

Probiotics in Aquaculture – Benefits to the Health, Technological Applications and Safety

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1. Introduction

Aquaculture is the fastest growing food-producing sector in the world at an average rate of 8.9% per year since 1970, compared with only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems over the same period (Subasinghe, 2005). Although aquatic food production through aquaculture is the fastest growing sector and vaccines are being developed and marketed in aquaculture, the disease is still a major problem in the aquaculture farming industry (Bondad-Reantaso *et al.*, 2005). During the last decades, chemical additives and veterinary medicines, especially antimicrobial agents, to prevent and control disease have been also applied in aquaculture (Wang and Xu, 2004; Cabello, 2006; Lupin, 2009). However, the risks associated with the transmission of resistant bacteria from aquaculture environments to humans, and the introduction in the human environment of nonpathogenic bacteria, containing antimicrobial resistance genes, and the subsequent transfer of such genes to human pathogens existed according to FAO (2005). Previous studies also show the aquatic bacteria can develop resistance genes as a consequence of exposure to antimicrobial agents (Smith *et al.*, 1994; Kim *et al.*, 2004; Sørum, 2006). Therefore, the need for alternative techniques is increasing and the contribution of probiotics may be considerable.

The use of probiotics in aquaculture is now widely accepted with an increasing demand for environment friendly aquaculture (Ringø and Gatesoupe, 1998; Gatesoupe, 1999; Sharma and Bhukhar, 2000; Irianto and Austin, 2002; Wang and Xu, 2006; Vine *et al.*, 2006; Wang, 2007; Denev *et al.*, 2009; Qi *et al.*, 2009). Nowadays, a number of preparations of probiotics are commercially available and have been introduced to fish, shellfish and molluscan farming as feed additives, or are incorporated in pond water (Moriarty, 1998; Wang *et al.*, 2005; Prado *et al.*, 2010). According to the claims of the producers, these products are effective in supporting the health of aquatic animals and are also safe. However, there are doubts with regard to the general concept of probiotics and to these claims on the other hand. Indeed, the current explanations and principles are still not enough to describe what

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probiotics actually are, where they come from, and what they can do (Wang *et al.*, 2008). Thus, there is clearly a need in increasing our knowledge of aquacultural animals and of effective preparation, technological applications and safety evaluation of probiotics. This chapter provided a summary of the status and challenges of probiotics application in aquaculture. In this chapter, the benefits to the health, technological application and safety evaluation were discussed. In addition, the probiotics information in aquaculture obtained from authentic and highly regarded sources was contained and listed.

2. Probiotics and gut microbiota

Three general modes of probiotics actions have been classified and presented by Oelschlaeger (2010) as follow: (1) Probiotics might be able to modulate the host's gut defences including the innate as well as the acquired immune system and this mode of action is most likely important for the prevention and therapy of infectious diseases but also for the treatment of inflammation of the digestive tract or parts thereof. (2) Probiotics can also have a direct effect on other microorganisms, commensal and/or pathogenic ones and this principle is in many cases of importance for the prevention and therapy of infections and restoration of the microbial equilibrium in the gut. (3) Finally, probiotic effects may be based on actions affecting microbial products, host products and food ingredients and such actions may result in inactivation of toxins and detoxification of host and food components in the gut. According to above summary, all three modes of probiotics actions are all likelihood associated with gut and/or gut microbiota. Therefore, it has become apparent that we are in fact dealing with another "organ", the so called "microbiotic canal" with the increased knowledge of the specific activity of the gut microbiota (Wolf, 2006). In general, the gut microbiota remain relatively stable throughout life once established although they can be influenced by several factors such as mode of delivery, hygiene and the use of antibiotics.

