used in fish nutrition and their effects

Identity of the probiotic	Species / Method of application	Effects	Reference(s)
Bacillus subtilis and *Bacillus licheniformis*	rainbow trout / Feed (*Onchorhynchus mykiss*)	Increased resistance to *Yersinia ruckeri*	Raida et al. (2003)
Bacilllus subtillis Lactobacillus delbriieckii	gilthead seabream / Feed	Stimulated cellular innate immune response	Salinas et al. (2005)
Bacillus spp. Photosynthetic bacteria	common carp / Feed (*Cyprinus carpio*)	Better digestive enzyme activities; Better growth performance and feed efficiency	Yanbo and Zirong (2006)
Bacillus spp.	rainbow trout / Feed *Onchorhynchus mykis*	Better growth performance and survival	Bagheri et al. (2008)
Bacillus subtilis (ATCC 6633) *Lactobacillus acidophilus*	Nile tilapia (*Oreochromis niloticus*)	Stimulated the gut immune system ; Enhance the immune and health status; Increased the survival rate and the body-weight gain;	Mesalhy et al. (2008)
Carnobacterium spp.	Atlantic salmon / Feed (*Salmo salar* L.)	Inhibited *A. salmonicida*, *V. ordalii* and *Y. ruckeri* ; Reduced disease	Robertson et al. (2000)
Carnobacterium maltaromaticum B26 *Carnobacterium divergens*	rainbow trout / Feed (*Onchorhynchus mykis*)	Enhanced the cellular and humoral immune responses	Kim and Austin (2006a
Carnobacterium divergens 6251	Atlantic salmon / Feed (*Salmo salar* L.)	*Carnobacterium divergens* is able to prevent to some extent pathogen-induced damage in the foregut.	Ringø et al. (2007)
Enteroccus faecium ZJ4	Nile tilapia / Water (*Oreochromis niloticus*)	Increased growth performance; Improved immune response	Wang et al. (2008a)
Lactobacillus rhamnosus	rainbow trout / Feed (*Onchorhynchus mykiss*)	Increased resistance to *Aeromonas salmonicida* ssp. *Salmonicida*; Reduced mortality from furunculosis	Nikoskelainen et al. (2001)
Lactobacillus rhamnosus (ATCC 53103)	rainbow trout / Feed (*Onchorhynchus mykiss*)	Enhanced Immune parameters; Stimulated Immune Response;	Nikoskelainen et al. (2003)
Lactobacillus rhamnosus (JCM 1136)	rainbow trout / Feed (*Onchorhynchus mykiss*)	Increased the serum lysozyme and complement activities	Panigrahi et al. (2004)
Lactobacillus rhamnosus	rainbow trout / Feed (*Onchorhynchus mykiss*)	Stimulated Immune Response	Panigrahi et al. (2005)

