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Review

The use of probiotics in aquatic organisms: A review

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The production of aquatic organisms for human consumption has been decreasing while its demand has increased. The decline of fish products from fisheries has been in part compensated by the aquaculture industry. The need for enhanced disease resistance, feed efficiency, growth performance and lower production costs of cultured organisms is substantial for various sectors of this industry. Different products have been used at some extent to prevent disease and as growth promoters; however, their inadequate application can create adverse disorders, environmental imbalances, and increase predisposition to disease. In the search of new disease control and prevention options, several studies have been carried out to test some functional additives (probiotics). Probiotics have been shown to improve energy expenditure derived from sources such as carbohydrates and increase the incorporation of protein for growth; and to increase the immunity and disease resistance of the host. The use of probiotics in aquaculture has high potential application at a commercial level; however, it has been poorly characterized and studied. This study aims to review the use and action of probiotics in the culture of aquatic organisms (bivalve, fish, shrimp); and the potential for further application of this in aquaculture production.

Key words: Aquatic organisms, functional additives, probiotic.

INTRODUCTION

The culture of aquatic products for consumption and aquariology is growing and set to increase dramatically, as a result of overfishing of the world's waters and an increasing demand of seafood; which opens an extensive range of opportunities for the aquaculture industry. However, as aquaculture production increases, culture intensification may amplify the risk of problems such as widespread epizootics, inadequate nutrient balance in the artificial diets, deterioration of environmental conditions, disease due to physiological stress, poor growth and increased mortality (El-Haroun et al., 2006; Rollo et al., 2006).

Currently, the purpose of the aquaculture industry is to increase growth and or survival performance, feed efficiency, and resistance of aquatic organisms, while reducing production costs (Ali, 2006). Hormones, antibiotics, ionophores, plant extracts, and some salt compounds have been used at some extent for disease prevention and as growth promoters (Lara et al., 2000); however, chemotherapeutic agents have been banned

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for disease management in aquaculture systems due to the emergence of antibiotic resistance genes and enduring residual effects in the environment (Nayak and Mukherjee, 2011). Furthermore, emerging antibiotic-resistant bacteria on aquaculture create the risk of transferring the antibiotic-resistance plasmid to human pathogenic bacteria (Das et al., 2008); thus, in the last years, the use of probiotics in the culture of aquatic organisms has increased with the demand for more environment-friendly aquaculture practices.

Functional additives, like probiotics, represent a new idea on aquaculture; where the addition of microorganisms on the diet has a positive effect on growth because of the better nutrient assimilation (carbohydrates, protein, etc.), as well as by diminishing mortality by disease, increasing antagonism to pathogens, and a better microbial balance in the intestine and the environment (Irianto and Austin, 2002). Probiotics have several definitions in aquaculture. They have been described as a live microorganism food supplement which improves the microbial balance of the host intestinal flora and or (Vine et al., 2006; Ziaei-Nejad et al., 2006) and which also providing a health benefit to the host (Crittenden, 2005); they have also been described as biologically active components, or single or mixed cultures of microorganisms capable of improving the health of the host (Ochoa-Solano and Olmos-Soto, 2006); as live microorganisms that improve disease resistance (Tacon, 2002); and as live microorganisms administered in adequate amounts that confer a health effect on the host (Gomez et al., 2007). These definitions reflect the use of microorganisms or their products (microbial cells element or cell free supernatant factors) in rearing and culture tanks and ponds, as biological control or for their capacity to modify the bacterial composition of aquatic animal´s intestine, water and sediment, or used with feed as health supplement and/or biological control.

Criteria for probiotic selection in aquaculture

Bacteria present in the aquatic environment may determine the composition and population of the microorganisms in biofilms (gut, skin mucus, gills and other aquatic animal tissues) and vice versa. One of the purposes of using probiotics is to help in the composition and balance between the pathogenic and non-pathogenic microorganisms found in the environment and the biofilm and/or microbiota of aquatic organisms.

Suitable probiotic selection and use may represent a beneficial effect on the aquatic organism, and for that reason, probiotic strains have been isolated from indigenous and exogenous microbiota of aquatic animals. Ideally, microbial probiotics should have a beneficial effect and not cause any harm to the host. Therefore, all strains have to be non-pathogenic and non-toxic in order to avoid undesirable side effects when administered to aquatic animals (Chukeatirote, 2003).