The gut microbiota with the epithelium and mucosal immune system orchestrate a network of immunological and nonimmunological defenses, providing both protection against pathogens and tolerance to commensal bacteria and harmless antigens (Sanz and Palma, 2009). The important role of commensal bacteria in development of optimally functioning mucosal immune system was demonstrated in germ-free animals (Tlaskalová-Hogenová, 2004). Therefore, the imbalance of gut microbiota has been linked to several diseases including inflammatory bowel diseases, periodontal disease, rheumatoid arthritis, atherosclerosis and allergy. So probiotics, that is, microbial strains that have beneficial effects on the host, are thought to benefit this intestinal ecosystem (Julio and Marie-Josée, 2011). In addition, some probiotics strains also induce the secretion of multiple antimicrobial materials by intestinal Paneth cells through cell-autonomous MyD88-dependent toll-like receptor activation (Vaishnavi *et al.*, 2008) and regulate the alterations of permeability related with infections, stress, and inflammatory conditions (Lutgendorff *et al.*, 2008). There is evidence that probiotics produce a protective effect on the gut microbiota and the beneficial effects of probiotics on several microbial disorders have been well reviewed (Gismondo *et al.*, 1999).

As for the aquatic animals such as fish and shrimp, the colonization of the gastrointestinal tract starts immediately after hatching and is completed within a few hours to 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 ecosystem (Balcázar *et al.*,

2006). This is attributed to competitive exclusion mechanisms and improved immune system development and maturation. Intake of probiotics has been demonstrated to modify the composition of the microbiota, and therefore assist in returning a disturbed microbiota (by antibiotics or other risk factors) to its normal beneficial composition (Gómez and Balcázar, 2008). As for the mechanisms during this physiological process, the production of antimicrobial substances, competition for nutrients or adhesion receptors, inhibition of virulence gene expression and enhancement of the immune response are all included (Irianto and Austin, 2002; Nikoskelainen, *et al.*, 2003; Vine *et al.*, 2004; Kim and Austin, 2006; Balcázar, *et al.*, 2007). However, the exact mechanism by which these probiotics do this is not known. Advances in the understanding of the mechanisms between gut microbiota and probiotics and how the immune system of aquatic animals generally responds to gut microbiota would be of great help to identify the molecular targets of probiotics and the biomarkers of their effects, and to provide sounder evidences on their benefits on physiologic conditions and immune-mediated disorders.

3. Probiotics effects in aquaculture: Benefits to the health

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 (Kesarcode-Watson *et al.*, 2008). Indeed, aquatic animals are quite different from the land animals and 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). A fairly constant habitat of resident microbiota in the gastrointestinal tract of terrestrial livestock is important, whereas most microbiota is transient in aquatic animals (Moriarty, 1990). Shift in intestinal microflora of Atlantic halibut (*Hippoglossus hippoglossus*) larvae during first feeding was studied and the results showed the transition from a prevailing *Flavobacterium* spp. intestinal flora to an *Aeromonas* spp./*Vibrio* spp. dominant flora occurred when first feeding commenced (Bergh *et al.*, 1994). It indicated that the gut microbiota of aquatic animals may change rapidly with the intrusion of microflora from water, live food and artificial diet. In addition, aquatic animal and microorganisms share the same ecosystem in the aquatic environment and it suggested that the interaction between the microbiota, including probiotics, and the host is not limited to the intestinal tract. Therefore, the definition of a probiotic for aquatic environments needs to be modified, which allows a broader application of the term “probiotic”. A probiotic is then defined by Verschuere *et al.* (2000) as 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.

Most probiotics used in aquaculture belong to the lactic acid bacteria, of the genus *Bacillus*, to the photosynthetic bacteria or to the yeast, although other genera or species have also been mentioned (Fig. 1). Many studies have reported promising results using a single beneficial bacterial strain as probiotic in the culture of many aquatic species (Gatesoupe, 1991; Noh *et al.*, 1994; Bogut *et al.*, 1998; Carnevali, *et al.*, 2006; Díaz-Rosales *et al.*, 2009; Li *et al.*, 2009; Zhou *et al.*, 2009; Tovar-Ramírez *et al.*, 2010; Wang and Gu, 2010; Zhou *et al.*, 2010; Wang, 2011). It is important to consider the possibility of using different species, as suggested by Noh *et al.* (1994) and Bogut *et al.* (1998). The effect of probiotics, photosynthetic