Lactobacillus delbrueckii subsp. *delbrueckii* (AS13B)	European sea bass / Feed (*Dicentrarchus labrax* L.)	Positive effects on welfare and growth; Increased body weight	Carnevali et al. (2006)
Lactobacillus rhamnosus GG	tilapia / Feed (*Oreochromis niloticus*)	Enhanced the growth performance and immunity	Pirarat et al. (2008)
Lactobacillus delbrueckii subsp. *bulgaricus*	rainbow trout / Feed *Onchorhynchus mykiss*	Enhanced humoral immune response	Tukmechi et al. (2007)
Lactobacillus rhamnosus (ATCC 53103); *Bacillus subtilis Enterococcus faecium*	rainbow trout / Feed *Onchorhynchus mykis*	Modulated cytokine production; Stimulated Immune Response	Panigrahi et al. (2007)
Lactococcus lactis subsp. *lactis; Lactobacillus sakei; Leuconostoc mesenteroides*	rainbow trout / Feed *Onchorhynchus mykis*	Stimulated phagocytosis; Enhanced the non-specific immunity	Balcázar et al. (2006a)
Lactococcus lactis ssp. *lactis*; *Lactobacillus sake Leuconostoc mesenteroides*	brown trout /Feed (*Salmo trutta*)	Modified the intestinal microbiota Stimulate the humoral immune response	Balcázar et al. (2007a)
Leuconostoc mesenteroides CLFP 196; *Lactobacillus plantarum* CLFP 238	rainbow trout / Feed *Onchorhynchus mykis*	Reduced fish mortality	Vendrell et al. (2008)
Micrococcus luteus	Nile tilapia / Feed (*Oreochromis niloticus*)	Enhanced the non-specific immune parameters; Improved resistance against *Edwardsiella tarda* infection	Taoka et al. (2006a)
Micrococcus luteus Pseudomonas spp.	Nile tilapia / Feed (*Oreochromis niloticus*)	Higher growth performance, survival rate and feed utilization ; Enhanced fish resistance against *Aeromonas hydrophila* infection	Abd El-Rhman et al. (2009)
Streptococcus faecium Lactobacillus acidophilus	Nile tilapia / Feed (*Oreochromis niloticus*)	Better growth performance and feed efficiency	Lara-Flores et al. (2003)
Saccharomyces cerevisae	Nile tilapia / Feed (*Oreochromis niloticus*)	Better growth performance and feed efficiency	Lara-Flores et al. (2003)
Live yeasts	European sea bass / Feed (*Dicentrarchus labrax*)	Better growth performance and feed efficiency	Tovar-Ramırez et al. (2004)
Saccharomyces cerevisiae strain NCYC Sc 47 (Biosaf® Sc 47)	rainbow trout / Feed *Onchorhynchus mykiss*	No significant effect on enzyme activities	Waché et al. (2006)
Saccharomyces cerevisiae var. *boulardii* CNCM I-1079 (Levucell® SB20)	rainbow trout / Feed *Onchorhynchus mykiss*	Stimulated enzyme activities	Waché et al. (2006)
Saccharomyces cerevisiae (DVAQUA®)	hybrid tilapia / Feed (*Oreochromis niloticus* ♀ × *O. aureus* ♂)	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)

Aquatic candidate probionts for larviculture have been isolated from healthy adults (Riquelme et al. 2000; Rengpipat et al. 2000; Gullian et al. 2004) or healthy larvae (Ringø et al. 1996; Gatesoupe et al. 1997; Ringø and Vadstein

1998; Vine 2004). It has been suggested that the efficacy of probiotics is likely to be highest in the host species from where they were isolated (Verschuere et al. 2000).

Commercial probiotic production should take into account beneficial traits of strain 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 manufacture 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. The reliable identification of probiotics requires molecular methods with a high taxonomic resolution that are linked to up-to-date identification libraries (Qi et al. 2009).

The *in vitro* tests with potential probiotic agents can, and should only be used as an indication of possible successes *in vivo*. Some assumptions regarding the *in vivo* mode of action based upon *in vitro* experiments may not hold. Therefore, the purpose of the *in vitro* experiments is to gain a better understanding of the potential of specific candidate probiotics before subjecting them to costly and time-consuming *in vivo* trials (Vine 2004). By the above reasons the effect of candidate probiotics for aquaculture should be tested not only *in vitro* but *in vivo* as well. It involves introducing a probiotic agent to the host under culture and then monitoring the growth, colonization, and survival. *In vivo* tests are important to evaluate the beneficial effects of probiotics e.g. enhanced nutrition, immune response, disease resistance, health, feed conversion and productivity (Sahu et al. 2008). All the above tests criteria are essential to select the candidate probiotics, but rearing experiments remain necessary to conclude that the strains are beneficial. The practical evaluation of the interest of probiotic treatments will require long-term surveys (Sahu et al. 2008).