Different modes of action or properties are desired on the potential probiotic, like antagonism to pathogens, the ability of probiotic cells to produce metabolites (like vitamins) and enzymes, colonization or adhesion properties, and the enhancement of the immune system (Ali, 2006; Swain et al., 2009) among others. On the other hand, a criterion to discard potentially harmful bacteria is the ability to produce toxins that induce lysis of host cells (Zamora-Rodríguez, 2003). Probiotics screening requires different selection strategies such as antagonism, production of beneficial compounds, and attachment and growth on various environments (Vine et al., 2004a); thus, the selection of some probiotics used on aquatic organisms is based in the following properties:

Antagonism

Bacterial antagonism is a common phenomenon in nature; therefore, microbial interactions play a major role in the equilibrium between competing beneficial and potentially pathogenic microorganisms, where microorganisms can produce different products that have inhibitory effects on microbial growth (Nayak and Mukherjee, 2011). Antagonism is used in probiotic products, and it is focused on counteracting the negative effects of pathogens (mainly bacteria) on aquatic organisms, while exerting their positive effects on host health (Gomez et al., 2007). Some probiotics with antagonistic effect are used for prevention and or to fight off bacterial disease, e.g. the use of lactic acid bacteria or yeast against *Flavobacterium psychrophilum*, the causative agent of the vertebral column compression syndrome, cause a decrease of this bacterium. *Vibrio* sp. and *Aeromonas* sp. have become the most pathogenic microorganisms in fish aquaculture used for the control of other pathogens. These pathogenic pseudomonads have received special attention as disease-protecting microorganisms and have been used as plant biocontrol. In recent years, there has been great interest in the use of lactic acid bacteria as disinfection agents (Gatesoupe, 2002; Venkat et al., 2004) and for the control of native microbiota such as *Aeromonas* and *Vibrio* spp. (Vazquez et al., 2005). *In vitro* antagonism tests, based on the natural phenomenon of antimicrobial metabolite production by some bacteria strains, are a frequent approach for screening probiotics. For example, antagonism of *Bacillus subtilis* (strain BT23) against *Vibrio harveyi* confers protection to *Penaeus monodon* (Vaseeharan and Ramasam, 2003). Guo et al. (2009) reported strong antagonisms of *Bacillus foraminis*, *Bacillus cereus biovar toyoi* and *B. fusiformis* against *Streptococcus inia*e and *Photobacterium damselae* subsp. *piscicida in vitro*, and increased larval survival of *Litopenaeus vannamei in vivo*. Antigenic components of diverse species of *Pseudomonas* exhibit different levels of antagonism against *Aeromonas hydrophila*. Cell free extracts of *Lactobacillus acidophilus*, *Streptococcus cremoris*, *Lactobacillus bulgaricus* (strains 56, 57) show a negative effect on growth of *Vibrio alginolyticus* in agar plate tests (Ajitha et al., 2004). The origin of the probiotic strain is an important element in antagonism tests. The microorganisms display different physiological or biochemical activities during their growth, which are based on their environments (fresh, seawater) and original source. These characteristics affect the probiotic potential for attachment sites and may create a false impression of the ability of probiotics to inhibit pathogens *in vivo* tests.

Competitive exclusion

Competitive exclusion is a process where an established microbiota prevents the colonization of a competing bacterial challenge for the same location. The objective of this type of probiotic products is to obtain a stable and equilibrated microbiota on culture, based on competition for attachment sites, nutrients, and production of inhibitory substances (Yan et al., 2002). Different strategies are displayed in the adhesion of microorganisms to those attachment sites as passive, hydrophobic and steric forces, electrostatic interactions, lipoteichoic acids, adhesins and specific adhesion structures (Salyers and White, 2002).

Some probiotics in aquaculture are designed to adhere on mucosal surfaces by a collection of microorganisms based on competitive exclusion factors (Farzanfar, 2004; Vine et al., 2004a, b). These factors are important for adhesion to intestinal epithelial cells or in the activation of immune system, thus helping the organism's health, intestinal homeostasis, and digestion (Gullian et al., 2004; Farzanfar, 2004; Panigrahi et al., 2004, 2005, 2011). These types of probiotic are extensively studied in fish; however, since these products were initially developed for vertebrates, their use in other aquatic organisms, like shrimp or bivalves, presents some problems. An example of this is the study by Beseres et al. (2005) which shows that *L. vannamei*, *Litopenaeus setiferus*, and *Farfantepenaeus aztecus* display a gut residence time of consumed feed from 45 to 90 min, which is a short time for bacteria adhesion or colonization.

Ziaei-Nejad et al. (2006) also show low colonization rates of *Bacillus* sp. on nauplius, zoea, mysis, and postlarvae (1-14) of *Fenneropenaus indicus*. However, some probiotic strains obtained from shrimp (Table 1) show different results; Rengpipat et al. (2000) used a *Bacillus* S11 obtained from *P. monodon,* which provided disease protection against *V. harveyi*. This effect was associated to an activation of the cellular and humoral immune defenses, and a possible competitive exclusion in the shrimp's gut. Li et al. (2008) used Arthrobacter XE-7, isolated from *P. chinensis* as a probiotic against *V. parahaemolyticus* with similar results.