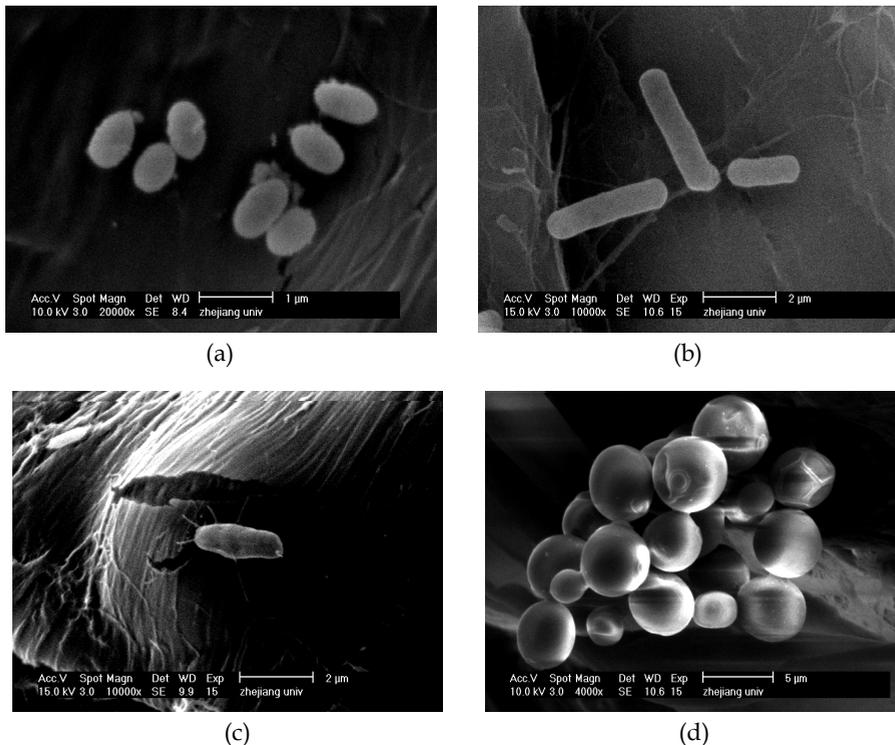


Fig. 1. The configurations of putative probiotics strains isolated and stored in our laboratory using scanning electron microscope (Philips XL30ESEM, Netherlands). a, *Lactococcus lactis*; b, *Bacillus coagulans*; c, *Rhodospseudomonas palustris*; d, *Saccharomyces cerevisiae*.

bacteria (*Rhodobacter sphaeroides*) and *Bacillus* sp. (*B. coagulans*), on growth performance and digestive enzyme activity of the shrimp, *Penaeus vannamei*, was investigated and the results showed that the effects were related with supplementation concentrations of probiotics and thus use of a 10 g/kg (wet weight) supplement of probiotics in shrimp diet was recommended to stimulate productive performance (Wang, 2007). A mixture of *Bacillus* probiotic bacteria (*Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilus*) was also evaluated in the gilthead sea bream (*Sparus aurata*) larviculture focusing on their effects on survival, growth and general welfare (Avella *et al.*, 2010). The data generated in this study show the benefit of the administration of *Bacillus* probiotic mixture in terms of stress response and growth and provide scientific and technical support for the implementation of sustainable development of sea bream aquaculture. Similar results were also observed in olive flounder supplemented with *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus sakei*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* as individual and mixed enriched diet (Harikrishnan *et al.*, 2011a). Lactobacil probiotics individually or mixed with Sporolac enriched diet were used to enhance the immune status, thereby improving the disease resistance in lymphocystis disease virus infected olive flounder (*Paralichthys olivaceus*) and the results showed that the better innate immune response and disease resistance were found in groups supplemented with mixed probiotics (Harikrishnan *et al.*,

2010). However, feeding experiments conducted on 600 *O. niloticus* using the diets containing single or mixed isolated probiotic bacteria show the different results in survival rates and the highest with fish fed diets supplemented with *B. pumilus* was observed, followed by a mixture of probiotics (*B. firmus*, *B. pumilus* and *C. freundii* in equal numbers), and then *C. freundii* (Aly *et al.*, 2008). It indicates that the beneficial effects of probiotics fed aquatic animals are associated with probiotic strains, isolation species, culture animals and water quality. Altogether, the data reported above may well explain the current trend to prefer alternative probiotics for the application in aquaculture.