The general concept that the use of probiotics in aquaculture may produce various beneficial effects has been proven beyond doubt (Balcázar et al. 2006; Kesarcodi-Watson et al. 2008). However, safety considerations neglected for a long time are now taken into account for the development and marketing of probiotics (Courvalin, 2006). Safety is the state of being certain that adverse effects will not be caused by an agent under defined conditions. New species and more specific strains of probiotic bacteria are constantly identified. It cannot be assumed that these novel probiotic organisms share the historical safety of tested or traditional strains. Prior to incorporating them into products, new strains should be carefully assessed and evaluated for both safety and efficacy. Probiotic manufacturers should apply modern molecular techniques to ensure that the species of bacteria used in their probiotics are correctly identified, for quality assurance as well as safety. The safety profile of a potential probiotic strain is of critical importance in the selection process. This testing should include the determination of strain resistance to a wide variety of common classes of antibiotics and subsequent confirmation of non-transmission of drug resistance genes or virulence plasmids (Moubareck et al. 2005). Evaluation should also take the end-product formulation into consideration because this can induce adverse effects in some subjects or negate the positive effects altogether. A better understanding of the potential mechanisms whereby probiotic organisms might cause adverse effects will help to develop effective assays that predict which strains might not be suitable for use in probiotic products.

Quality control of probiotics in aquaculture will become an important issue. With the increased use of molecular methods for the definitive analysis of the bacterial components of probiotic products and for *in vivo* validation, it is expected that both the probiotics quality and functional properties can significantly be improved. This type of research can aid for the development of adequate technology for the evaluation of the efficiency and safety of microbial agents as probiotics in aquaculture (Qi et al. 2009).

4. Prebiotics – The concept

4.1. History, definition and principles

The term *prebiotic* comes from the Greek words “pro” and “bios" meaning “before life”. The probiotic concept was introduced about fifteen years ago by Gibson and Roberfroid (1995). A prebiotic was originally defined as “A non-digestible foods ingredient(s) that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health” (Gibson and Roberfroid 1995). In the past decade a large number of studies have demonstrated that prebiotics have great potential as agents to improve or maintain a balanced intestinal microbiota to enhance health and wellbeing. The European market for health promoting prebiotics is growing rapidly (FAO 2007). However, a prebiotic effect has been attributed to many food components, sometimes without due consideration to the criteria required. In particular, many food oligosaccharides and polysaccharides (including dietary fiber) have been claimed to have prebiotic activity, but not all dietary carbohydrates are prebiotics. There is, therefore, a need to establish clear criteria for classifying a food ingredient as a prebiotic. Such classification requires a scientific demonstration that the ingredient: (1) resists gastric acidity, hydrolysis by enzymes and GI absorption; (2) is fermented by the intestinal microflora; (3) stimulates selectively the growth and/or activity of intestinal bacteria associated with health and wellbeing (Gibson et al. 2004).

Gibson and colleagues (2004) have recently reviewed their original prebiotic concept in the light of much research that has been published in the past decade, and in particular the three key aspects of their definition: (1) resistance to digestion; (2) fermentation by the intestinal microflora; and (3) a selective effect on the flora that promote health. Their updated definition is: "A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health". The key words in both definitions are "selective" and "benefit/improve...host...health". Therefore, a prebiotic substrate must be particularly readily available to some groups of bacteria of which lactobacilli and bifidobacteria are considered indicator organisms that are beneficial to intestinal health, but less available to potentially pathogenic bacteria, such as toxin-producing *Clostridia*, proteolytic *Bacteroides* and toxygenic *E. coli* (Manning and Gibson 2004). In this manner, a "healthier" microbiota composition is obtained whereby the bifidobacteria and/or lactobacilli become predominant in the intestine and exert possible health promoting effects. The principal concept associated with both of these definitions is that the prebiotic has a selective effect on the intestinal microbiota which results in an improvement in health of the host. The definitions arose from observations that particular dietary prebiotics as functional ingredients bring about a specific modulation of the GI ecosystem, particularly increased numbers of beneficial bacteria, and decreased numbers of potential pathogenic species, which associated with improved host health.

According to FAO experts "A prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota" (FAO 2007). The definition has the following characteristics: 1) Component - not an organism or drug; a substance that can be characterized chemically; in most cases this will be a food grade component; 2) Health benefit - measurable and not due to absorption of the component into the bloodstream or due to the component acting alone; and over-riding any adverse effects; 3) Modulation - show that the sole presence of the component and the formulation in which it is being delivered changes the composition or activities of the microbiota in the target host. Mechanisms might include fermentation, receptor blockage or others.