Immune stimulation

Some probiotics produce substances that have the ability to alert the immune system against pathogen agents. These immunomodulators which increase the immune response (Rendón and Balcazar, 2003) can be extracted from the cell walls of microorganisms such, as Gram-negative bacteria (lipopolysaccharides), Gram positive bacteria (peptidoglycan) and fungi (β-1,3-glucan). These immunostimulants can be applied by immersion and injection; however, the most practical method for the administration of these immunostimulating substances is by integration to the feed. There are several studies which have tried to explain the different mechanisms by which probiotics stimulate the fish immune system, which is immune cells, antibodies, acid phosphatase, lysozyme, and antimicrobial peptides. Panigrahi et al. (2005) and Goncalves et al. (2011) demonstrated the increase of innate immune parameters such as lysozyme, and phagocyte activity with the use of *Lactobacillus rhamnosus* or their cell wall components on *Oncorhynchus mykiss* and as prophylactic factor to low stress in *Oreochromis niloticus,* respectively. Song et al. (2006) observed an increase in acid phosphatase and lysozyme activity in *Miichtys miiuy* fed with *Clostridium butyricum* indicating a stimulated immune system. Rodríguez et al. (2007) reported an enhancement of larval survival and WSSV resistance in *Penaeus vannamei* treated with probiotics. On the other hand, acquired immunity has not been observed in shrimp, but several studies have demonstrated the development of an immune response. Rengpipat et al. (2000) showed that *Bacillus* sp. can provide disease protection by activating *P. monodon* immune defenses. Balcazar et al. (2004) show that the administration of a mixture of *Bacillus* sp. and *Vibrio* sp. promotes the resistance of juveniles of *L. vannamei* against *V. harveyi*. *Vibrio* cells, lipopolysaccharide (LPS), peptidoglycan (PG), β 1-3 glucan, fucoidan, laminarin, and yeast glucans have been experimentally tested in small-scale culture and their results suggest that they can be used as an important element in the control of disease, because of their effect on shrimp or crustacean immunostimulation (Gullian et al., 2004; Pais et al., 2008. It has been shown that β 1 to 3 glucans improve resistance against various infectious diseases in fish, bivalves and shrimp (Rodríguez et al., 2007).

Adhesion

Probiotics are part of the resident microbiota and contribute to the health and well being of their host. The ability of some strains to adhere to mucus in the gastrointestinal tract, epithelial cells and other tissues is a common characteristic used in the probiotic selection because it is associated with bacteria colonization

Table 1. Probiotic used in aquaculture industry.

Identity of the probiotic	Origin	Used on	Application	Mode of action	Doses	Reference
Arthrobacter sp. (XE-7)	*P. chinensis*	*P. chinensis*	Water	Antagonism to *Vibrio* sp, *V. parahaemolyticus*.	10^6 cfu mL^{-1}	Li et al. (2005, 2008)
Alteromonas sp. (CA2)	Adult of *Crassostrea gigas*	Larvae of *C. gigas*	"	Better survival	10^5 - 10^6 "	Douillet and Langdon (1993)
Aeromonas media (A199)	Fresh water	*C. gigas*	"	Antagonism to *V. tubiashii* and better survival	10^4 cfu mL^{-1}	Gibson et al. (1998)
A. hydrophila, V. fluvialis, *Carnobacterium* sp	*O. mykiss* gut	O. *mykiss*	Premix with feed	Better survival, immunostimulation and enhanced lysozyme activity	10^6-10^8 cell g^{-1} of feed	Irianto and Austin (2002)
Bacillus sp. (S11)	*P. monodon gut*	*P. monodon*	Premix with feed	Antagonism to *V. harveyi* and water quality	10^{10} cfu mL^{-1}	Rengpipat et al. (2003)
"	"	"	"	"	10^{10} cfu g^{-1} of feed	"
"	"	"	"	Antagonism to *V. harveyi* and Immunostimulation	10^2 "	Rengpipat et al. (2004)
"	"	"	"	Antagonism to *V. harveyi*	10^{10} "	Meunpol et al. (2003)
" (P64)	*L. vannamei*	*L. vannamei*	Water	"	10^7 cell mL^{-1}	Gullian et al. (2004)
" (BT23)	Shrimp culture pond	*P. monodon*	"	"	10^6 - 10^8 "	Vasseharan and Ramasan (2003)
Bacillus sp.	Commercial product	*L. vannamei*	"	Better survival and growth	10^4 - 10^5 "	Moriarty (1998)
"	"	*Or. niloticus*	Feed	Better growth, survival and digestion	10^8 cfg g^{-1} of feed	Shelby et al. (2006)
"	"	*P. monodon*	Water	Bacterial population and health status of shrimp	?	Dalmin et al. (2001)
Identity of the probiotic	Origen	*Used on*	Application	Mode of action	Doses	Reference
Bacillus sp. (B2), *Vibrio* sp. (C33), *Pseudomonas* sp. (11),	Argopecten purpuratus culture water	*Larvae of A. purpuratus*	Natural survival Experiment in mass culture	Agree larvae survival without antibiotic supply	103 cfu mL^{-1}	Riquelme et al. (2001)
Bacillus sp., *Saccharomyces* sp.	Commercial product	*P. monodon*	Water	Water quality	108 + 105 cfu mL_{-1}	Matias et al. (2002)
Bacillus sp., *Nitrobacter* sp., *Nitrosomonas* sp.	"	"	"	"	108 "	"