Additionally, a large number of studies have combined probiotics with prebiotics, 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 (Gibson *et al.*, 2004). Thus the synbiotics, as a combination of probiotics and prebiotics, have been studied to expect the synergistic effects. Nowadays, there are several recognized functional prebiotic oligosaccharides such as fructooligosaccharides (FOS), mannan oligosaccharides (MOS), insulin, β -glucan, and xylo-oligosaccharides (XOS) in use around the world. The effect of dietary application of a commercial probiotic (*Bacillus* spp.) and MOS, used singularly and combined, on the survival, growth performance and feed cost-benefit of European lobster (*Homarus gammarus*) larval was assessed and the results in this study strongly suggest that the dietary combination of *Bacillus* spp. and MOS is cost effective when used to promote survival and provides the added benefits of improved growth performance, compared to their individual supplementation (Daniels *et al.*, 2010). Similar results have been reported on shrimp, *Litopenaeus vannamei*, and the disease resistance was also improve by enhancing immunity, as well as presumably modulating microflora in the shrimp's gut (Li *et al.*, 2009). It suggested that the combined application of probiotics and prebiotics is an interesting prospect for replacement of growth-promoting chemotherapeutics in the aquaculture industry and could be a useful tool in the rearing of certain aquatic animals. Recently, herbs and probiotics are combined in diet and treated as one of the promising alternative tools to supplement and supplant antibiotics, chemicals or vaccines (Sahu *et al.*, 2008; Nayak, 2010). According to Harikrishnan *et al.* (2011b), administration of probiotics (*Lactobacillus sakei* BK19) and herb (*Scutellaria baicalensis*) can effectively minimize the mortality and restore the altered hematological parameters and enhancing the innate immunity in *O. fasciatus* against *Edwardsiella tarda*, which indicate a promising role to prevent diseases and disease outbreaks in aquaculture. Similar results were also determined in olive flounder, *Paralichthys olivaceus*, against *Streptococcus parauberis* and the enhanced growth, blood biochemical constituents, and nonspecific immunity were observed in the groups treated with probiotics and herbals mixture supplementation diet (Harikrishnan *et al.*, 2011c). Further investigations on the interaction between probiotics and other functional additives at molecular level are warranted in aquaculture.

4. Manufacture and safety evaluation of probiotics

The continuing expansion of interest in probiotic bacteria has led to an increase in manufactured functional foods and feeds containing these bacteria. Given the natural and/or intestinal origin of these microorganisms, the challenges these putative probiotics face in order to be in a highly viable state throughout processing, manufacture, and storage are enormous. Environmental stresses such as temperature, acid, exposure and osmotic

pressure, oxygen have important effects on probiotics survival and activity both in product and animal gut. However, like all bacteria, probiotic bacteria retain a broad arsenal of molecular mechanisms to combat the often lethal environmental stresses encountered during processing and following ingestion and therefore the comprehensive appreciation of these mechanisms should inevitably lead to the design and manufacture of probiotic cultures, which retain greater viability through to the target site in the intestine (Corcoran *et al.*, 2008). Environmental stress responses in *Lactobacillus*, which have been investigated mainly by proteomics approaches, are reviewed by De Angelis and Gobbetti (2004) and the physiological and molecular mechanisms of responses to heat, cold, acid, osmotic, oxygen, high pressure and starvation stresses are described. As for the proteomics approaches, the technique primarily bases on two-dimensional gel electrophoresis (2-DE) (Kellner, 2000). The intensity of an individual spot indicates how much the cell has produced of that actual protein and thus it has facilitated the rapid characterization of thousands of proteins in a single polyacrylamide gel for the molecular mechanism studies of probiotics. Such studies associated with the cellular processes and metabolism mechanisms available to probiotic bacteria to facilitate survival in various stressful conditions can lead to production of designer probiotic strains with enhanced viability in feed systems and efficacy following ingestion for aquatic animals. Additionally, several other factors including the physiologic state of the probiotics, the chemical composition of the product and possible interactions of the probiotics with the starter cultures must be considered to ensure the abilities of probiotics in aquaculture.