There are several recognized functional prebiotic oligosaccharides in use around the world: Fructooligosaccharides (FOS), Mannanoligosaccharides (MOS), Xylooligosaccharides (XOS), Inulin, ß-glucan (Qiang et al. 2009). The most prebiotics are indigestible, but fermentable carbohydrates. (Ouwehand et al. 2005; Mussatto and Mancilha 2007; Wang 2009). The main advantage of prebiotic oligosaccharides is that they are natural functional ingredients. Their incorporation in the diet does not require particular precautions, and their authorization as food/feed additives may be more easily obtained, in spite of some concerns about their safety and efficacy (Gatesoupe 2005).

The field of prebiotic research is still young, yet the progress made in elucidating the beneficial health effects of specific prebiotics is significant not only for humans and animals, but for fish species as well. During the last ten years a number of studies have investigated the effects of prebiotics on health and productivity of poultry (Patterson and Burkholder 2003; Kocher et al. 2005; Denev et al. 2005, 2005a, 2006: Denev 2008; Choct 2009; Janardhana et al. 2009); pigs (Awati, 2005; Pettigrew et al. 2005; Roselli et al. 2005; Xu et al. 2005; Pierce et al. 2006; Kogan and Kocher 2007; Bindelle et al. 2008); calves (Terre et al. 2007; Heinrichs et al. 2009), rabbits (Mourao et al. 2006) and pets (Strickling et al. 2000; Kocher and Tucker 2005). Independently that the studies with prebiotics in aquaculture are limited, they are important for improving growth performance, immunomodulation and resistance to diseases of various fish species, shrimp and other aquatic organisms.

4.2. Prebiotics in fish aquaculture

4.2.1. Modes of action

In recent years there has been great interest in the use of prebiotics in fish aquaculture (Li and Gatlin 2004, 2005; Staykov et al. 2005, 2007; Genc et al. 2007a, 2007b; Yilmaz et al. 2007; Grisdale-Helland et al. 2008; Hai and

Fotedar 2009). Various mechanisms have been proposed to explain their specific action, such as selective stimulation of beneficial microbiota, improvement of immune functions, disease resistance, survival, growth performance and feed efficiency.

4.2.1.1. Selective stimulation of beneficial microbiota

Originally, prebiotics were chosen to stimulate beneficial probiotic microbiota in the GI ecosystem. According to Gatesoupe (2005) the case is different in fish, where many opportunistic bacteria can utilise a wide range of carbohydrates. Some of these strains may be capable of metabolising the oligosaccharides, but the effect on the host fish remains uncertain. The pathogenic strains are highly specialised, and unlikely to benefit from the prebiotic. However, the continuous supply of the substrate in the intestine may create the risk that the pathogen could acquire the ability to use either the native compound or its degraded products. It is wise to introduce the prebiotic in the diet with discernment, and many experiments will be necessary with microbial survey, before practical applications in hatcheries and fish farms.

According to updated prebiotic concept not all dietary carbohydrates are prebiotics (Gibson et al. 2004). Prebiotics (FOS, MOS, GOS, and XOS) are functional substrates that can only be consumed by a limited number of beneficial bacteria (lactobacilli and bifidobacteria). Among the group of beneficial bacteria present in the GI tract are those that most utilize prebiotic oligosaccharides being considered as the only microorganisms able to beneficially affect the host's health. Numerous human and animal feeding studies have shown that they selectively stimulate one or a limited number of beneficial bacteria thus causing a selective modification of the host's intestinal microbiota. (Teitelbaum and Walker, 2002; Mussatto and Mancilha 2007; Venter 2007). This clearly built upon the success of prebiotics for microbiota management approaches.

Many prebiotics (FOS, MOS) have been investigated for nutritional manipulation of the GI ecosystem of humans and animals, because they facilitate and support the symbiotic relationship between host and its microbiota (Newman 2004; Ferket, 2004; Venter 2007). FOS and MOS are two classes of prebiotic oligosaccharides that are beneficial to enteric health, but they do so by different means. For example, dietary supplementation of prebiotic oligosaccharides has been shown to provide a nutrient source for beneficial bacteria and may promote the maintenance of bifidobacteria and certain LAB in the gut of humans and animals (Mussatto and Mancilha 2007; Moura et al. 2007; Denev, 2008). FOS influence enteric microflora by 'feeding the good bacteria', which competitively excludes the colonization of pathogens and thus improving animal health and growth performance (for reviews see Ferket 2004; Newman 2004).