Table 1. Probiotic used in aquaculture industry (continuation).

Bacillus sp., *S. cerevisiae*, *Nitrosomonas* sp., *Nitrobacter* sp.	“	*P. vannamei*	“	Water quality and bacteria control	104 - 109 “	Wang et al. (2005)
Spore of *B. subtilis, B. licheniformis, B. polymyxa, B. laterosporus, B. circulans*	“	*F. indicus*	Water supply and feed supplement	Digestion	106 - 107 “	Ziaei-Nejad et al. (2006)
B. pumilus, L. acidophilus, B. subtilis, Saccharomyces sp., *A. oryzae*	B. pumilis isolated from gonads of O. niloticus + Commercial product	*Or. niloticus*	Feed supplement	Growth, feed performance, and enhance immunity	106 and 1012 cfu kg^{-1} of feed	Aly et al. (2008 b)
B. licheniformis, B. subtilis	Commercial product	“	“	Growth performance	0.5 - 2.0% of the product	El-Haroun et al. (2006)
B. licheniformes, B. subtilis, L. acidophilus, S. cerevisiae	“	*Carnegiella strigata*	Water supply	Diminish the stress in larval transport, improve the health and survival rate	10 mg L-1 of the product	Carvalho-Gomes et al. (2008)
B. circulans (PB7)	Catla catla gut	*Ca. catla fingerlings*	Feed supplement	Growth and feed performance, and enhance immunity	104 - 106 cfu mL^{-1}	Bandyopadyyay et al. (2009)
B. subtilis	*Cirrhinus mrigala* gut	*Poecilia reticulata, Po. sphenops, Xiphophorus helleri, X. maculates*	“	“	10^5 - 10^8 “	Ghost et al. (2008)
B. subtilis	*Cirrhinus mrigala* gut	*Labeo rohita*	“	Enhanced innate immune	10^7 cfu g^{-1} of feed	Kumart et al. (2008)
B. subtilis (B10), *B. coagulans* (B16), *Rhodopseudomonas palustris*	Isolated form *Cyprinus carpio*	*Or. niloticus*	Dissolved on water	Growth performance and enhance immunity	10^7 cfu mL^{-1}	Zhou et al. (2009)
B. toyoi, B. cereus	Commercial product	*Dentex dentex* L.	Feed supplement	Growth performance, survival and increase the liver proteolytic activities	0.5, 1.0 and 2 g kg^{-1} of the products	Hidalgo et al. (2006)
B. pumilus, B. firmus and *Citrobacter freundii*	*Or. niloticus* gut	*Or. niloticus*	“	Resistance to *Aeromonas hydrophila* infection	10^7 - 10^9 cfu mL^{-1}	Aly et al. (2008a)
B. pumilus, *B. sphaericus*, *B. subtilis*	*P. monodon*	*P. monodon*	Feed	Antagonism to *V. harveyi* and immunostimulation	$10^{11\text{-}12}$ cfu g^{-1} of feed	Purivirojkul et al. (2005)
C. maltaromaticum (B26), *C. divergens* (B33)	*O. mykiss* gut	*O. mykiss*	Feed supplement	Antagonism to *A. salmonicida, Y. ruckeri,* Gram – and + bacteria	>10^7 “	Alavandi et al. (2004)
Debaryomyces hansenii (AY1)	*Haliotis midae gut*	*H. midae*	“	Better Growth and survival to V. anguillarum infection	106 to 107 “	Macey and Coyne (2005)

Table 1. Probiotic used in aquaculture industry (continuation).