Although the probiotic species such as *Lactobacillus acidophilus* have been safely used for a long time, the safety aspects have always to be considered and possible adverse effects should continuously be evaluated as illustrated by literature (Salminen *et al.*, 1998). However, a growing number of diseases that appeared with the worldwide development of aquaculture may be assigned to distinct bacteria belonging to the genera *Streptococcus*, *Lactococcus*, *Vagococcus* and *Carnobacterium*, but, in most cases, the clear mechanisms have not been found (Ringø and Gatesoupe, 1998). In addition, safety considerations regarding antimicrobial resistance neglected for a long time are now taken into account for the development and marketing of probiotics (Courvalin, 2006). The question whether genetic exchange may occur between probiotics and gut microflora or pathogens is raised because the genes can be transferred between microorganisms. As a result, the antibiotic multi-resistance existent of probiotics shows the possible insecurity caused by the possibility of resistance genes transfer from probiotic strains to bacterial pathogens or from aquatic commensals to probiotics. According to O'Brien *et al.* (1999), it is important to differentiate between intrinsic resistance and that mediated by special genetic elements when evaluating the antibiotic resistance profiles among different species and strains. Indeed, safety is the state of being certain that adverse effects will not be caused by an agent under defined conditions. Therefore, feeding of novel probiotics to healthy aquatic animals is not only concerned with efficacy but safety even though lactobacilli and bifidobacteria are generally regarded as safe. With the development of molecular biology and other advanced modern techniques, the critical, tailored approaches such as cell culture to safety evaluation of probiotics can ensure that healthy benefits are accessible to aquatic animals. The epithelial cells of tilapia (*Oreochromis nilotica*) were isolated and primarily cultured as the cells model to evaluate the probiotic, *Rhodopseudomonas palustris*, through the morphologic characters, cells viability, livability and permeability (Wang and

Xu, 2007). This study shows cell culture is one of the promising approaches to safety evaluation of probiotic in the future.

5. Future probiotics for aquaculture

The important role of the gut flora in the maintenance of health and in the prevention of disease is well recognized (Holzapfel and Schillinger, 2002). Use of probiotics is likely to be the most natural and safe means for improving gut flora balance to prevent bacterial pathogens by competing for essential nutrients or attachment sites (Chukeatirote, 2003). As for aquatic animals gut flora, the continuous interaction with the environment, the body system and intrinsic microorganisms is very complex. Although the explosion in recent years of publications dealing with probiotic organisms has been increased, central and vital information is still needed and therefore more advanced methods should be developed to assess the changes in the composition of the gut flora and their mutual interaction with the metabolism of aquatic animals. Currently, probiotics may serve to partially replace the presently reduced or even prohibited application of nutritive antibiotics or chemotherapeutics in animal nutrition and in fulfillment of health claims in man and animals (Reuter, 2001). According to Kesarcodi-Watson *et al.* (2008), a probiotic for the new, effective and safe products in aquaculture must possess certain properties as follow: (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, and (5) it should preferably not contain virulence resistance genes or antibiotic resistance genes. These properties should be considered during the manufacture process and safety evaluation of novel probiotics. Then the future will provide targeted probiotic bacteria accord with above properties for specific use with carefully controlled studies on clearly defined selected strains. In addition, an increasing demand for alternative to antibiotics products applied in aquaculture indicates a bright future for probiotics and a number of better commercial probiotics will be available, particular directed at larval culture.

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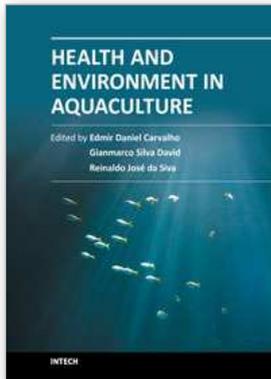
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Aquaculture has been expanding in a fast rate, and further development should rely on the assimilation of scientific knowledge of diverse areas such as molecular and cellular biology, and ecology. Understanding the relation between farmed species and their pathogens and parasites, and this relation to environment is a great challenge. Scientific community is involved in building a model for aquaculture that does not harm ecosystems and provides a reliable source of healthy seafood. This book features contributions from renowned international authors, presenting high quality scientific chapters addressing key issues for effective health management of cultured aquatic animals. Available for open internet access, this book is an effort to reach the broadest diffusion of knowledge useful for both academic and productive sector.

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