The nutritional properties of prebiotics are related directly to the physiological changes they induce in the host. Bacterial metabolites are probably the main effectors of most observed effects. The most important metabolites are the short-chain fatty acids (SCFA) as acetate, propionate and butyrate. Prebiotic consumption cans double the pool of SCFA in the GI tract. These SCFA acidify the GI environment, which is beneficial for the development of probiotic bacteria, and detrimental to the growth of harmful species (Blaut, 2002; Venter 2007).

On the other hand unlike FOS, MOS is not used as a substrate in microbial fermentation, but it still exerts a significant growth-promoting effect by enhancing the animal's resistance to enteric pathogens. For example Bio-Mos® (Alltech Inc., KY, USA) as the commercial source of MOS has been used in most of the published research literature. According to Ferket (2004) Bio-Mos® enhances resistance to enteric disease by different means. One of them is that it inhibits colonization of enteric pathogens by blocking bacterial adhesion to gut lining. Adhesion of pathogens to the epithelium surface of the gut (colonization) is believed to be the first critical stage leading to infection (Moran 2004). Many potential pathogens as *E. coli, Salmonella* and *Vibrio* spp. as well as commensally bacteria attach to the mucosal surface of the intestine and that attachment is the key to their proliferation. They have mannose specific lectins on the cell surface that recognize specific sugars and allow the cell to attach to that sugar (Panigrahi and Azad 2007). Early studies using mannose in the drinking water of broiler chicks demonstrated that this therapy could reduce colonization rate of *S. typhimurium*. Purified mannose and MOS have been successfully used to prevent bacterial attachment to the host animal by providing the bacteria a mannose-rich receptor that serves to occupy the binding sites on the bacteria and prevent colonization in the animal. Gram-negative pathogens with the mannose-specific Type-1 fimbriae attach to the MOS instead of attaching to intestinal epithelial cells and they move through the gut without colonization (Panigrahi and Azad 2007) used a chick model to demonstrate that MOS (Bio-Mos®) could significantly reduce the colonization of *E. coli* and *Salmonella* spp. Animal trials in other species show similar benefits in reducing pathogen concentrations. It has been proposed that pathogenic bacteria bind to MOS in the intestinal lumen rather than to the mucosal surface, and therefore fail to proliferate so extensively, which enhancement of intestinal integrity. The control of bacteria-mediated attachment has been proposed as a possible means of reducing enteric infection.

In summary, the information about specific biological functions and action of prebiotic oligosaccharides in the GI ecosystem of fish and other aquatic organisms is very limited. Moreover, the interactions between prebiotics and the indigenous microbiota are still poorly understood. The above hypotheses need to be tested by further studies with different fish species to confirm these earlier findings. A lot more fundamental research has to be done to develop mechanisms to verify, models to certify and methods to quantify the beneficial effects of prebiotics in this area of interest. Finding ways to exploit this knowledge in fish aquaculture is the current challenge.

4.2.1.2. Improvement of immune functions and disease resistance

The immune system of the fish is the primary defense mechanism against infectious disease. Immunity in fish like that in all other vertebrates plays a major role in protection against pathogens. It can be either non-specific, which is an innate defense mechanism or an acquired specific immunity. Immune systems of most fish are very similar, and have certain similarities to mammals. Fish, however, rely more on non-specific defense mechanisms than mammals do (Swain et al. 2007). The non-specific immune system of fish consists of several key humoral and cellular components that provide innate protection against infection, regardless of the pathogen type (Magnadóttir 2005; Whyte 2007). To date, several studies have demonstrated the benefits of immunostimulants on the fish immune system (Amara et al. 2004; Huttenhuis et al. 2006; Kunttu et al. 2009). However, there is very little information regarding the effect of prebiotics in fish although they have been successfully used in terrestrial animals - pigs, poultry and calves. Numerous studies have investigated the effect of prebiotic MOS on their humoral and cellular immunity. Whilst the exact mechanisms have not been completely elaborated, significant evidence has been accumulated to propose that MOS plays a multi-purpose role in immune modulation (for reviews see Ferket, 2004; Moran, 2004).