Debaryomyces hansenii (CBS 8339)	*O. mykiss* gut	*Sparus aurata*	"	Immunostimulation at cellular level	10^6 "	Reyes-Becerril et al. (2008)
Enterococcus faecium (ZJ4)	Piglet gut	*Or. niloticus*	Dissolved on water	Growth performance and Immunostimulation	10^7 cfu mL^{-1}	Wang et al. (2008)
Kocuria SM1	*O. mykiss* gut	*O. mykiss*	Feed supplement	Immunostimulation and protection against *V. anguillarum*	10^8 cfu g $feed^{-1}$	Sharifuzzaman and Austin (2009)
Lactobacillus sp. (NS6.1)	*Nodipecten subnodosus*,	*C. corteziensis*	Water	Better growth and survival	10^4 cell mL^{-1}	Campa-Cordova et al. (2009)
Lactobacillus bulgaricus (NCIM 2056, NCIM 2057)	NCIM collection	*F. indicus*	Feed	Antagonism to *V. alginolyticus*	10^6 "	Ajitha et al. (2004)
L. acidophilus (NCIM 2285)	NCIM collection	*F. indicus*	Feed	Antagonism to *V. alginolyticus*	10^6 "	"
L. delbrueckii delbrueckii AS13B)	*Dicentrarchus labrax* gut	*D. labrax*	Artemia naupli and Rotifers as vector	Better body weight	10^5 cell mL^{-1}	Carnevali et al. (2006)
L. delbrueckii delbrueckii	"	*D. labrax, S. aurata*	Oral administration	Better growth, decreased cortisol levels, and immunostimulation	"	Abelli et al. (2009)
L. delbrueckii delbrueckii (AS13B)	"	*D. labrax*	Rotifers and Artemia as vector	Gut integrity, an increase of T-cells and acidophilic granulocytes	10^5 cell cm^3	Picchietti et al. (2009)
L. delbrueckii ssp. Lactis, *B. subtilis*	CECT collection	*S. aurata*	Oral administration	Increase of phagocytic and cytotoxic activity	10^7 cfu g^{-1}	Salinas et al. (2005)
L. fructivorans (AS17B), *L. plantarum* (906)	*S. aurata* gut, and human faeces, respectively	*S. aurata*	Rotifers and Artemia as vector	No inflammatory effect after tissue damage	10^{10} cfu g^{-1} of feed	Picchietti et al. (2007)
L. sakei (CLFP 202), Lactococcus lactis ssp. lactis (CLFP 100), Leuconostoc mesenteroides CLFP 196	*CLFP collection*	*O. mykiss*	Feed supplement	Reduced the severity of furunculosis, and enhanced humoral and cellular immune response	106 "	Balcazar et al. (2007)

Table 1. Probiotic used in aquaculture industry (continuation).

L. rhamnosus (JCM 1136)	JCM collection	"	Feed	Immunostimulation	10^{11} "	Panigrahi et al. (2005, 2011)
L. rhamnosus, *Enterococcus faecium* and *B. subtilis*	JCM and ATCC collection	"	"	"	10^{9} "	Panigrahi et al. (2007, 2011)
Photosynthetic bacteria and *Bacillus* sp.	*Cy. carpio* culture pond	*Cy. carpio*	Feed supplement	Growth performance and increment the enzyme activities	10^{10} cfu Kg^{-1}	Yanbo and Zirong (2006)
Pseudomonas sp. (PS-102)	Lagoon	*P. monodon*	Water and intramuscular injection	Antagonism to *Vibrio* sp.	10^{5-8} cfu mL^{-1}	Vijayan et al. (2006)
Pseudomonas sp. (PM 11)	*P. monodon*	"	Water	Immunostimulation	10^{3} "	Alavandi et al. (2004)
P. aeruginosa (YC58) + *Burkholderia cepacia* (Y021)	*L. vannamei*, *C. corteziensis* (respectively)	*C. corteziensis*	"	Better survival	10^{4} "	Campa-Cordova et al. (2009)
S. cerevisiae, *S. exigus*, *Phaffia rodozoma*	Commercial product	*L. vannamei*	Premix with feed	Immunostimulation and protection to *Vibrio harveyi*	1% of feed diet	Scholz et al. (1999)
S. cerevisiae	"	Hybrid of *O. niloticus* x *O. aureus*	Feed supplement	Better growth and immunostimulation	0.125, 0.25, 0.5 and 2 g kg^{-1} of product	He et al. (2009)
S. cerevisiae	Commercial live bakers´yeast	*O. niloticus*	"	"	0.25, 0.50, 1.0, 2.0 and 5.0 g yeast kg^{-1} diet	Abdel-Tawwab et al. (2008)
S. cerevisiae, E. faecium, L. acidophilus, L. casei, L. plantarum, L. brevis	Commercial product,	*O. mykiss*	"	Enhance the utilization of soybean meal as protein source	0.1% of the product in the diet	Sealey et al. (2009)
S. cerevisiae (NCYC Sc 47/g), *S. cerevisiae* var. *boulardii* (CNCM I-1079)	NCYC and CNCM collection	"	"	Increment the digestive enzymes	10^{6} cfu g^{-1} of diet	Weché et al. (2006)
Shewanella putrefaciens (Pdp11)	*S. aurata*	*Senegalese sole*, *Solea senegalensis*	Oral administration	Reduce the attachment to skin and intestinal sole mucus of *V. harveyi*, better fish survival	10^{8} cfu g^{-1}	Chabrillon et al. (2005)
Shewanella putrefaciens (Pdp11)	"	*S. aurata*	"	Reduce the attachment to mucus of *L. anguillarum*, better fish survival	10^{8} cfu g^{-1} of fish	Chabrillon et al. (2006)
Streptococcus cremoris	NCIM collection	*F. indicus*	Feed	Antagonism to *V. alginolyticus*	10^{6} cell g^{-1} of feed	Ajitha et al. (2004)