Recently Staykov et al. (2005, 2007) have reported that MOS (Bio-Mos®, Alltech Inc., KY, USA) incorporated into a standard commercial extruded diets (2 kg/t) significantly increased serum lysozyme levels ($P < 0.001$) as well as complement activity ($P < 0.05$) and reduced mortality of rainbow trout *Oncorhynchus mykiss* raised in net cages and in raceway systems. Similar results were obtained with common carp *Ciprinus carpio* (Staykov et al. 2005). These results demonstrated that prebiotic MOS have a positive effect on non-specific immune response, enhanced resistance to diseases and survival of fish. Similar effects have been published from other researchers. Zhou and Li (2004) reported significant improvement of several indicators of immune status of carp fed a diet supplemented with MOS. Other study clearly demonstrated the association of improved immune status and resistance to diseases in fish fed Bio-Mos® (Sweetman and Davies 2006). Torrecillas et al. (2007) reported the ability of MOS to improve immune status and disease resistance of sea bass (*Dicentrarchus labrax*) in cohabitation and inoculative challenge tests with the pathogen *Vibrio alginolyticus*. In other study Torrecillas et al. (2007a) observed also that supplementation of the diet with prebiotic MOS enhanced the immune functions (phagocytic activity of leucocytes and the bacterial activity of the sera) of European sea bass. The immune parameters in the MOS fed groups are statistically significant, compared to the untreated control.

During the last five years many studies in finfish aquaculture demonstrated as well that supplementation of Bio-Mos® in the diet has stimulated immune activity and promoted resistance to bacterial infection of different fish species: common carp (*Cyprinus carpio*; Staykov et al. 2005c, 2006b); rainbow trout (*Salmo gairdneri irideus* G.; Staykov et al. 2005a, 2005b, 2006, 2006a); channel catfish (*Ictalurus punctatus*; Welker et al. 2007); rainbow trout (*Oncorhynchus mykiss*; Staykov et al. 2009); Marron (*Cherax tenuimanus*; Sang et al. 2009); juvenile western king prawns (*Penaeus latisulcatus*; Hai and Fotedar, 2009); European sea bass (*Dicentrarchus labrax*; Terova et al. 2009) and etc. However, full understanding of the mode of action of Bio-Mos® on the immune activity of fish awaits further research.

In the past decade many studies have demonstrated that not only MOS, but inulin (Cerezuela et al. 2008) and glucans (Cook et al. 2001; Paulsen et al. 2001; Bagni et al. 2005; Mai et al. 2006; Kumari and Sahoo 2006; Misra et al. 2006; Skjermo et al. 2006; Ai et al. 2007; Wang et al. 2007; Djordjevic et al. 2009; Rodriguez et al. 2009) are effective natural immunostimulants as well. They enhanced immunity and disease resistance of different fish species as well.

4.2.1.3. Effects on fish health and productivity

Controlled experiments took place at Trakia University, Stara Zagora, Bulgaria with carp (*Cyprinus carpio*) (Staykov et al. 2005c) in which 0.2% prebiotic MOS (Bio-Mos®, Alltech Inc., KY, USA) was incorporated into a standard commercial extruded diet (23.5% protein and 5.4% lipid). From a start weight of approximately 140 g, fish given Bio-Mos® grew to an average weight of 480 g vs. 430 g in controls, an 11.6% higher weight gain ($P < 0.001$). Feed conversion ratios (FCR) were improved with Bio-Mos® (1.69 vs. 2.05 in controls), by 17.6% ($P < 0.01$). Lower mortalities were also observed in the Bio-Mos®-fed fish (1.92% vs. 3.59% for the control ($P < 0.001$).

Juvenile carp reared in tanks at the University of Osijek in Croatia showed similar improvements in weight gain in response to Bio-Mos® (Culjak et al. 2006). The diet used in these trials was 39.91% crude protein and 4.51% lipid; and Bio-Mos® was added at 0.6%. The fish grew from an average weight of 5.28 g to 31.23 g in controls vs. 38.73 g in the Bio-Mos® treatment, a 24% higher weight gain ($P < 0.01$). Bio-Mos® improved FCR from 2.06 to 1.60 ($P < 0.05$); and mortality from 50.0 to 16.7% ($P < 0.01$).

Similar studies were conducted at Trakia University, Stara Zagora, Bulgaria with rainbow trout (*Oncorhynchus mykiss*) with supplementation of prebiotic Bio-Mos® (0.2%) in standard commercial extruded feeds. The rainbow trout was raised in net cages and raceways (Staykov et al. 2007). In the net cages, at the end of the six week trial period, the mean body weight of fish receiving prebiotic was 13.7% higher, compared to the control groups ($P < 0.01$). The supplementation of prebiotic in the diet significantly decreased FCR ($P < 0.05$) and mortality ($P < 0.01$). In the raceways, the final body weight of the rainbow trout, receiving MOS was significantly improved by 9.97% compared with the control group ($P < 0.01$). The FCR and mortality in the experimental group was significantly lower ($P < 0.01$) in comparison with the untreated control. These results demonstrated that prebiotic mannan-oligosaccharides can be an effective tool for improving the growth performance, health status and feed efficiency of rainbow trout grown in a variety of production systems.

The addition of prebiotic MOS (Bio-Mos®) to the diet of other freshwater species such as European catfish (*Silurus glanis)* juveniles (Bogut et al. 2006) has shown similar improvements in growth from 22 to 76 g in the control groups and 83 g in the Bio-Mos® groups, a 9.7% higher average body weight ($P < 0.01$). The FCR was also lower by 11.6% ($P < 0.01$) and mortality decreased from 28.33 to 16.67% ($P < 0.01$). These data support findings of Hanley et al. (1995) who also demonstrated that hybrid red tilapia juveniles, fed 0.6% prebiotic (Aqua-Mos™, Alltech Inc., KY, USA) in their hatchery diets had a 22.5% improved survival with a 27.2% increase in weight gain.

In Turkey, Hossu et al. (2005a, 2005b) determined the effects of prebiotic Bio-Mos® on digestibility of the diet and growth performance of gilthead sea bream *Sparus aurata* in a commercial scale production. Bio-Mos® (0.2%) significantly improved digestibility of the diet, weight gain, final body weights, feed efficiency, and reduced mortality rate, compared with fish receiving control diet.

Hai and Fotedar (2009) investigated the effects of dietary immunostimulants (Bio-Mos® and β-1,3-D-glucan) on the growth, survival and immune responses of juvenile western king prawns (*Penaeus latisulcatus*). Continuous supplementation with the above immunostimulants (84 days) showed considerable improvement in the growth, FRC, survival and immune response of the prawns.

Bio-Mos®, produced by Alltech, Inc. is the most thoroughly researched of the mannan oligosaccharide products now available. According to Sweetman and Davies (2006) it has been shown to be an effective tool for fish producers increasing performance and health status of a number of important commercial species. Acting in a prophylactic manner it provides multiple benefits when incorporated in to aqua feed diets. Bio-Mos® improves the GI morphology and therefore its function through an increased absorptive surface and better absorptive capability and interacts with the immune system in a modulatory manner, and alters enzymeactivity promoting the better utilization of dietary nutrients therefore improving performance characteristics and immune function The combination of all these benefits results in better performance, livability and disease resistance and therefore gives a more cost effective fish production.