Table 1. Continuation.

Streptomyces sp	Estuary sediment	*P. monodon*	"	Water quality and bacteria control	2-10 g of dry mat/kg feed	Das et al. (2006)
Vibrio midae (SY9), *Cryptococcus* sp. (SS1)	*H. midae* gut	*H. midae*	Growth study and challenge with *V. anguillarum*	Better activity of intestinal enzyme	10^6 to 10^7 cfu g^{-1}	Macey and Coyne (2005)
Tetraselmis suecica (CS-187)	CSIRO collection	*F. indicus*	Feed	Antagonism to *Vibrio* spp.	10^4 cell mL^{-1}	Regunathan and Wesley (2004)
Vibrio alginolyticus	*L. vannamei*	*L. vannamei*	Water	"	10^{3-5} "	Vandenberghe et al. (1999)
"	Beach sand	"	Feed and bath (10 min)	Antagonism to *V. parahaemolyticus*	10^6 "	Garriques and Arevalo (1995)
" (Ili)	"	"	"	Antagonism to *V. harveyi*	10^7 "	Gullian et al. (2004)
V. fluvialis (PM 17)	*P. monodon*	*P. monodon*	"	Immunostimulation	10^3 "	Alavandi et al. (2004)
Vibrio proteolyticus (VP)	?	*Paralichthys oilvaceus*	Feed supplementation	Stimulation of apparent nitrogen digestibility	10^{10} "	Schrijver et al. (2000)
Vibrio sp. (P62, P63)	*L. vannamei*	*L. vannamei*	Water	Antagonism to *V. harveyi*	10^7 "1	Gullian et al. (2004)
Yeast, Grobiotic-A	?	*O. mykiss*	Feed	Better survival after challenge with IHNV	2% of feed inclusion	Sealey et al. (2007)

(Farzanfar, 2004; Crittenden et al., 2005). The main objective for adhesion is to deliver a high and significant level of bacteria in the host and preventing them from being flushed out by the movement of food through the digestive tract. By attaching to the intestinal mucosa, probiotics can extend their time within the gut thereby influencing the gastrointestinal microbiota of their host (Rengpipat et al., 2003; Alavandi et al., 2004). Adhesion of probiotic bacteria to the intestinal mucosa has been shown to enhance their antagonistic activity against pathogens. The attachment ability of some bacteria have been tested *in vitro* and *in vivo,* and results suggest that the pathogen was displaced by the potential probiotic, based on the ability of probiotics to attach to the mucus, where pathogen growth in the digestive tract might be suppressed by the candidate probiotic presence (Farzanfar, 2004; Vine et al., 2004b). This characteristic is highly associated with competition for essential nutrients, space, etc. In a healthy gut, attachment may allow the probiotic to exert its beneficial effects whilst in a diseased gut it may reduce the possibility of pathogen translocation when the host's defense mechanisms are impaired. Different strains of acid lactic bacteria, like *Enterococcus faecium* and *Lactobacillus* sp.; and other groups of bacteria Gram-positive and Gram-negative as *Bacillus* sp., *Vibrio* sp., have been tested and used as probiotic for the ability of adhesion (Irianto and Austin, 2002; Rengpipat et al., 2003; Ajitha et al., 2004; Vine et al., 2004a, b; Swain et al., 2009).