During the last ten years a large number of well conducted trials and several reports have documented the effects of different prebiotics on fish health and productivity: MOS (Zhou and Li 2004; Zegarra et al. 2005; Daniels 2005; Daniels et al. 2006; Sweetman and Davies 2006; Torrecillas et al. 2006; Peterson et al. 2007; Grisdale-Helland et al. 2008; Staykov et al. 2005, 2006, 2006a, 2006b, 2009); FOS (Hui-yuan et al. 2007; Grisdale-Helland et al. 2008); XOS (Xu et al. 2009); Grobiotic™ (Li and Gatlin 2004; 2005; Li 2005) and etc. These studies have shown that the use of prebiotics for improvement of fish health and performance are one of the best-documented applications. Additional evaluations are required to obtain data that will result in optimal health benefits and productivity. Further microbiological, physiological, immunological, nutritional, and environmental research is needed to identify modes of action and applications for these promising functional ingredients in fish aquaculture.

5. Conclusion and future trends

Science-based knowledge on pro- and prebiotics has increased in recent years. No doubt exist that in the last decade we have greatly expanded our knowledge about pro- and prebiotics as important natural ingredients in finfish aquaculture. They have numerous beneficial effects: improved activity of GI microbiota and enhanced immune status, disease resistance, survival, growth performance and feed utilization. As functional dietary products pro- and prebiotics have much potential to increase the efficiency and sustainability of aquacultural production.

Therefore, comprehensive research to more fully characterize the intestinal microbiota of prominent fish species, mechanisms of action of pro- and prebiotics and their effects on intestinal ecosystem, immunity, fish health and performance is warranted. Not enough is known of the composition, dynamics, metabolism and activity of the GI microbiota in health and diseases. Particular efforts should be made to characterize the probiotic properties of intestinal bacteria, mechanisms of their benefits and host-microbe interactions to facilitate the selection of effective probiotics, according the needs of different fish species. In addition, more detailed knowledge of the pathogenic agents in aquaculture, their virulence factors and their interactions with the host would be of great importance. Knowledge of proliferation and invasion sites of the fish pathogens would assist in determining whether a water borne or food borne vehicle is the most appropriate. Such understanding is required for further technological developments.

Also the knowledge on the role of the intestinal microbiota in the development and function of immune response needs more investigations. Well-designed studies are required to evaluate the potential of pro- and prebiotics for optimizing the immune functions, to increase resistance to diseases and responsiveness to vaccines. Also, there is need for establishing dose-response relationships. Carefully controlled nutrition trials, under real conditions, need to be carried out to determine the beneficial effects of pro- and prebiotics before substantial health and production claims can be made.

It is clear that the further development of this field reliable testing methods are needed. One example is through the application of up to date molecular procedures to improve our knowledge of the gut microbiota, its interactions with the fish host and its role in maintenance of health. Advances of these areas will ultimately provide us with very sophisticated dietary tools to manipulate this important ecosystem and improve fish health and performance. The development and validation of research methods, *in vitro*, *ex vivo* and *in vivo* test models, have also provided important information to understand the mechanisms of action behind the effects.

On the other hand, a combination of a pre- and probiotic, termed a symbiotic, is receiving much attention at present in the since this association is thought to improve the survival, activity and efficiency of probiotic bacteria in the GI ecosystem. Symbiotics represent a very new concept for aquaculture. To the best of our knowledge, evaluation of these products has not been conducted to date in aquatic species. However, much more further research with different fish species are required to characterize the mechanisms of action and symbiotics effects of pro- and prebiotics on microbial ecology of the GI tract. These studies are essential for development of efficient management strategies to manipulate GI microbiota of fish and enhance their health and production.

Safety assessment is an essential phase in the development of the new up to date pro- or prebiotic supplements for fish. All pro- and prebiotic products must be evaluated for their safety before being used in fish nutrition. Instead, a multidisciplinary approach is necessary, involving contributions from ihtiologists, microbiologist, biochemists, physiologists, immunologists, pathologists, toxicologists, nutritionists, and ecologists. However, future development of pro- and prebiotics for fish aquaculture requires stringent guidelines for safety assessment of these products. The above problems should be a high research priority in the finfish aquaculture during the forthcoming years.

Abbreviations: GI- Gastrointestinal; LAB- Lactic acid bacteria; AGP- Antibiotic growth promoters; MOS- Mannanoligosaccharides; FOS- Fructooligosaccharides; XOS – Xylooligosaccharides.

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