Disease prevention and control

Some probiotic are used as an environmentally friendly method that impacts the growth of aquatic pathogens and enhances the growth of beneficial bacteria, leading to improved water quality and healthier aquatic organisms. Brunt et al. (2007) used different bacteria stains (10^8 cells g^{-1} of feed) and detected a better survival rate of rainbow trout (*O. mykiss*) infected with *Aeromonas salmonicida*, *Lactococcus garvieae*, *Streptococcus iniae*, *Vibrio anguillarum*, *Vibrio ordalii* or *Yersinia ruckeri*. Different bacteria species have been isolated from aquatic organism's culture water,sediment pond, gastrointestinal tract, skin mucus, and gills. Those probiotics are based in the principle of competitive exclusion, enzyme production, stress resistance, and or immunostimulation, which are the main preventive methods against aquatic pathogen (Gatesoupe, 2002; Gullian et al., 2004; Ali, 2006; Ninawe and Selvin, 2009). However, the positive result of some potential probiotic strains against fish, crustacean, or bivalve pathogens, tested under *in vitro* conditions, was different when those probiotics were used *in vivo* infection tests under laboratory conditions, or when used in a commercial production setting (Nimrat and Vuthiphandchai, 2011). Therefore, more research is needed to evaluate the effect of probiotics as diseases control in aquaculture.

Digestive processes

In order to understand the function and potential contribution of probiotics towards the health and well-being of aquatic organisms, in-depth knowledge of the digestive tract as an ecosystem is required (Yang-Bo et al., 2008). The digestive tract of aquatic organisms is an open system in constant contact with the surrounding environmental water. Compared to water, the digestive tract is an ecosystem richer in nutrients and therefore more suitable for the growth of the majority of bacteria. Gastrointestinal bacteria (GIT) take part in the decomposition of nutrients, providing the macroorganisms with physiologically active materials, such as enzymes, amino acids, and vitamins (Moriarty 1998; Ramirez and Dixon, 2003).

An important effect of the use of probiotics that has not been extensively studied, but which has demonstrated a significant effect, is feed efficiency and the growth promotion of aquatic animals by probiotic supplementation (Gatesoupe, 2002; Lara-Flores et al., 2003). Probiotics applied to the feed, reach the intestine of the animals and improve their health (Das et al., 2006). The probiotics transit through the stomach, attaching to the intestine and use a large number of carbohydrates for their growth, producing a range of relevant digestive enzymes (amylase, protease and lipase) that increase the digestibility of organic matter and protein, resulting in a higher growth (Lara-Flores et al., 2003; El-Haroun et al., 2006). In finfish, the use of probiotics (acid lactic bacteria and yeast) has demonstrated beneficial effects on growth performance, feed efficiency and digestibility of organic matter and protein (Schrijver and Ollevier, 2000; Lara-Flores et al., 2003).

In some cases this beneficial effect was attributed to the capacity of the probiotic to stimulate and or produce some enzymes on the intestinal tract. For example, Lara et al. (2000) observed a high activity of alkaline phosphatase in Nile tilapia (*Oreochromis niloticus*) when probiotics were administered in the diet; and that this high activity reflected a possible development of brush border membranes of enterocytes, stimulated by the probiotic, which can be an indicator of carbohydrate and lipid absorption and explain the higher weight gain and the better feed conversion. In shrimp, the beneficial effects of probiotics in the nutritional and digestive process have been reported (Farzanfar, 2004; Lin et al., 2004; Ziaei-Nejad et al., 2006), where *Bacillus* sp. and spores of *B. subtilis*, *Bacillus licheniformis*, *Bacillus polymyxa*, *Bacillus laterosporus*, *Bacillus circulans* have been used. Ziaei-Nead et al. (2006) examined the effects of *Bacillus* sp. on *F. indicus* at different shrimp stages. In pond, probiotic treated shrimp showed a significantly higher activity of amylase, total protease, and lipase. The addition of *Bacillus* sp. in *L. vannamei* diets shows a significantly higher apparent digestibility of some essential nutrients as phosphorus (Lin et al., 2004). Hai et al. (2009) reported an improvent on the health of juvenile western king prawns when they were treated with *Pseudomonas synxantha* and *Pseudomonas aeruginosa* as a supplement in the formulated feed. There is ample research about the application of probiotic strains in shrimp, which indicate an apparent increase of some digestive enzymes; however, the short gut-residence time of feed on shrimp (45 to 90 min) (Beseres et al., 2005) difficult the evaluation of this parameter. It is important to point out that the attachment ability of certain probiotics observed *in vitro* cannot be assumed to have a similar effect *in vivo* (Guo et al., 2009).

Water and pond quality

*A*quaculture is developed at different levels of production (extensive to super-intensive), where their artificial conditions accumulate organic matter (unutilized feed, fecal matter and dead organisms) in the pond bottom (Matias et al., 2002, Wang et al., 2005, Zhou et al., 2009). These products decrease water quality, increase pollution when pond effluents are discharged in ecosystems, and damage the aquaculture settings. Some products have been used as probiotics in aquaculture ponds, where better overall growth and water quality were observed (Das et al., 2006; Zhou et al., 2009). However and according to a previous definition of probiotic (Ali, 2006), only products used directly in the organism are true probiotics, while the products applied in pond are considered as bioremediators.

Directly, a probiotic may produce a positive affect in water quality when this generate a better organism digestion, which reduces the nutrient excretion (protein principally) to pond water and while generating a better growth of the aquatic organisms cultured. Table 1 display some *Lactobacillus* sp. products with lactic acid production, which are mixed with feed and produce a positive affect in aquatic organism's digestion and water quality. The main idea of bioremediators microorganisms as probiotic products in aquaculture ponds is that some bacteria are more efficient in the transformation of the organic matter to their elemental constituents (C, O, N, H, P, Si) during the culture cycle, which generates a stable phytoplankton and organism growth. However, some studies have used bacteria such as *Bacillus* sp., *Nitrobacter* sp., and *Nitrosomonas* sp. in the aquaculture pond with unclear results. Matias et al. (2002) used that microbiota on a *P. monodon* pond with not significant difference in water quality between the experimental and control ponds (no microbial product supply); but Wang et al. (2005), using the same microorganisms in *L. vannamei* ponds, reported a reduction of the nitrogen and phosphorus levels. The best evidence about the positive use of this type of products is observed in biofiltration systems; where bacteria associated to nitrification process are inoculated in this system.

Closed recirculation systems (CSR)

The CRS represent a production alternative which has been used on an experimental, pilot, and commercial scale to increase the aquaculture production and water quality; and decrease disease, antibiotic, and chemotherapeutant treatments (Gonzalez-Gonzalez et al., 2009). CRS efficiency requires biofiltration where microbial communities are managed, and where probiotic and/or bioremediators strains represent a tool in this management process. However, in spite of the use of biofilters being a common practice in CRS, the use of probiotic and/or bioremediators strains is not so common, with only some bacteria available commercially and used principally in aquarium settings. Research on shrimp (*L. vannamei*) and fish (*Paralichthys olivaceus*) reared in CRS (Xiongfei et al., 2005; Taoka et al., 2006) using probiotics show that cultured organisms exhibited a better growth and survival than control groups. Furthermore, at least in fish (*P. olivaceus*) probiotic-fed fish displayed a better stress and immune response compared to control. The microbial ecology of CRSs along with the shrimp production cycle and its association to biofiltration processes are poorly evaluated areas, where a better understanding will probably help in the advancement of probiotic use in CRSs at pilot or commercial scale.

Author opinion

The positive effects of probiotics on aquatic animals and or their environment is poorly understood, where some effects such as: i.- Adhesion, colonization, or competitive exclusion; ii.- Growth, survival, and health of aquatic animals; or iii.- Water or pond quality; are only evidenced with the isolation of the probiotic strain after it is applied to the system. Sometimes, this type of information affects the real potential of a probiotic designed for the aquaculture industry. However, different species-specific probiotics have been thoroughly researched and show a potential for their use in aquaculture. The use of probiotic offers a suitable alternative for the stabilization of the gut environment and the enhancement of the immune system which in turn help in the health of aquatic organisms. Aquatic health research represents an interesting opportunity for the future application of probiotics in aquaculture, which associated with good management practices may help to increase aquaculture production.

The probiotic strains used on farms and aquatic laboratories need attention and research. Some probiotic strains are developed with specific adhesions or colonization properties, antimicrobial product production, or synergistic action; which could represent a possible environmental risk if they are supplied indiscriminately to the aquaculture industry. The use of those probiotic strains is necessary to reach an understatement before massive application on aquaculture.

The literature evidence shows that a probiotic can have different results depending on the organism (fish, crustaceans, bivalves), application area (intestinal biofilm, water, sediment) and culture conditions (extensive, intensive, CRS). This suggest that the future progress of probiotic research could be directed to: (i) A better understanding of the bacterial ecology and target organs for probiotics, (ii) the strain source, doses and their respective effects on a particular host, (iii) specific mechanisms of adhesion and or colonization, (iv) the activation of the host immune system, and (v) the environmental effect on culture conditions at pilot scale.

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